Suspended and Attached Growth Cellular ATP Food-To-Microorganism Ratios for Real-Time Reduction in Ammonia Oxidation Detection

by

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BScE – Chemical, University of New Brunswick, 2019

A Report Submitted in Partial Fulfillment
of the Requirements for the Degree of

Master of Engineering

in the Graduate Academic Unit of Civil Engineering

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This report is accepted by the Dean of Graduate Studies

THE UNIVERSITY OF NEW BRUNSWICK

April, 2024

Abstract

A packaged dairy plant waste resource recovery facility in southern USA is experiencing long periods of inefficient ammonia removal. To investigate, alternative food-to-microorganism (F:M) ratios using suspended cellular ATP (cATP), attached growth ATP (agATP), and theoretical oxygen demand (ThOD) were used. The system concentrations of ThOD and suspended cATP were key to this systems issues. This study also challenges the conventional assumption that attached growth biomass protects treatment efficiency from surges in organic loading. The percentages of agATP in comparison to suspended cATP in 2022 and 2023 were only 7.2 ± 1.8% and 13 ± 5.0%, respectively. 16S Next-generation sequencing (NGS) confirmed the differences as the top 85% of the suspended biomass community represents only 6% of the attached biomass community while the top 85% of the attached biomass community represents only 5% of the suspended biomass community.

Acknowledgements

I would like to express my appreciation to my supervisor, Dr. Kripa Singh, P. Eng., without his supervision this report would not have been possible. I am grateful for the support, guidance, and mentorship he provided me throughout this report.

I would like to thank Dr. Jordan Schmidt and Kristine Walker from LuminUltra Technologies Ltd. for their support throughout my Master of Engineering journey. Their encouragement and assistance were invaluable to the completion of this study.

Finally, I would like to thank my partner, Jason, and my family, who have supported me every step along the way.

Table of Contents

List of Tables

List of Figures

List of Abbreviations and Acronyms

agATP	Attached Growth Adenosine Triphosphate
AOX	Ammonia-Oxidizing
ASP	Activated Sludge Process
cATP	Cellular Adenosine Triphosphate
COD	Chemical Oxygen Demand
DAF	Dissolved Air Flotation
dATP	Dissolved Adenosine Triphosphate
DN	Denitrifying
F:M	Food-to-Microorganism Ratio
HDPE	High-Density Polyethylene
IFAS	Fixed film Activated Sludge
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
NGS	Next Generation Sequencing
NOX	Nitrite-Oxidizing
RAS	Return Activated Sludge
sCOD	Soluble Chemical Oxygen Demand
SND	Simultaneous Nitrifying and Denitrifying
tATP	Total Adenosine Triphosphate
ThOD	Theoretical Oxygen Demand
ThNOD	Theoretical Nitrogenous Oxygen Demand

TKN	Total Kjeldahl Nitrogen
WAS	Waste Activated Sludge
WRRF	Waste Resource Recovery Facility

1. Introduction

Ammonia removal is a major concern within wastewater treatment today due to significant challenges created when insufficient treatment occurs. Excess ammonia in surface water can cause eutrophication and the reduction of dissolved oxygen which is cause for concern for effluent disposal of wastewater into the environment due to nitrification (Tan, Tan, Tea, & Li, 2006) (Ibrahim, Sabeen, Noor, & Ahmad Mutamim, 2020). The importance of sufficient wastewater treatment has led to an increase in continuous research around preventative action toward endangering the environment.

A packaged dairy plant water resource recovery facility (WRRF) in southern USA has been experiencing intermittent reductions in ammonia oxidation over several months. It became apparent when the effluent ammonia concentration from the WRRF continuously exceeded the expected range. The reduction in ammonia oxidation has not been easily explained using traditional wastewater treatment monitoring parameters. The WRRF consists of Dissolved Air Flotation (DAF) primary clarification, anoxic-aerobic Integrated Fixed-film Activated Sludge (IFAS) system, and DAF secondary clarification. Internal recycle, return activated sludge (RAS), and waste activated sludge (WAS) are also used in the system to allow for total nitrogen removal, organics removal, and to maintain healthy microorganisms. On top of regular monitoring parameters, an additional monitoring program was implemented from May of 2022 to June of 2023 to test for suspended cATP, attached cATP (agATP), and Theoretical oxygen demand (ThOD) in the aerobic bioreactor to investigate the system concerns. In 2022 a period of inefficient ammonia removal was observed and in 2023 a period of stable ammonia removal was observed.

A common control parameter in activated sludge systems is the Food-to-Microorganism ratio (F:M ratio). Operators have often relied on this parameter to optimize the treatment of the influent wastewater and to improve efficiency of oxidation (Mason, Watson, Forth, & Schmidt, 2019). There are general guides found throughout industry for the F:M ratio and the accepted traditional range is 0.2-0.6 kg BOD/kg MLSS/d (Mason, Watson, Forth, & Schmidt, 2019). However, every process is different and each F:M ratio is wastewater and process water specific meaning the F:M ratio must always be adjusted. This study will be investigating an alternative F:M ratio that will respond in real-time to reductions in ammonia oxidation to F:M ratios using common parameters like sCOD and MLSS. Instead of waiting days for commonly used substrate results like BOD or $cBOD_5$, cATP and ThOD can be used. ThOD in this study was calculated using the addition of sCOD and theoretical nitrogenous oxygen demand (ThNOD), thus incorporating ammonia oxidation into the equation. LuminUltra Technologies Ltd. ATP testing provides reliable and accurate measurements of cATP, all living biomass in a bioreactor within a matter of minutes. ThOD, suspended cATP, and agATP were used in the alternative F:M ratios to show a direct relationship with inefficient ammonia removal.

To maintain an efficient activated sludge system other parameters and factors should be considered. This could be parameters like influent ammonia concentration, substrate loading, and factors like the microbial communities present. A healthy microbial community is necessary for efficient treatment. Microbial populations on attached growth and suspended growth were investigated in this report in attempts to further understand their treatment capabilities and relative distributions. This study questions the common assumption that attached growth biomass protects the wastewater treatment efficiency from complications related to surges in concentration.

1.1 Problem Statement

Several industries today release effluent wastewater directly into the environment. Therefore, it is key to treat wastewater efficiently to protect fish, fish habitat, and human health. To ensure communities and the environment are protected, regulations and guidelines are set in place. In Canada, industries are required to follow Wastewater System Effluent Regulations, under the Fisheries Act (Government of Canada, 2024). For an industry to stay operational, it must continuously meet all guidelines for their specific area, which means process efficiency needs to be optimized.

Process efficiency is a common factor within plant operations that should always be improved, and water resource recovery is no exception. Traditional wastewater monitoring fails to explain losses in ammonia oxidation which can occur due to changes in characteristics of influent wastewater. Changes in influent wastewater can happen due to surges in concentration, like ammonia or nitrogen, which can negatively impact the ammonia removal efficiency of suspended and attached growth systems. Surges in concentration can directly impact IFAS systems which results in a decrease in process efficiency, thereby affecting wastewater discharge quality. Additionally, the relative distribution and efficiency of attached and suspended growth is not truly known. The conventional assumption is that attached growth protects the efficiency of wastewater treatment systems from influent surges in organic materials, however, this assumption is questioned.

IFAS systems are used globally throughout industry and by using alternative F:M ratio parameters, this study can show the benefit of using the non-conventional parameters to adjust to common system issues, like ammonia removal. This WRRF in southern USA and any activated sludge system suffering with decreases in nitrification would benefit from this report. The report will show and provide industries a possible alternative to adjust treatment in real-time to reductions in ammonia oxidation. Industries can use this information to improve their processes to become more efficient, with more consistency, and production of higher quality treated water for safe discharge.

Furthermore, several activated sludge systems use media to improve removal efficiencies, however, the true effectiveness of removal is not very well known. This study may put the conventional assumption about the efficiency of attached growth in question as cATP and 16S NGS will be used to evaluate the true active biomass population. Therefore, several industries, design engineers, and operators could benefit from the findings in this report.

1.2 Research Target and Objectives

This research was done to investigate the relationships and patterns of inefficient ammonia removal to alternative F:M ratios using ThOD, suspended cATP, and agATP. On top of this, to investigate trend comparisons along with relative distributions between attached growth and suspended growth biomass during treatment. With a better understanding of these relationships and trends, the overall goal was to find countermeasures that can be used to identify and improve inefficiencies in ammonia removal at WRRF's based on the results of this study. These goals were accomplished through the following four objectives:

1. To analyze data collected on top of regular process parameters from the packaged dairy plant WRRF in southern USA which include suspended cATP, agATP, and ThOD.
2. To identify trends between ammonia removal and alternative F:M ratios using ThOD, suspended cATP, and agATP.
3. To determine the optimum relationship between ammonia removal and an alternative F:M ratio.
4. To assess and compare the relative distribution and effectiveness of suspended growth and attached growth during day-to-day treatment.

1.3 Assumptions

The following assumptions have been made for this study:

- The cATP tests done for suspended growth will represent the overall composition of the active suspended growth within the aerobic bioreactor.
- The agATP tests done for attached growth will represent the overall composition of the active attached growth within the aerobic bioreactor.
- There were no cATP or agATP losses throughout the tests.
- The ammonia and sCOD data collected and used in the ThOD calculation will represent the overall composition of the ThOD within the aerobic bioreactor.
- All operators completed the ATP tests as per the LuminUltra Technologies Ltd. instructions, with no modifications.
- The suspended biomass 16S NGS sample will represent the overall suspended biomass population within the aerobic bioreactor.
- The attached biomass 16S NGS sample will represent the overall attached biomass population within the aerobic bioreactor.

1.4 Research Scope and Restrictions

This study was focused on determining the relationship of alternative F:M ratios with ammonia removal performance during the water treatment process. The relationships between alternative F:M ratios using suspended cATP, agATP, and ThOD to ammonal removal was be evaluated. cATP tests were completed by operators at the packaged dairy plant WRRF in southern USA as per test the LuminUltra Technologies Ltd. instructions. Ammonia removal efficiency was the only aspect reviewed by the alternative F:M ratios. Suspended growth and attached growth changes in this plant were only compared to one another. Additionally, the chemicals used during the water treatment process by the WRRF are outside the scope of this research.

1.5 Expected Outcomes

The following are the expected results:

- Ammonia removal inefficiency will be directly related to periods of high F:M.
- Consistent ammonia removal will have a direct relationship with a consistent F:M.
- The alternative F:M ratios that includes cATP, agATP, and ThOD will magnify process changes relative to inefficient ammonia removal in comparison to F:M ratios using sCOD and MLSS.
- Suspended biomass will have more sensitivity to process changes than attached biomass.

2. Literature Review

When excess ammonia is released into the environment at high enough levels it can have negative impacts on the environment and aquatic life. Excess ammonia within the environment can cause over-fertilization and eutrophication (Government of Canada, 2024). This is an environmental issue as eutrophication increases plant and algae growth, like blue-green algae which is harmful to humans and animals (Vu, et al., 2023). Excess algae growth also results in the depleting of dissolved oxygen from water and oxygen rich water is essential to marine life. Aquatic organisms in high levels of ammonia also have a difficult time excreting the toxicant, which can potentially lead to death (United States Environmental Protection Agency, 2024). Many WRRF's do not have ammonia reduction as the primary objective. However, the increase in climate and environmental concerns to reduce eutrophication has led to more research into plant optimization for efficient ammonia removal. (Government of Canada, 2024).

WRRF biological processes like ASP are commonly used for BOD removal in wastewater treatment. These biological processes can also be optimized to remove nitrogen and ammonia compounds from wastewater. Figure 1 is a standard ASP configuration for nitrogen removal. The anoxic and aeration tanks can be separate or combined in one tank and separated by a baffle. There are several modifications and tank orientations that can be made for the ASP process to optimize treatment. For example, nitrified recycle from the end of the aeration tank to the beginning of the anoxic tank is a common modification added to increase the amount of nitrogen removed. Another modification that is commonly done, is to add carrier media to the aeration tank to make it an IFAS process. The benefit of an IFAS system is that it not only allows the removal of ammonia, but it also allows for an increased organic loading.

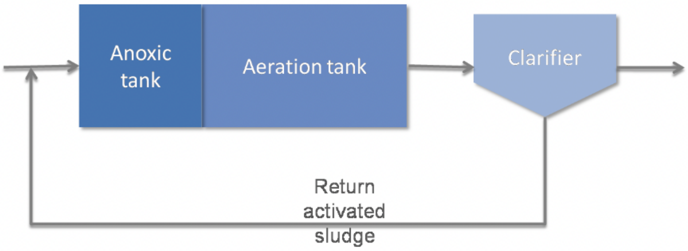


Figure 1: Standard ASP Modification for Nitrogen Removal (Curtin, Duerre, Fitzpatrick, & Meyer, 2011)

Ammonia removal occurs through nitrification and denitrification. During the aerobic section, if organic nitrogen is present, it is converted to ammonia through ammonification. All ammonia is then available for nitrification which occurs in two steps. In step one, ammonia is oxidized and converted to nitrite by nitrifying bacteria, often *Nitrosomonas.* Oxygen acts as the electron acceptor and ammonia acts as the electron donor. Another nitrifier, often *Nitrobacter,* is then used to oxidize nitrite to nitrate in step two. pH, alkalinity, and DO are environmental factors that need to be considered to promote positive nitrification rates. Additionally, efficient nitrification is completed by providing a long enough SRT to allow for nitrifiers to grow. (Rajesh Banu, et al., 2020)

Denitrification occurs in anoxic conditions, where nitrate is reduced to nitrogen gas by heterotroph bacteria like *Pseudomonas* (Larroche, Sanroman, Du, & Pandey, 2016). During denitrification, organic carbon is needed for the conversion and an additional carbon source is commonly added for efficient denitrification. Common carbon sources added are organic matter from the aerobic tank, methanol, and ethanol. (Rajesh Banu, et al., 2020)

To achieve an efficient activated sludge system, there must be a healthy microbial population in the aeration tank. *Nitrosomonas* and *Nitrobacter* mentioned previously are just two of the most common microbes in the process that assist in nitrification. Nitrification is completed through the assistance of several microbes like Ammonia-Oxidizing (AOX), Nitrite-Oxidizing (NOX), Denitrifying (DN), and Simultaneous Nitrifying and Denitrifying (SND) (Steeve, et al., 2022). To maintain a healthy and active microbial community, there must be a good quality and quantity of influent nutrients available. A healthy biomass population is necessary to allow flocs of biomass to form from the suspended growth which can then be removed through liquid-solid separation. Furthermore, in IFAS systems, it allows for the attached growth to create biofilm on the attached media. There has been an increase in research being completed on suspended growth and attached growth to optimize operations. It has been found that the size, shape, and volume of media can improve water treatment (Ibrahim, Sabeen, Noor, & Ahmad Mutamim, 2020). The conventional assumption is that attached growth systems protect WRRF outcomes from variable organic loading by reducing the amount wastewater treated by suspended growth (Ibrahim, Sabeen, Noor, & Ahmad Mutamim, 2020). This indicates that treatment is impacted by the change in the relative distribution created by the attached growth.

To service and monitor activated sludge systems, F:M ratio is often used. Operators often rely on the F:M ratio to optimize the oxidation of organic material by simply adjusting the biomass. The ease of use of this ratio is why it is used widely by operators throughout industry. The F:M typical range that is accepted by municipal and industrial industries is 0.2 – 0.6 kg BOD/kg MLSS/d, however the ratio must be adjusted to site-specific conditions (Mason, Watson, Forth, & Schmidt, 2019). Therefore, the optimal F:M range per site can vary which lacks consistency. The F:M ratio is calculated by the following equation (Metcalf & Eddy Inc, Tchobanoglous, Burton, Tsuchihashi, & Stensel, 2014):

$$\frac{F}{M} = \frac{total\ applied\ substrate\ rate}{total\ microbial\ biomass} = \frac{QS_o}{VX}$$

Where:

Q = influent wastewater flowrate, m^3/day

S_o = influent substrate concentration, g/m^3

V = aeration tank volume, m^3

X = biomass concentration in the aeration tank, g/m^3

The food portion of the F:M ratio is commonly calculated using biochemical oxygen demand (BOD). Chemical oxygen demand (COD) or soluble chemical oxygen demand (sCOD) are frequently substituted for BOD. BOD and COD measure the oxygen consumption which can be correlated to the influent organic loading. However, these methods can come with drawbacks. BOD testing takes 5 days to produce, and these tests are known to have interferences that can lead to inaccurate results (Mason, Watson, Forth, & Schmidt, 2019). When time-sensitive decisions are needed due to swift changes in influent organic loading, these limitations can make the F:M ratio difficult to use in real-time. Although COD only takes hours to produce test results, COD cannot distinguish between biodegradable and non-biodegradable compounds (Mason, Watson, Forth, & Schmidt, 2019). The incorporation of inorganic compounds can lead to inaccurate ratios.

ThOD can also be used as an alternative to the food portion of the F:M calculation. ThOD uses stoichiometry to determine how much oxygen is required to oxidize compounds. Therefore, it can be used to correlate specific targets, like ammonia. ThNOD can be used with influent sCOD as a food alternative to further review the treatment efficiency of ammonia removal. This creates a direct relationship with ammonia as through nitrification, 1 mg/L of ammonia requires 4.57 mg/L of oxygen to oxidize ammonia to nitrate, this can be seen through the total oxidation reaction of nitrification (Davis, 2010):

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$$

The microorganism portion of the F:M ratio is usually calculated using MLSS or MLVSS. The concentration of either parameter does not accurately depict what is going on throughout the system. MLSS cannot distinguish between biomass and other particulate matter. MLVSS cannot distinguish between active biomass and dead biomass or inert organics. Therefore, if there is a toxicity event throughout the system leading to excessive dead biomass, it would go undetected. The health of active biomass is dependent on the amount of food available to the system. If food becomes scarce, the amount of active biomass would decrease due to starvation. In this scenario, this leads to poor system representation through MLSS, as it would remain constant.

Another parameter, cATP, the true indicator of active biomass can be used as an alternative for the microorganism portion of the ratio. ATP is the energy carrier of all living organisms and can be broken down into three areas: cellular ATP (cATP), dissolved ATP (dATP), and total ATP (tATP). cATP measures only the living portion of cells, measuring the active biomass (Schmidt, et al., 2020). dATP is found outside of the living cells, measuring the cells that are dead or have just recently died and tATP is the sum of cATP and dATP. Therefore, cATP can be used within the F:M ratio to be a true indication of active biomass and detection of changes within the biomass can be seen in real-time (Mason, Watson, Forth, & Schmidt, 2019).

Understanding the importance behind the F:M ratio can lead to increased treatment performance. Using an alternative F:M ratio using ThOD and cATP can reduce the amount of ineffective substitute parameters used. Additionally, by incorporating ThNOD into ThOD, this creates a direct link into ammonia removal efficiency which is helpful if a plant suffers with continuous reductions in ammonia oxidation. The timelines of system upsets can be reduced if routine monitoring is completed because of real-time results.

This can lead to cost savings as it can reduce the amount of time needed for troubleshooting. Routine real-time monitoring can also decrease the treatment variability leading to more cost savings as it reduces the additional countermeasures needed to subside inefficient treatment.

The true effectiveness of suspended and attached growth is not well known. During changes in influent wastewater, like an increase in organic loading or a toxicity event, it is unclear which biomass growth is more sensitive. cATP allows for each biomass growth to be reviewed independently, but also compared to one another. Additionally, NGS is another method to compare each growth. As mentioned previously, AOX, NOX, DN, and SND are four nitrogen organism related functional groups that can be identified using 16S NGS. The percentage of nitrogen related organisms per growth can be reviewed to see how effective each growth is in relation to ammonia removal. In 2022, Steeves, et al. found that consistent complete nitrification could be achieved for a refinery wastewater treatment facility in southwestern United States by stabilizing the desired microbial communities. Prior to plant modications, the refinery assumed complete nitrification was occuring, when cATP testing and 16S NGS prooved otherwise (Steeve, et al., 2022).

There are other functional groups besides nitrogen related that can be reviewed and compared to investigate the true effectiveness of each growth. Slime formers are prokaryotes that are cabable of producing extracellular polysaccharide polymers which can cement themselves in the formation of biofilms (LuminUltra Technologies Ltd., 2023). Therefore, slime-forming is a functional group that can cause a reduction in treatment efficency and it is possible to see which growth is more prone to this metabolic functional group.

3. Methodology

There were two phases completed to achieve the goals of this study. The first phase was to implement a monitoring program; thus, organization, delineating and categorizing all data were essential. The second phase of this process includes completing a statistical analysis of the data collected to identify trends and relationships between an alternative F:M ratio using ThOD, suspended cATP, and agATP to ammonia removal. Concurrently the suspended biomass and attached biomass relative distributions and community data trends and differences will be identified to further understand their effectiveness within the process.

3.1 Materials and Methods

A monitoring program was implemented at the packaged dairy WRRF to do a complete investigation into the reduction of ammonia removal. As mentioned earlier, the WRRF consists of DAF primary clarification, anoxic-aerobic IFAS system, and DAF secondary clarification. Figure 2 shows a simplified process flow diagram of the WRRF with the effluent packaged dairy plant wastewater (process water), internal nitrified recycle, RAS and WAS streams. The monitoring program implemented added additional parameter monitoring on top of traditional process parameters like ammonia, pH, flow, MLSS, and sCOD. The additional monitoring program consisted of adding daily cATP testing in the aerobic bioreactor of both suspended growth and attached growth. The cATP test on suspended growth to provide the living biomass within the bulk liquid (suspended cATP) and the cATP test on the attached growth (agATP) to provide the living biomass on the carrier media. The monitoring program implemented started in May of 2022 and lasted until June of 2023.

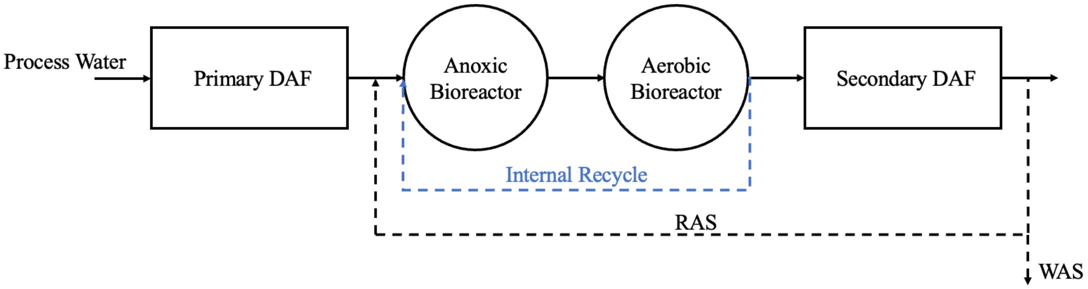


Figure 2: Simplified Process Flow Diagram

To accomplish the additional monitoring program, suspended cATP and agATP were measured using 2nd Generation ATP® testing that was provided by LuminUltra Technologies Ltd. This benchtop commercially available ATP technology was specifically designed for wastewater application testing. cATP is measured through introducing luciferase to a process sample in a test tube. Luciferase is an enzyme that is naturally occurring from fireflies, and when it is introduced to cATP, light is produced. Light is produced due to the reaction that occurs which is then measured using the PhotonMaster™ luminometer. The amount of light produced from the reaction with luciferase dictates how much cATP is present in the sample as there is a direct relationship. Figure 3 shows a prepared sample within a test tube being put into the PhotonMaster™ luminometer for measurement. It also shows other equipment required to complete the full ATP test. (LuminUltra Technologies Ltd., 2024)

Figure 3: 2nd Generation ATP® Testing Equipment (LuminUltra Technologies Ltd., 2024)

Samples of cATP and agATP were taken daily and as mentioned previously, suspended growth samples were taken directly from the bulk liquid. As for the attached growth samples, 3 carrier medias were used as the sample size for all samples of agATP. The aerobic bioreactor within the IFAS system is 50% full of the carrier media, roughly 345 m^3. Figure 4 is the media used in this WRRF, the HDPE Nutricell DTi3 biofilm carriers that have a diameter of 0.83 inches, void space of 79.6%, and an active surface area of 630 m^2/m^3 (Veolia Water Technologies, 2024). The samples of agATP were placed directly into a lyses buffer removing the need for swabbing, reducing biomass losses. The suspended cATP and agATP results found were used to derive the respected F:M ratios in this study.

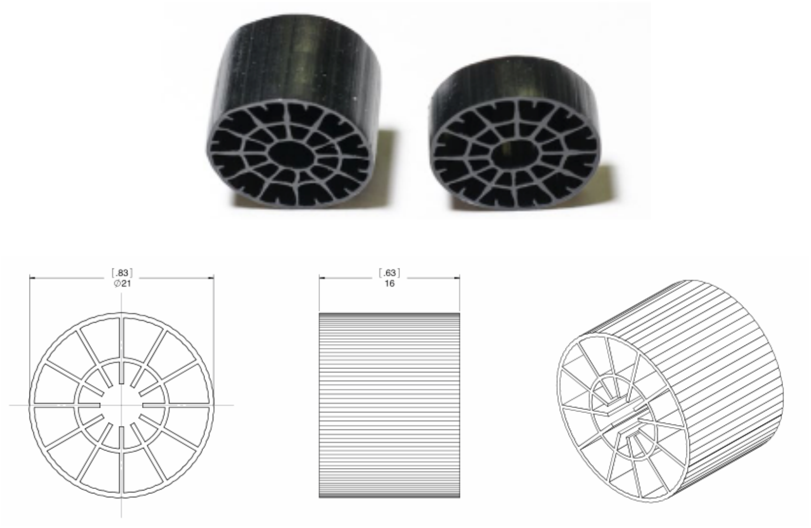


Figure 4: Nutricell DTi3 Biofilm Barriers (Veolia Water Technologies, 2024)

To analyze the microbial communities of suspended growth and attached growth, 16S NGS was completed through LuminUltra Technologies Ltd., their Microbial Community Analysis services. Through these services, NGS results provide a completed overview of the microbial activity within the process as the 16S provides the differentiation and identification of all microbe’s present within a sample (LuminUltra Technologies Ltd., 2024). This test was completed only twice in 2023. The two tests were completed during a period of efficient ammonia removal, once for the suspended growth and once for the attached growth.

Once all data was collected, compiled, and categorized, the second phase could begin. All data was collected and complied into Microsoft Excel. This included normal WRRF parameters, the additional monitoring program parameters implemented, and the 16 NGS results. Some of the normal WRRF parameters included COD, influent wastewater flowrate, ammonia, TKN, pH, turbidity, and MLSS, and as mentioned earlier, the additional monitoring program included ThOD, cATP, and agATP. The ThOD in this study was completed through the addition of ThNOD and sCOD. The 16S NGS results included the microbe's population, which was broken down into Kingdom, Phylum, Class, Order, Family, and Genus. The NGS results also included the categorized data of the sample, some examples of what metabolic functional groups included in this were: Nitrate-Reducing, Nitrite-Oxidizing, Nitrite-Reducing, and Slime-Forming.

Once all data was compiled and organized into Microsoft excel, a deep dive was completed through data analysis which included using graphs, pivot tables, and other Microsoft tools and equations. All analysis, comparisons, and results found in this study were completed in Microsoft Excel.

4. Analysis and Results

All data collected from the packaged dairy WRRF in southern USA was analyzed and investigated. The findings are presented in the following sections and as mentioned previously, the data was collected over the span of 2022 to 2023. Throughout the investigation, a period of continuous inefficient ammonia removal was observed over roughly three months in 2022, from August to October. In 2023, consistent ammonia removal was observed, therefore, a three-month period was used to compare against the inefficient removal timeline. The efficient removal timeline in 2023 that will be used for the comparison in this report is from March to May.

4.1 Ammonia Removal and COD Removal in 2022 and 2023

From the beginning of August to the end of October in 2022 shows that COD removal remained consistent and efficient, as shown in Figure 5. This was not the case for ammonia removal, where it reached a low of 10% removal in late August and a low of 26% removal in late October. While the reduction in ammonia oxidation in August only lasted for a couple weeks, the second inefficient ammonia removal period lasted all of October and even the last week of September. As for the 3-month period in 2023 shown in Figure 6, COD removal and ammonia removal remained consistent and at almost 100% removal for the entire timeline.

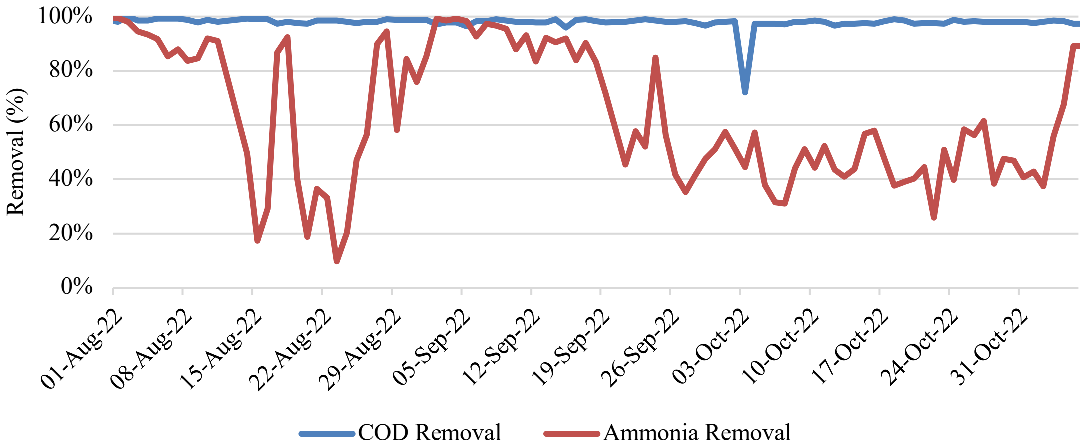


Figure 5: Ammonia Removal and COD Removal in 2022

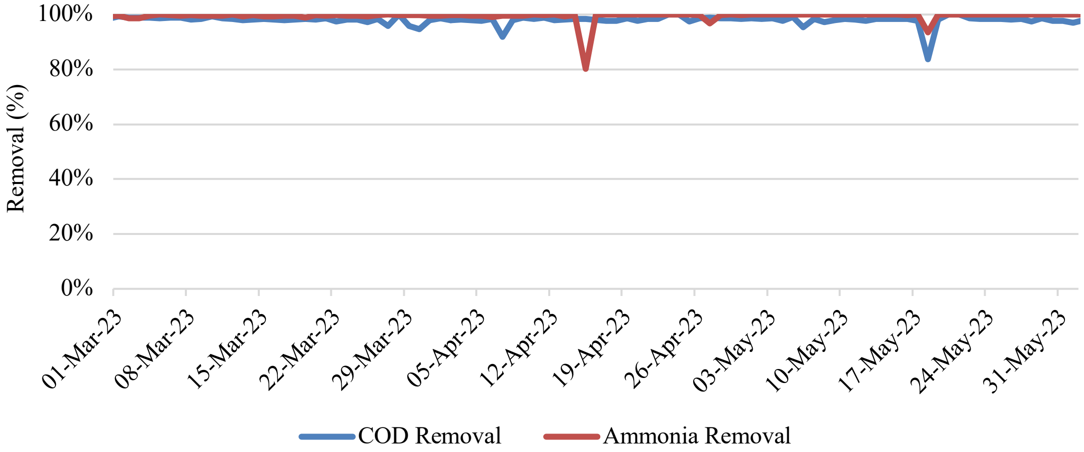


Figure 6: Ammonia Removal and COD Removal in 2023

4.2 F:M Ratios in 2022

The investigation into the 2022 inefficient ammonia removal continued by calculating both F:M ratios using suspended cATP and agATP. All F:M ratios calculated using suspended growth can be found in Figure 7 and all F:M ratios calculated using attached growth can be found in Figure 8. Furthermore, a sample F:M ratio calculation can be found in Appendix A.

4.2.1 Suspended Growth F:M Ratios

The three F:M ratios calculated in Figure 7 and compared to ammonia removal were completed using sCOD, ThOD, suspended cATP, and MLSS. The results show that inefficient ammonia removal is directly related to periods of high F:M. When comparing the response of sCOD to ThOD, it is observed that ThOD further magnifies the changes. The F:M calculated using MLSS does react to the ammonia removal trends, but it is less evident than the F:M ratios calculated using cATP. These results further indicate the benefit of using cATP for real-time process control.

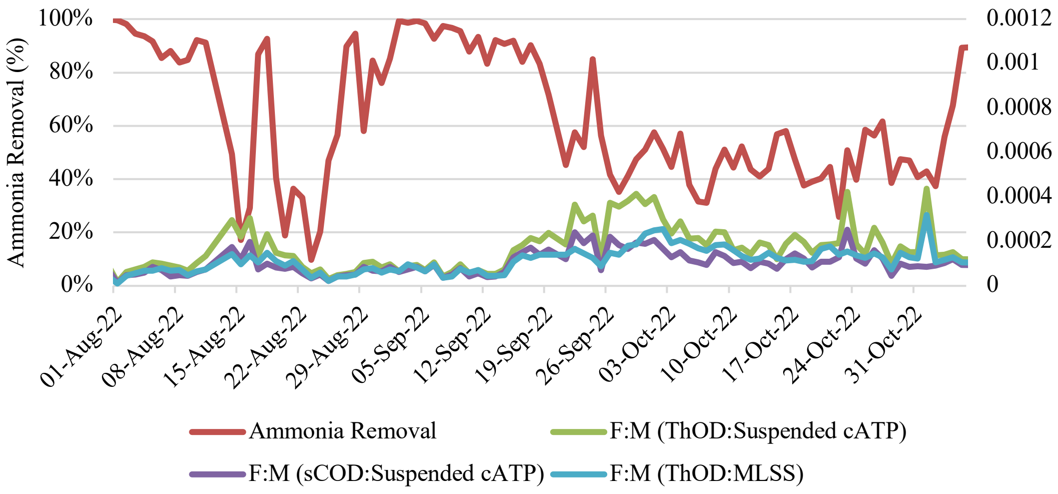


Figure 7: Suspended Growth F:M Ratios in 2022

4.2.2 Attached Growth F:M Ratios

Two F:M ratios were calculated to compare against the response to ammonia removal using agATP, sCOD, and ThOD. Figure 8 indicates that reductions in ammonia oxidation are directly related to periods of high F:M, showing another consistent relationship. Similarly, to the suspended growth trends, it is observed that the trends are further magnified by using ThOD in comparison to sCOD.

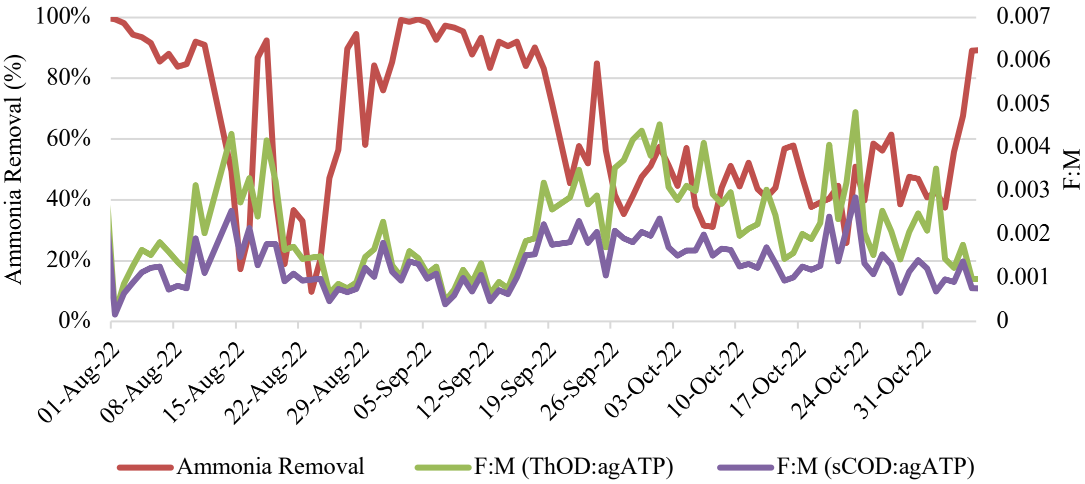


Figure 8: Attached Growth F:M Ratios in 2022

4.3 F:M Ratios in 2023

While 2023 showed consistent ammonia removal, F:M ratios were still calculated to confirm the relationship with ammonia removal. F:M ratios were calculated again using suspended cATP and agATP. F:M ratios calculated using suspended growth can be found in Figure 9 and F:M ratios calculated using attached growth can be found in Figure 10.

4.3.1 Suspended Growth F:M Ratios

The suspended growth F:M ratios were all calculated using the same methods and parameters as done for the 2022 suspended growth F:M ratios. The trends of the F:M ratios were like those in 2022, where ThOD and suspended cATP magnify process trends in comparison to sCOD and MLSS, respectively. However, the overall results observed in Figure 9 were unexpected, as the ammonia removal did not change with the F:M changes. This was another reason why this three-month period in 2023 was chosen, as while it was during a time of consistent ammonia removal, it also had the most fluctuations in F:M values. This left the question of whether decreases in ammonia removal can be detected using an alternative F:M ratio alone. However, it is important to note that this system is largely oversized for the current demand.

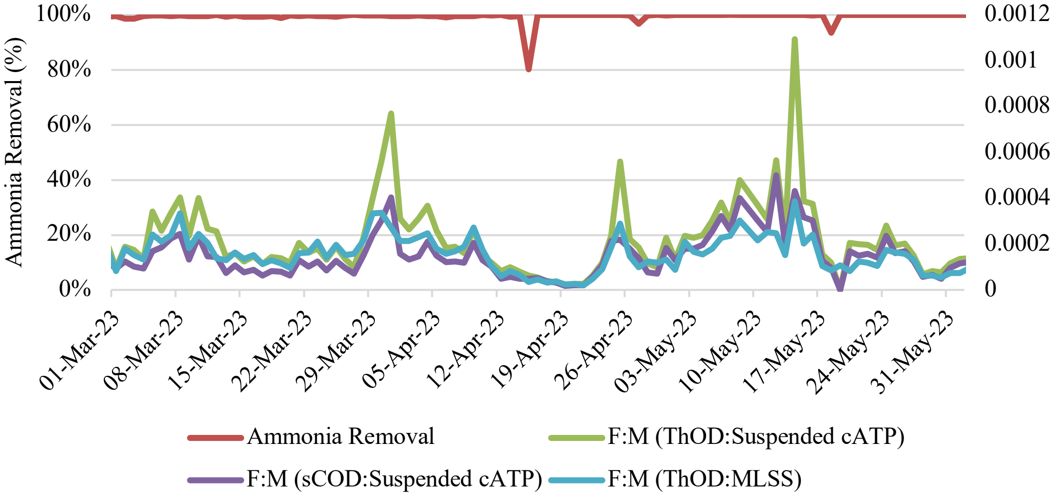


Figure 9: Suspended Growth F:M Ratios in 2023

4.3.2 Attached Growth F:M Ratios

The two attached growth F:M ratios were calculated using the same methods and parameters as done for the 2022 attached growth F:M ratios. Again, while the ThOD showed more sensitivity to process changes in comparison to sCOD, the overall results were unexpected. The ammonia removal did not react with the huge fluctuations in the F:M ratios using agATP. This is when further investigation was pursued.

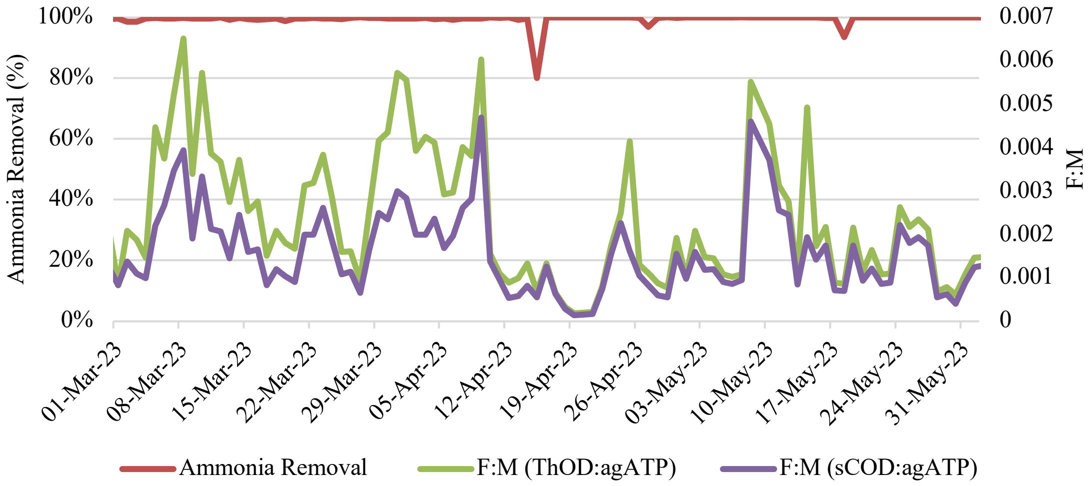


Figure 10: Attached Growth F:M Ratios in 2023

4.4 sCOD Loading, Flow, and Concentration

After the unexpected results from 2023, the sCOD loading, influent wastewater flowrate, and concentrations of sCOD, ThOD, and suspended cATP were reviewed. The influent wastewater flowrate for 2022 and 2023 can be found in Figure 11 and the sCOD loading for 2022 and 2023 can be found in Figure 12. Additionally, the concentration ratios calculated for 2022 and 2023 can be found in Figure 13 and Figure 14, respectively. The concentration ratios were calculated using suspended growth only.

4.4.1 Flow in 2022 and 2023

Figure 11 shows there is a notable change in the influent flow from 2022 and 2023. The average flows in the 3-month period in 2022 and 2023 were 79,648 ± 13,721 gal/day and 98,288 ± 9394 gal/day, respectively. This is a 19% difference with no observed complications, only treatment improvements. As mentioned previously, the system is largely oversized for its current use. Bringing the system size into consideration; it was now assumed that the hydraulic retention time is not an issue.

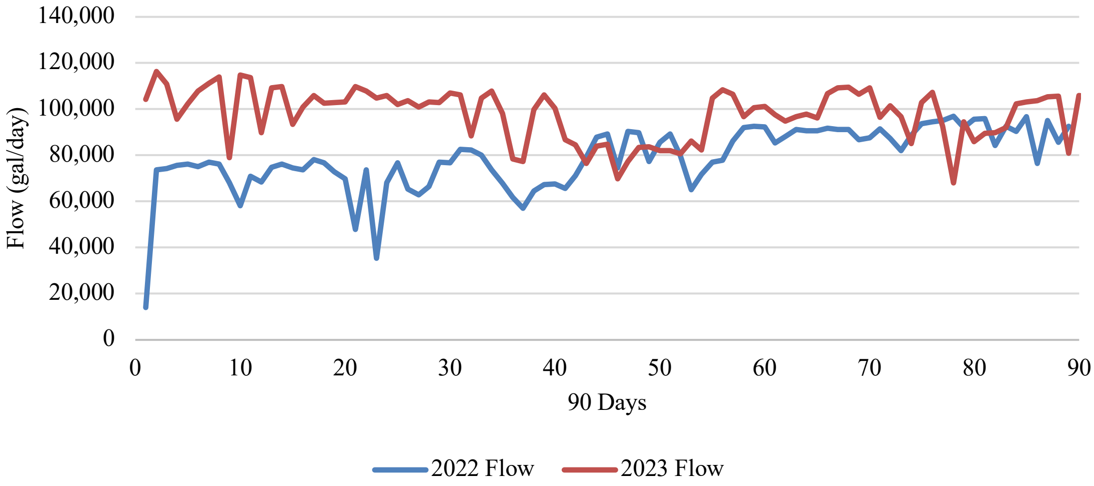


Figure 11: Flow in 2022 and 2023

4.4.2 sCOD Loading in 2022 and 2023

Figure 12 shows similar trends as Figure 11. The average sCOD loadings in the 3-month period in 2022 and 2023 were 248,176,492 ± 88,180,125 mg/day and 296,733,351 ± 119,389,876 mg/day, respectively. This is a 16% difference and again, there were no notable impacts to treatment from the increase, only improvements.

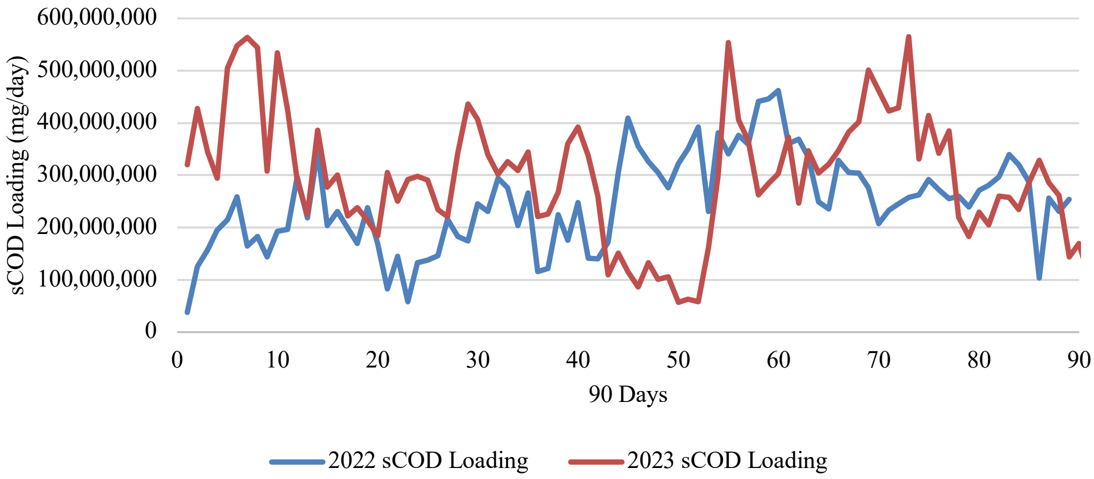


Figure 12: sCOD Loading in 2022 and 2023

4.4.3 Concentration Ratios in 2022 and 2023

The notable increases in both influent wastewater flowrate and sCOD loading from 2022 to 2023 with the added information of the system size justified the new assumption. At this point in the investigation, the new assumption is that hydraulic retention time is no longer a factor. This indicates that the ThOD, sCOD, and cATP concentrations could be a bigger factor for the WRRF. Figure 13 and Figure 14 show ThOD and sCOD to suspended cATP with ammonia removal in 2022 and 2023, respectively.

Figure 13 shows that there is a direct relationship between the concentration ratios of sCOD and ThOD to suspended cATP and ammonia removal. When a spike in ThOD to suspended cATP ratio is observed, a reduction in ammonia removal follows. This trend is continuous as it occurs in August and September. The ratio using ThOD clearly magnifies the cause-and-effect relationship in comparison to the ratio using sCOD.

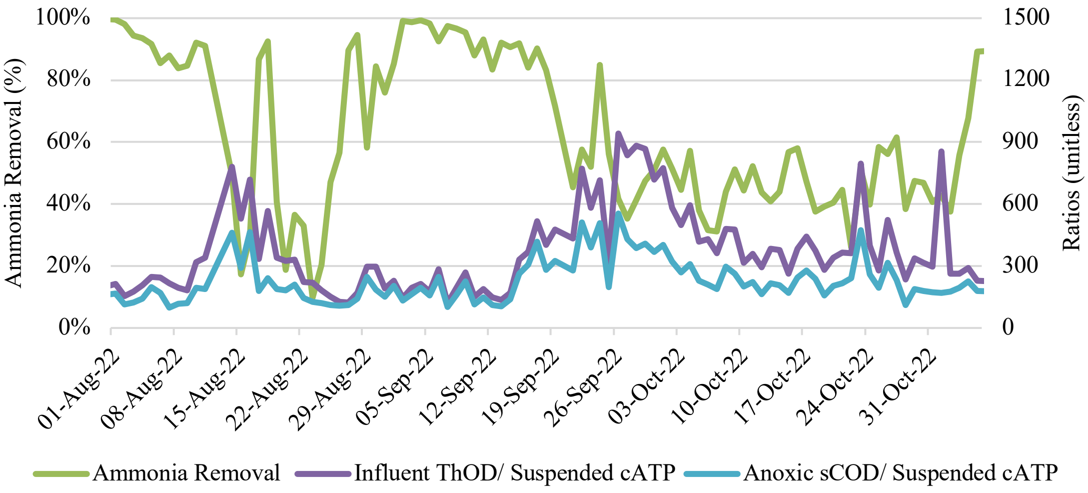


Figure 13: Concentration Ratios Response to Ammonia Removal in 2022

In 2023, there are still spikes in the ThOD and sCOD to suspended cATP concentration ratios, however, the ratio comparisons are different. In 2022 there was a clear magnification from ThOD, and this is seen in 2023, but not to the same extent. An increase in sCOD loading was observed, but this also indicates that the addition of

ThNOD was minimal in 2023. This could also indicate that the microbial communities from 2022 to 2023 may be different and should be investigated further.

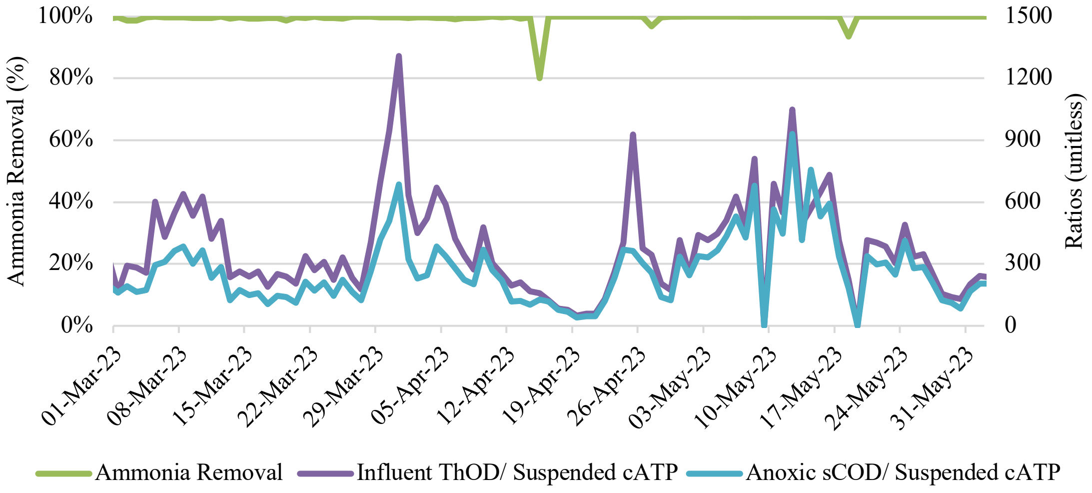


Figure 14: Concentration Ratios Response to Ammonia Removal in 2023

4.5 Attached Growth vs Suspended Growth

During the investigation, the difference in F:M ratio values using suspended cATP and agATP were apparent. The F:M ratios using suspended cATP were significantly smaller than the F:M ratios using agATP. Therefore, Figure 15 and Figure 16 were created. Figure 15 and Figure 16 show the attached biomass, suspended biomass, and agATP percentage for 2022 and 2023, respectively.

4.5.1 Attached Biomass, Suspended Biomass, and agATP Percentage in 2022

The average attached biomass, suspended biomass, and agATP percentage in the 3-month period in 2022 were found to be 0.20 ± 0.05 kg, 2.6 ± 0.60 kg, and 7.2 ± 1.8%, respectively. It is evident from Figure 15 that the active attached growth remains consistent throughout treatment. This indicates that the changes in influent wastewater flowrate, and sCOD and ThOD concentrations have little impact on the active attached growth. Whereas the changes in active suspended growth indicate that this growth has a higher sensitivity to influent characteristic process changes. Based on the agATP percentage, the suspended biomass has far more microorganisms to assist in treatment and the agATP percentage changes are primarily from the changes in active suspended biomass.

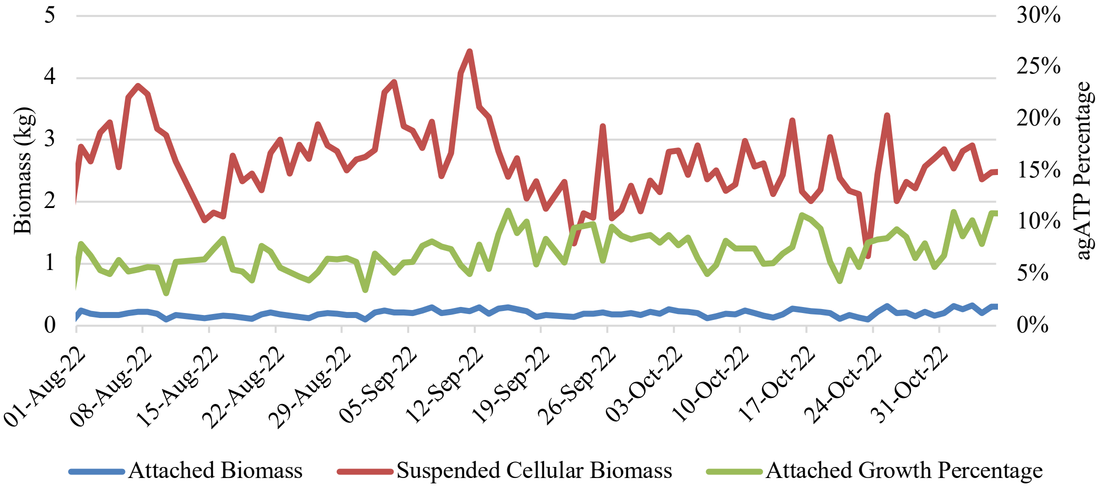


Figure 15: Attached Biomass, Suspended Biomass, and agATP Percentage in 2022

4.5.2 Attached Biomass, Suspended Biomass, and agATP Percentage in 2023

The average attached biomass, suspended biomass, and agATP percentage in the 3-month period in 2023 were found to be 0.23 ± 0.09 kg, 1.6 ± 0.42 kg, and 13 ± 5.0%, respectively. The results and trends observed from Figure 16 were like those from Figure 15. Although there were increases in influent wastewater flow and sCOD loading in 2023, there was an overall slight decrease in active suspended growth which led to a higher average agATP percentage. The agATP percentages are still influenced primarily from the changes in active suspended growth and are small in value. This leads to the thought that the microorganisms are playing a larger role in the efficient 2023 ammonia removal.

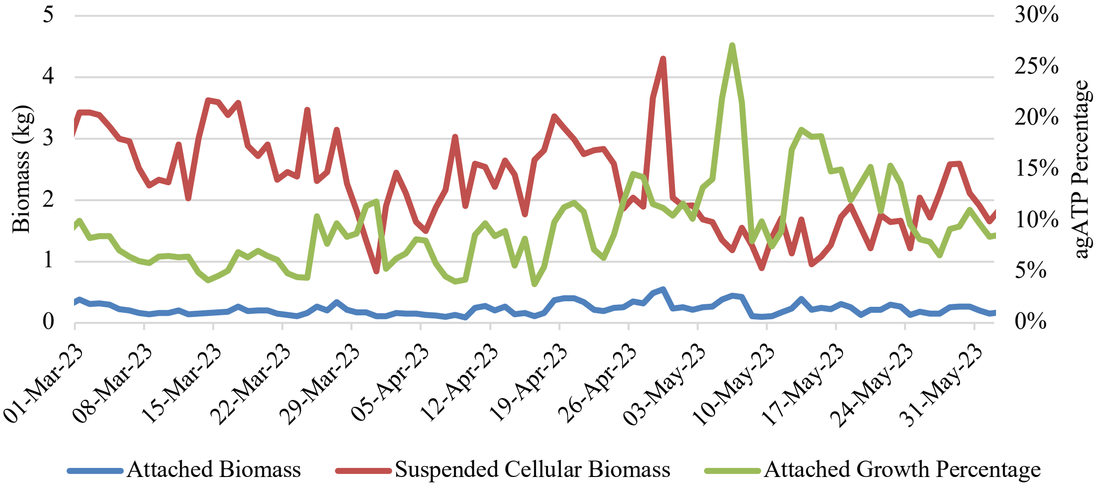


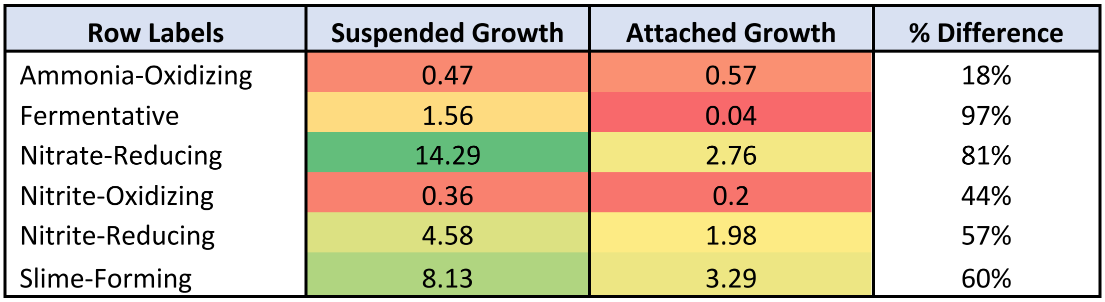
Figure 16: Attached Biomass, Suspended Biomass, and agATP Percentage in 2023

4.5.3 Microorganism Communities

Throughout the investigation, it was evident that the microbial populations of the suspended growth and attached growth could have a larger role on the influence of efficient ammonia removal through nitrification. This prompted the 2023 16S NGS testing on both growths. The notable metabolic functional groups which have more relevance to this study were pulled from the report created by LuminUltra Technologies Ltd. and put in Table 1. All functional group abundances that appeared in the NGS results for suspended growth and attached growth can be found in Appendix B and Appendix C, in Table 2 and Table 3, respectively. Heat maps have been added to all tables for visual assistance. The histograms for the full functional groups relative abundance percentages for suspended and attached growth can also be found in Appendix B and Appendix C, in Figure 18 and Figure 19, respectively. The histograms were pulled directly from the LuminUltra NGS report and assisted with the microbial community group comparisons.

Table 1 shows that the suspended growth has a much larger presence of nitrogen related functional groups which is clear by the heat mapping and the percent differences. Out of the four-nitrogen related functional groups, two have over 50% difference and one has over 40% difference. This indicates that the suspended growth is more invaluable to nitrogen and ammonia compounds removal. It is interesting to note that AOX was the only group from Table 1 that had a higher abundance for attached growth.

Table 1: Attached Growth and Suspended Growth Microbial Abundance Community Comparison



Row Labels	Suspended Growth	Attached Growth	% Difference
Ammonia-Oxidizing	0.47	0.57	18%
Fermentative	1.56	0.04	97%
Nitrate-Reducing	14.29	2.76	81%
Nitrite-Oxidizing	0.36	0.2	44%
Nitrite-Reducing	4.58	1.98	57%
Slime-Forming	8.13	3.29	60%

The Slime-Forming and Fermentative functional groups were added into Table 1 as these groups had notable percent difference results. Suspended growth showed to have a higher presence of slime-forming microorganisms than attached growth. In this case, 2023, this level did not impact the efficiency of ammonia removal. The fermentative percent difference was found to be 97%, this is a notable difference.

A deeper dive into the abundances of each growth revealed that the top 85% of the suspended biomass community represents only 6% of the attached biomass community. Additionally, the top 85% of the attached biomass community represents only 5% of the suspended biomass community. This finding led to investigating the top 20 family abundance differences in attached growth and suspended growth, Figure 17. The 4 notable family differences from Figure 17 were *Burkholderiaceae*, *Sphingomodaceae*, *Rhodocyclaceae*, and *Rhodanobacteraceae*. The full list of all genus abundances relative to *Burkholderiaceae*, *Sphingomodaceae*, *Rhodocyclaceae*, and *Rhodanobacteraceae* can be found in Appendix D. The genus abundances relative to *Burkholderiaceae* and

Sphingomodaceae can be found in Table 4 and the genus abundances relative to *Rhodocyclaceae* and *Rhodanobacteraceae* can be found in Table 5.

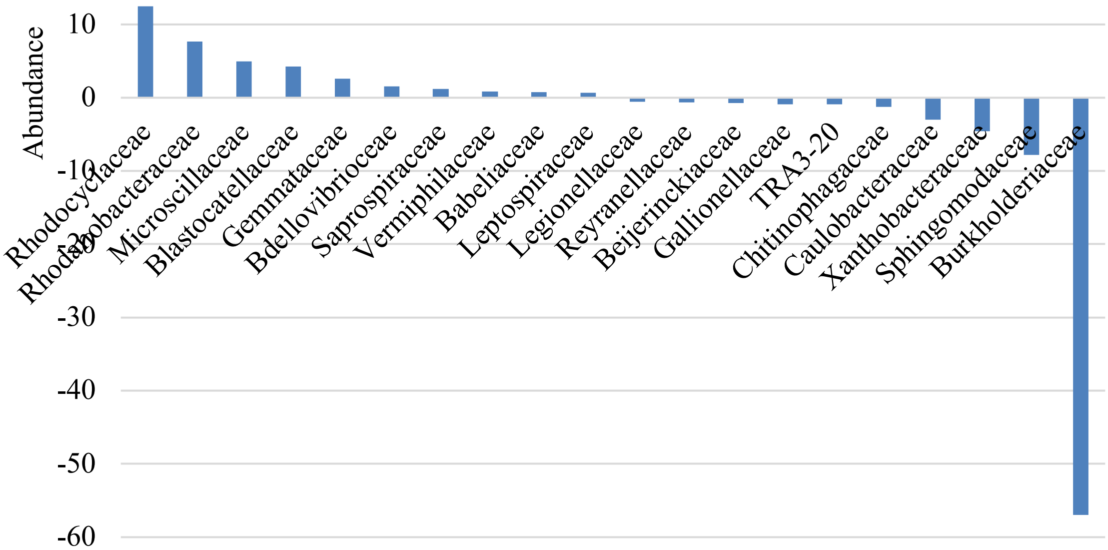


Figure 17: Top 20 Family Abundance Differences in Suspended Growth and Attached Growth

5. Discussion

A packaged dairy WRRF in southern USA experiencing intermittent ammonia oxidation nitrification reductions was investigated over the period between 2022 and 2023. The WRRF consists of DAF primary clarification, anoxic-aerobic IFAS system with internal nitrified recycle, and DAF secondary clarification. The alternative F:M ratios with ThOD, suspended cATP, and agATP were thought to be the optimum relationships for detecting decreases in ammonia removal in real-time based on the 2022 results. The alternative F:M ratios magnified the cause-and-effect relationships with ammonia removal in comparison to the ratios using MLSS and sCOD. This was expected because of the incorporation of ThNOD and cATP, a direct relationship with ammonia oxidation and active biomass, respectively. However, the results from 2023 showed that there were still fluctuations in the alternative F:M ratios while efficient ammonia removal remained constant. The 2023 results were unexpected, but this indicated that other factors could be key into understanding the systems issues.

It was discovered that the system is largely oversized for its current demand. Influent wastewater flow, sCOD loading, and the sCOD, ThOD, and suspended cATP concentrations were then reviewed. The average influent wastewater flow and sCOD loading from 2022 to 2023 increased by 19% and 16%, respectively. Although there were increases in both no treatment impacts were observed, only improvements. With this information, the following assumption was made and justified: the hydraulic retention time is not a factor for this system. If hydraulic retention time is no longer a factor, this indicates that ThOD, sCOD, and suspended cATP concentrations could be influencing the system. Thus, influent wastewater flowrate and volume were removed from the F:M ratio.

The concentration ratios using ThOD, sCOD and suspended cATP in 2022 showed a direct relationship with ammonia removal. Once a reduction in ammonia removal was observed, increases in the ratios were seen. ThOD clearly magnified the changes, but again this is expected because of the incorporation of ammonia oxidation. The 2023 concentration ratios results were once again unexpected. There were still several severe fluctuations in the concentration ratios, but with consistent efficient ammonia removal. However, when comparing the concentration ratios from 2022 and 2023, a slight difference was noticed. Both concentration ratios in 2023 had little difference, meaning the addition of ThNOD was minimal. This also prompted the next investigation, as this could indicate that there could have been different microbial populations in 2022 than in 2023.

During the investigation, the difference in agATP and suspended cATP values were very evident. This was noticed during the first F:M calculations. Therefore, both growths were also compared. It was expected that the suspended biomass would have more sensitivity to process changes than attached biomass, but it was unexpected to see how little attached growth would respond to influent characteristic process changes. Additionally, the percentages of agATP in 2022 and 2023 of 7.2 ± 1.8% and 13 ± 5.0%, are extremely low. If the system is oversized, this begs the question if the addition of media is truly needed.

While it is reassuring that the active biomass amount remains constant to influent organic wastewater changes, is it worth the extra operational cost and time.

Although it was too late to test and investigate the past 2022 microbial populations, it was possible to complete 16S NGS in 2023. 16S NGS was able to complete a deeper dive into the comparison between the attached biomass and suspended biomass. The 4-nitrogen related functional groups showed that suspended growth had much higher abundance levels. This information paired with the agATP percentages, and an oversized system indicates that for this plant the media may not be needed. The fermentation and slime-forming groups were also reviewed. It should be noted that the 97% difference in fermentation is a notable difference, indicating the metabolic activity of converting sugars primarily occurred in the suspended growth. Slime formers also showed to be more prone to suspended growth, but for this system and the abundance paired with efficient COD and ammonia removal indicate it is at an acceptable level.

The differences found from the 6 functional groups reviewed led to investigating the top 20 family differences between attached growth and suspended growth. *Burkholderiaceae*, *Sphingomodaceae*, *Rhodocyclaceae*, and *Rhodanobacteraceae* were found to be the notable differences. The Midas field guide was used to investigate the top 4 family differences through the report genus results; it was found that:

- *Rhodocyclaceae* family have metabolisms primarily of aerobic heterotrophs and possible nitrite-reduction.

- *Rhodanobacteraceae* family have metabolisms primarily of aerobic photoheterotrophs and chemoheterotrophs.
- *Sphingomodaceae* family have metabolisms primarily of aerobic heterotrophs and fermentation.
- *Burkholderiaceae* family have metabolisms primarily of aerobic heterotrophs and possible fermentative and nitrite-reducing.

One notable genus from the top 20 family differences was *Thauera.* The abundance of genus *Thauera* was 87% of *Rhodocyclaceae* in suspended growth. There was no *Thauera* abundance in the attached growth. *Thauera* are denitrifiers, but they can also be implicated in slime formers (Aalborg University Denmark, 2024). This coincides with the metabolic functional group findings, that suspended growth have more nitrogen related and slime-forming functional groups.

Overall, the results showed that the alternative F:M ratios using ThOD, suspended cATP, and agATP can be used to review ammonia removal. Whether just using the ratio with suspended cATP or agATP, it is also important to understand what is happening in the system and to understand how the system works. This systems size allowed for the assumption that hydraulic retention time had no influence on the system, therefore, ThOD, sCOD, and suspended cATP concentrations were a bigger factor. Microbial activity was another factor that had a big impact on efficient ammonia removal, but it would be beneficial to see the microbial communities during a period of poor ammonia removal.

6. Conclusion and Recommendations

6.1 Conclusion

A packaged dairy plant WRRF in southern USA that consists of DAF primary clarification, anoxic-aerobic IFAS system, and DAF secondary clarification was experiencing long periods of inefficient ammonia removal. Traditional monitoring parameters failed to explain the reduction in ammonia removal, therefore, an additional monitoring program that included ThOD, suspended cATP, and agATP was implemented. The system was monitored from May of 2022 to June of 2023 and during this time two periods were observed. A 3-month period of poor ammonia removal was observed in 2022 and a 3-month period of stable ammonia removal was observed in 2023. ThOD, cATP, and suspended cATP were used to calculate the alternative F:M ratios to find a direct relationship with ammonia removal efficiency.

The results showed that inefficient ammonia removal could be detected through the alternative F:M ratios in real-time. The alternative ratios magnified the relationship with ammonia removal in comparison to ratios with sCOD, and MLSS. This magnification was expected due to ammonia oxidation being incorporated into the ratio. However, 2023 results proved that other factors along with understanding the system implemented should considered when system treatment becomes inefficient.

This system is oversized for the current demand. Therefore, it was assumed that the hydraulic retention time is not a factor indicating sCOD, ThOD, and cATP concentrations were playing a larger role at this WRRF. The average influent wastewater flow and sCOD loading from 2022 to 2023 increased by 19% and 16%, respectively, further justifying the assumption. The alternative F:M ratios were then converted to concentration ratios and the results showed a continuous trend with ammonia removal. Decreases in ammonia removal were paired with spikes in SCOD and ThOD to suspended cATP concentration ratios.

Suspended biomass and attached biomass relative distributions and microbial populations were also reviewed. The percentages of agATP in 2022 and 2023 were 7.2 ± 1.8% and 13 ± 5.0%, respectively. The suspended growth was found to be sensitive to process changes, whereas attached biomass remained consistent. The findings led to 16S NGS testing to discover more about each growth's microbial populations. It was discovered that each growth has very different communities. The top 85% of the suspended biomass community represents only 6% of the attached biomass community while the top 85% of the attached biomass community represents only 5% of the suspended biomass community. It was also found that the suspended biomass had higher abundances for nitrogen related metabolic functional groups. This indicates that the suspended growth in this system has more microorganisms available to remove nitrogen and ammonia compounds.

6.2 Recommendations for Further Research

Further research should be completed into the differences of suspended and attached growth. This study only had one set of 16S NGS data for each growth. Therefore, more tests should be completed to confirm the findings. On top of this, more tests should be completed during a period of inefficient ammonia removal, as this data could indicate where the issue is. The microorganisms present in a system directly impact the process efficiency. By comparing which nitrogen related functional groups are lacking during inefficient removal, changes in system operations could be recommended to prevent or reduce future ammonia oxidation losses.

The relevance of adding attached growth to a system when size constraint is not an issue should also be further researched. This study found that the agATP percentages and the nitrogen related functional groups abundances were small in comparison to suspended biomass. It is questioned whether the additional costs and additional treatment associated with adding attached media are needed, especially if a system is oversized.

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Appendix A – Sample F:M Ratio Calculation

Example is for ThOD : suspended cATP and the data is from May 10, 2023, where:

Q = 96,432 gal/day

Volume = 180,000 US gal

X= 2,051 mg/mL

sCOD = 1,159 mg/L

NH3-N = 55.5 mg/L

S_o = ThOD = sCOD + ThNOD

$$S_o = 1{,}159\ \frac{mg}{L} + \left(55.5\ \frac{mg}{L}\right)(4.57) = 1{,}412.635\ \frac{mg}{L}$$

(4.57 is known from stoichiometry, see Literature Review)

From Literature Review: $\frac{F}{M} = \frac{total\ applied\ substrate\ rate}{total\ microbial\ biomass} = \frac{QS_o}{VX}$

$$\frac{F}{M} = \frac{\left(96{,}432\ \frac{gal}{day}\right)\left(3.78\ \frac{L}{gal}\right)\left(1{,}412.635\ \frac{mg}{L}\right)}{(180{,}000\ gal)\left(3.78\ \frac{L}{gal}\right)\left(2{,}051\ \frac{mg}{mL}\right)\left(1{,}000\ \frac{mL}{L}\right)} = 0.000341\ \frac{kg\ ThOD}{kg\ Suspended\ cATP{-}day}$$

Appendix B – LuminUltra Technologies Ltd. Suspended Growth 16S NGS Results

Table 2: Full Suspended Growth Functional Group Abundance Results

Group	IFAS Suspended Abundance
Acid-Producing	3.72
Ammonia-Oxidizing	0.47
Carbon-Fixing	13.71
Chlorate, Perchlorate-Reducing	0.06
Fermentative	1.56
Hydrocarbon-Degrading	13.86
Iron-Oxidizing	1.93
Iron-Reducing	0.56
Manganese-Oxidizing	2.48
Methane-Oxidizing	0.04
Methanogen	0.02
Nitrate-Reducing	14.29
Nitrite-Oxidizing	0.36
Nitrite-Reducing	4.58
Potential Pathogens	0.4
Slime-Forming	8.13
Sulfate-Reducing	0.51
Sulfur-Oxidizing	3.1
Sulfur-Reducing	0.51

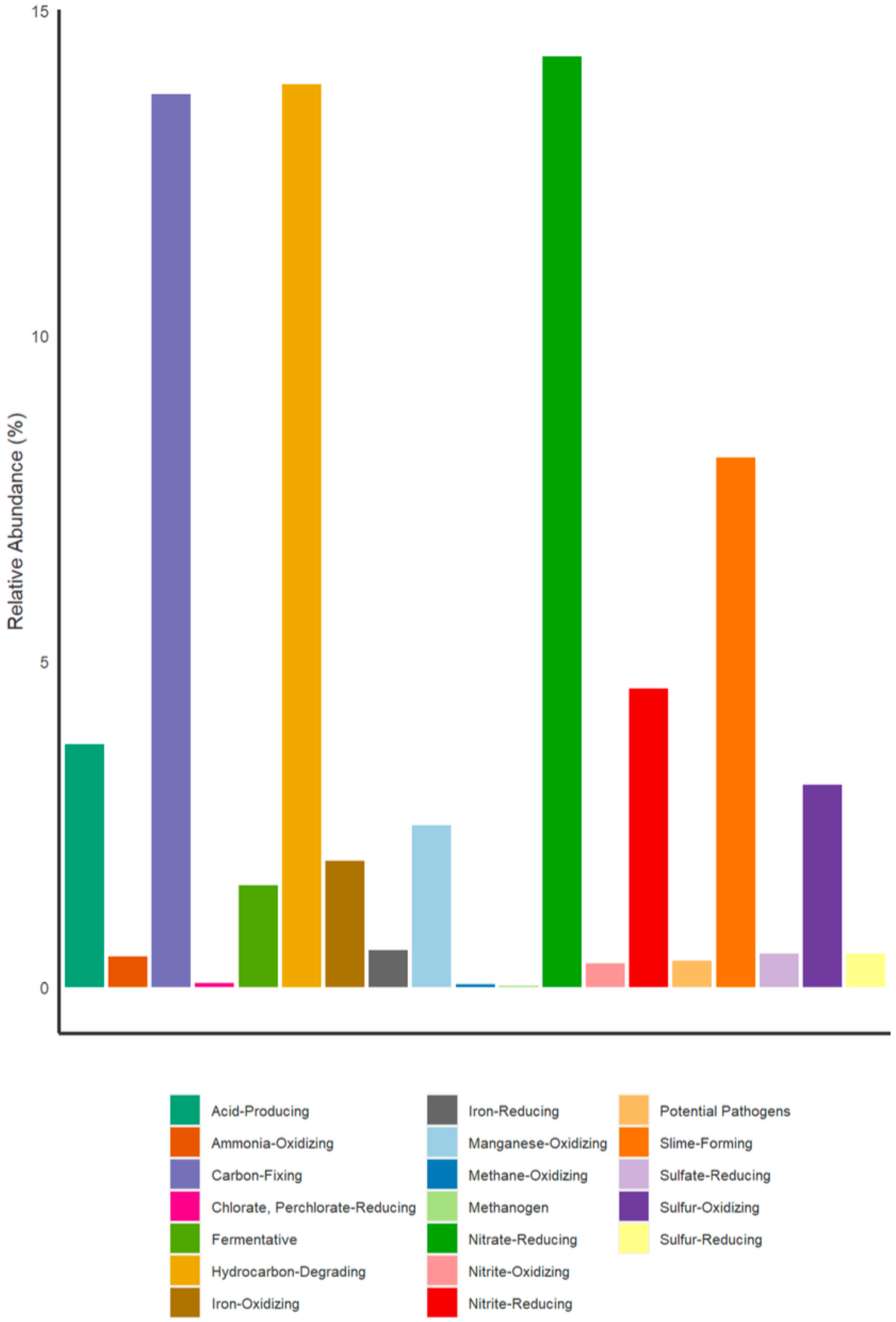
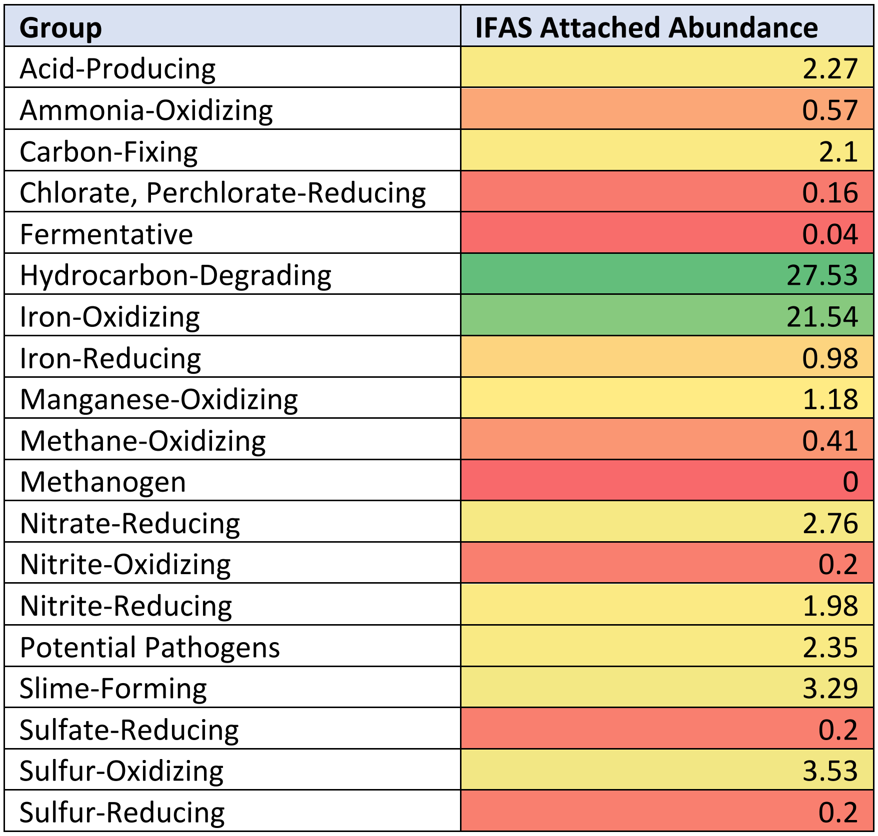


Figure 18: Full Suspended Growth Relative Abundance Percentage Results

(LuminUltra Technologies Ltd., 2023)

Appendix C – LuminUltra Technologies Ltd. Attached Growth 16S NGS Results

Table 3: Full Attached Growth Functional Group Abundance Results



Group	IFAS Attached Abundance
Acid-Producing	2.27
Ammonia-Oxidizing	0.57
Carbon-Fixing	2.1
Chlorate, Perchlorate-Reducing	0.16
Fermentative	0.04
Hydrocarbon-Degrading	27.53
Iron-Oxidizing	21.54
Iron-Reducing	0.98
Manganese-Oxidizing	1.18
Methane-Oxidizing	0.41
Methanogen	0
Nitrate-Reducing	2.76
Nitrite-Oxidizing	0.2
Nitrite-Reducing	1.98
Potential Pathogens	2.35
Slime-Forming	3.29
Sulfate-Reducing	0.2
Sulfur-Oxidizing	3.53
Sulfur-Reducing	0.2

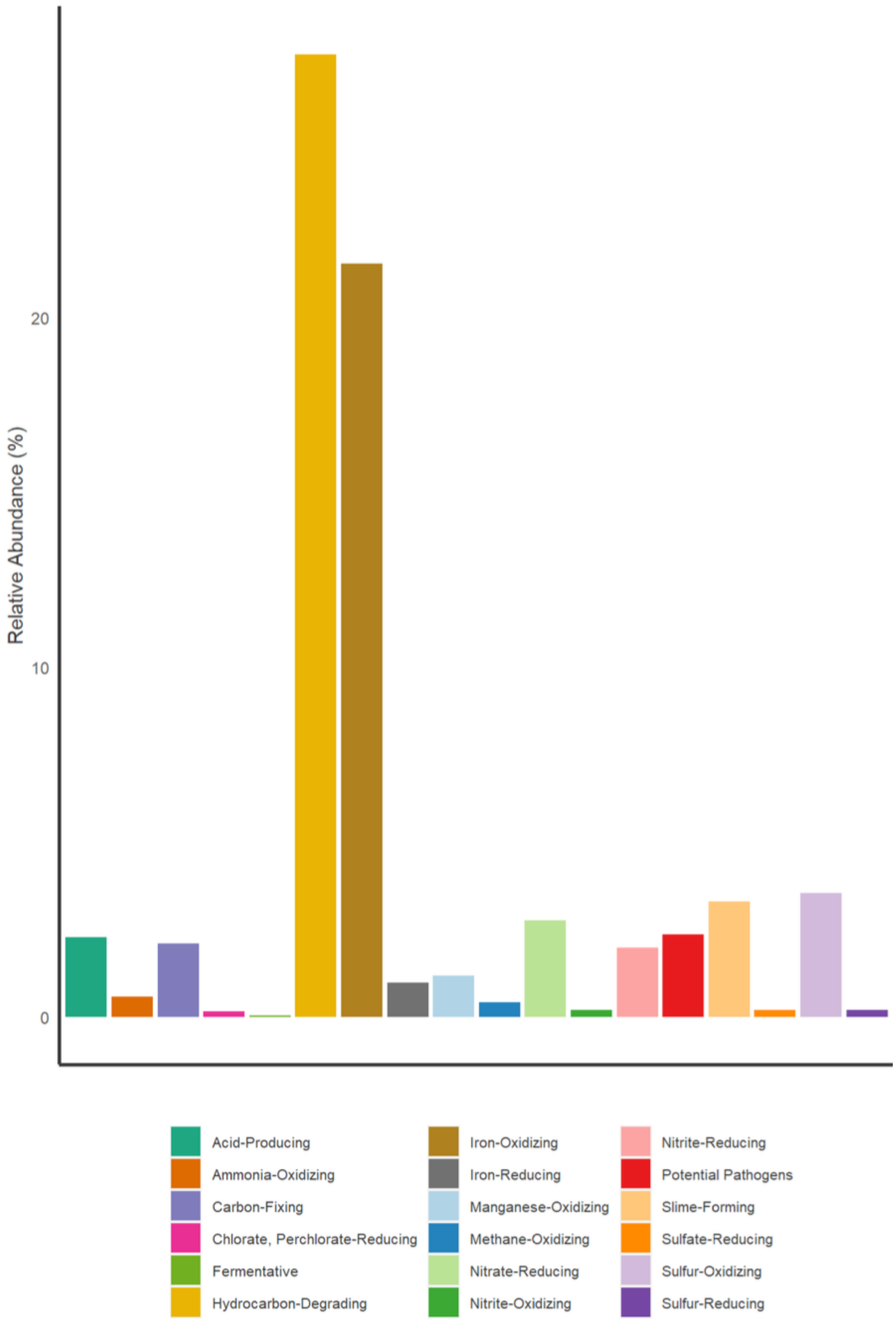


Figure 19: Full Attached Growth Relative Abundance Percentage Results

(LuminUltra Technologies Ltd., 2023)

Appendix D – LuminUltra Technologies Ltd. Attached Growth and Suspended Growth Genus 16S NGS Results for *Burkholderiaceae*, *Sphingomodaceae*, *Rhodocyclaceae*, and *Rhodanobacteraceae*

Table 4: NGS Genus Abundance Results Relative to *Burkholderiaceae* and *Sphingomodaceae*

Family	Genus	Attached Growth Abundance	Suspended Growth Abundance
Burkholderiaceae	Malikia	0.16	0.96
Burkholderiaceae	Lautropia	0	0.04
Burkholderiaceae	Inhella	0	0.02
Burkholderiaceae	Ralstonia	0	0.02
Burkholderiaceae	Sutterella	0	0.02
Burkholderiaceae	Tepidimos	0.04	0
Burkholderiaceae	Undibacterium	0.04	0
Burkholderiaceae	Noviherbaspirillum	0.08	0
Burkholderiaceae	Hydrogenophaga	0.24	0
Burkholderiaceae	Limnobacter	1.37	0.02
Burkholderiaceae	Acidovorax	1.58	0.02
Burkholderiaceae	Curvibacter	7.49	0.04
Burkholderiaceae	Aquabacterium	17.81	0.09
Burkholderiaceae	Unknown	32.05	2.63
Sphingomodaceae	Sphingorhabdus	0.04	0.11
Sphingomodaceae	Sphingobium	0.04	0.02
Sphingomodaceae	Altererythrobacter	0.04	0
Sphingomodaceae	Porphyrobacter	0.2	0.04
Sphingomodaceae	Rhizorhapis	0.37	0
Sphingomodaceae	Unknown	1.16	0.61
Sphingomodaceae	Sphingomos	0.62	0
Sphingomodaceae	Sphingopyxis	1.29	0.09
Sphingomodaceae	Novosphingobium	5.49	0.61

Table 5: NGS Genus Abundance Results Relative to *Rhodocyclaceae*, and *Rhodanobacteraceae*

Family	Genus	Attached Growth Abundance	Suspended Growth Abundance
Rhodanobacteraceae	Dokdonella	0	5.24
Rhodanobacteraceae	Unknown	0.04	2.63
Rhodanobacteraceae	Rhodanobacter	0.04	0
Rhodanobacteraceae	Tahibacter	0.04	0
Rhodanobacteraceae	Fulvimos	0.12	0
Rhodocyclaceae	Thauera	0	11.6
Rhodocyclaceae	Zoogloea	0.04	0.89
Rhodocyclaceae	Unknown	0.58	0.89
Rhodocyclaceae	Denitratisoma	0	0.02
Rhodocyclaceae	Azonexus	0.08	0
Rhodocyclaceae	Azospira	0.12	0
Rhodocyclaceae	Methyloversatilis	0.12	0

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