Shortcut Nitrogen Removal Decision Tools for Wastewater Treatment

by

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ABSTRACT

Nitrogen removal and energy reduction in wastewater treatment are shared goals. Approaches to achieve those goals include the techniques of shortcut nitrogen removal utilizing nitrite shunt, nitritation, deammonification, biocatalyst, and simultaneous nitrification-denitrification. The practice of those techniques is newer in the industry of wastewater treatment but continues to develop, along with the understanding of the biological and chemical activities that drive those processes. The kinetics and stoichiometry of traditional and shortcut nitrogen removal reactions are generally well understood to date. However, the thermodynamics of those processes are complex and deserve additional research to better understand the dominant factors that drive cell synthesis. Additionally, the implementation of nitrogen shortcut techniques can reduce the footprint of wastewater treatment processes that implement nitrogen removal at least 5 percent and can reduce operating costs by between 12 and 26 percent annually. Combined, nitrogen shortcut techniques can contribute to significant reduction in the long-term cost to operate, due to lower energy and consumable requirements, faster reaction times resulting in shorter solids retention times, and improvement efficiency in nitrogen removal from wastewater. This dissertation explores and defines the dominant factors that contribute to the success of efficiencies in traditional and shortcut nitrogen removal techniques, focusing on the natural microbiological processes. The culmination of these efforts was used to develop decision matrices to promote consideration of nitrogen shortcut techniques by practitioners during conceptual planning, design, and optimization of wastewater treatment facilities.

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CHAPTER 1

INTRODUCTION

1.1 Environmental Risks

Nitrogen and nitrogen compounds play an important role in natural cycles in the environment and pose risks of negative impacts regarding wastewater management. Various concentrations and forms of nitrogen compounds can result as pollutants, which transform based on time, environmental conditions, and by the sources from which they originate. There are four major categories of impacts of nitrogen on natural environments, including contamination of groundwater, oxygen depletion, toxicity, and eutrophication of hydrologic systems, each of which is briefly discussed in the context of nitrogen removal and management in sections below. The sources of discharges that contribute to those impacts are either point sources, such as industrial and wastewater treatment discharges, or are from non-point sources such as wet and dry deposition, urban and rural runoff, agriculture runoff, and animal waste runoff or discharges.

The magnitude of contribution to the nitrogen budget from each various source has been exponentially increasing over the past century and increasing the actual or potential impacts as further described in Section 1.3 below. However, the contribution from the type of sources varies, based on local and regional composition of sources, configuration, regulation, and the extent of the implementation of pollution mitigation strategies.

It is important to note and quantify the overall nitrogen budget and sources for an area or region, at least in terms of a mass balance, to be able to quantify the actual or potential benefit from implementing nitrogen management, and discharge reduction strategies. As an example, a nitrogen mass balance was conducted of two different regions, with a different make-up of sources, for the same time to demonstrate how nitrogen loading can vary. The results of that comparison are depicted in Figure 1 and Figure 2 below.

Those results reveal that in the North American, San Francisco Basin region, the nitrogen input from municipal wastewater treatment discharges accounted for the majority, or 31.4 percent of nitrogen loading. Improvements in nitrogen reduction and removal from such point-sources may greatly reduce the impact from discharges with excessive nitrogen concentrations, which can lead to impacts to natural habitats and human health.

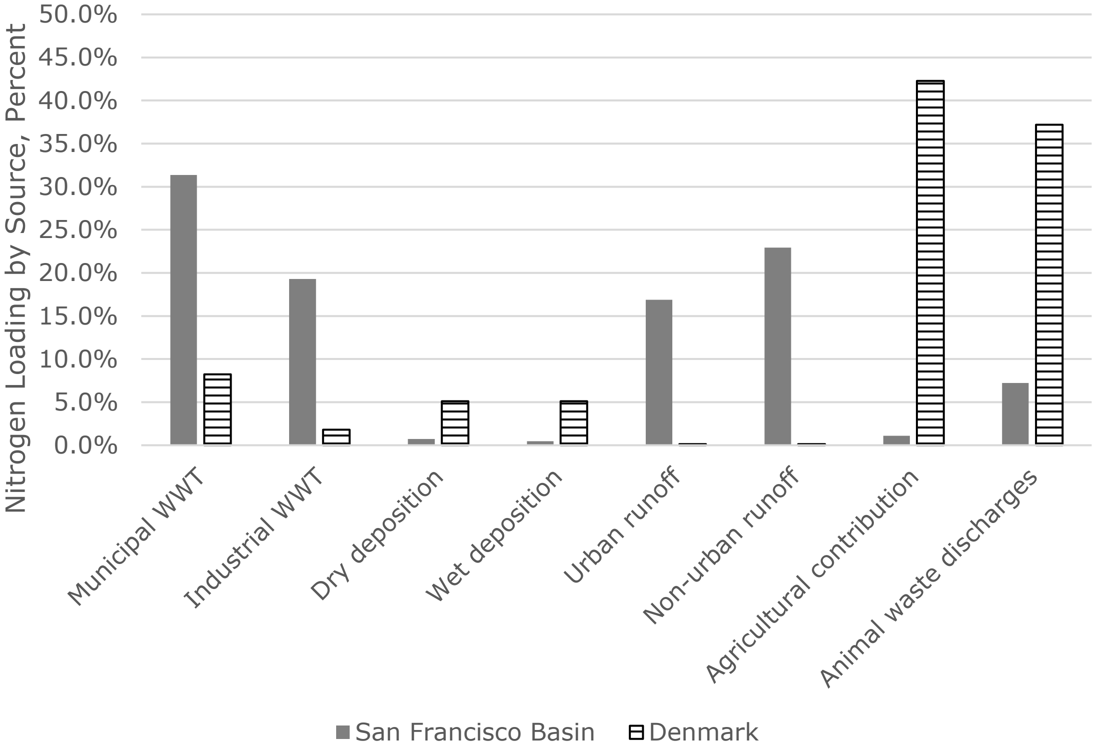

Figure 1 Nitrogen Loading by Source, Percentage (Halling-Sørensen, Jørgensen, 1993)

When compared with the European example of Denmark, as depicted in Figure 2 below, the nitrogen contributions were approximately five times greater from non-point sources, including agricultural and animal waste discharges, as compared with Municipal wastewater treatment discharges. Therefore, nitrogen reduction and removal from wastewater treatment will still provide benefit but is minor compared with potential benefits from reducing the impacts from non-point sources.

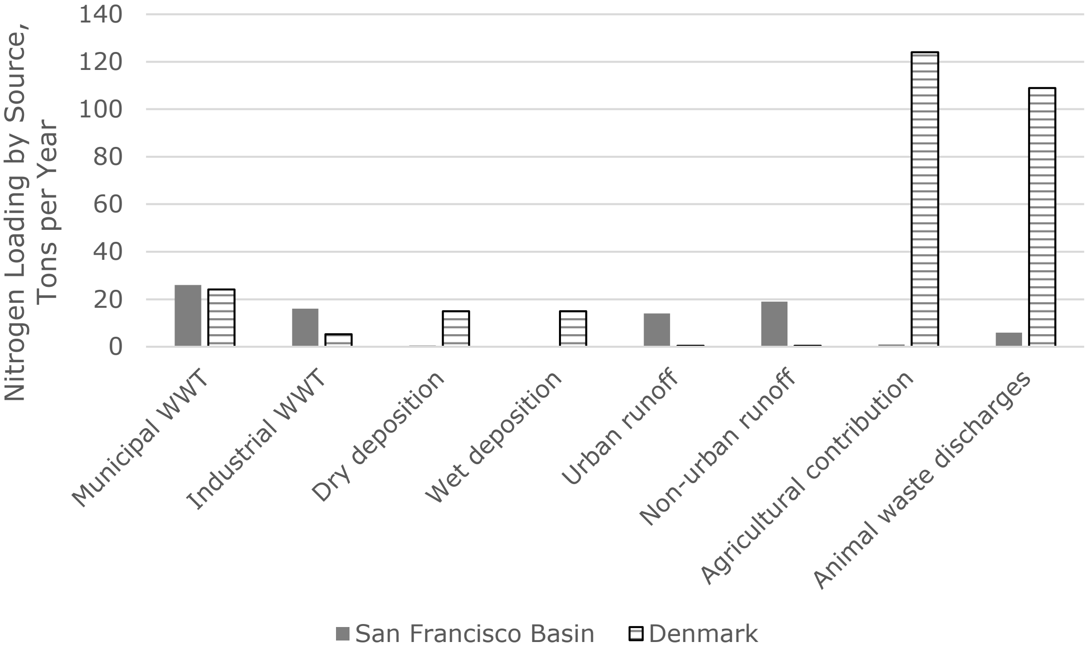

Figure 2 Nitrogen Loading by Source, 1,000 Tons per Year (Halling-Sørensen, Jørgensen, 1993)

The focus of nitrogen removal techniques explored in this research are mainly limited to point-sources and may support significant benefits for areas and regions with large contributions from Municipal and industrial wastewater treatment discharges. However, potential savings and optimization of anthropogenic implementation of biological nitrogen removal processes are also applicable to mitigate non-point source solutions if such sources were culminated, in addition to an overall reduction of nutrient loading.

Eutrophication is due to the discharge of nutrients, including phosphorous and nitrogen compounds to hydrologic systems, which leads to algae (phytoplankton) blooms. The concern of eutrophication is related to exponential increase of such nutrient loading, above natural concentrations, which leads to excessive algae concentrations and the later decomposition, which consumes oxygen, and reduces available free oxygen concentrations.

Bodies of water with relatively high concentrations of dissolved oxygen and low nutrients often have a nitrogen to phosphorus ratio (N:P) greater than 10. Algae uses four to 10 times more nitrogen than phosphorus. However, nitrogen accumulates to a less degree than phosphorus, due to natural denitrification processes, and is generally less available than phosphorus compared to demand of algae, even in the presence of discharges from municipal or industrial wastewater waters, which generally has an N:P ratio of 3:1.

Adequate dissolved oxygen concentrations are vital to sustain plant, animal, and microbiological processes in aquatic environments. Generally, a concentration of at least 5 mg/L is required, at approximately 56% saturation at 20 degrees Celsius. Concerns of oxygen depletion with an abundance of nitrogen compounds or phosphorus concentrations in aquatic environments, as organic matter decomposes, ammonia is formed, which is used as an energy source for certain microbiology, in the presence of dissolved oxygen, to produce nitrate ions (NO_3^-) in accordance with the Equation 1 below.

$$NH_4^+ + 2O_2 = NO_3^- + H_2O2 + H^+$$

Equation 1 Nitrification Reaction (Halling-Sørensen, Jørgensen, 1993)

This reaction yields nitrate, which can be, or can lead to toxicity, in addition to hydrogen peroxide and a positive ion, both potentially negatively affecting the pH of the water. Thus, the formation of nitrogen compounds, including ammonium ions (NH_4^+), nitrate ions, and nitrite ions (NO_2^-), either from the decomposition of organic matter, and/or in addition to concentrations of nitrogen compounds from the discharges from municipal and industrial wastewaters, contribute to the contamination of groundwater, oxygen depletion, toxicity, or eutrophication of hydrologic systems.

Ammonium ions can contribute to the overabundance of the growth of aquatic plants, including algae, leading to dissolved oxygen completion, and is toxic as ammonia (NH_3). The presence of nitrate ions has similar concerns for the overabundant growth of aquatic plants, dissolved oxygen completion, toxicity and methemoglobinemia. Nitrite ions also have similar concerns for the overabundant growth of aquatic plants, dissolved oxygen completion, and toxicity.

Ammonium ions are a preferred nutrient or energy source for certain biological, which are converted to ammonia in the presence of waters with higher pH. The distribution of the ammonia species in water is highly dependent on the pH as depicted in Figure 3 below. This depicts that a basic pH results in a higher percentage of gaseous ammonia (NH_3), and acidic pH results in a higher percent Ammonium (NH_4^+). The pH range of most wastewaters at wastewater treatment plants is between 6.0 and 9.0 standard units (su). Therefore, the bulk of the Ammonia-N will be in the Ammonium ion form.

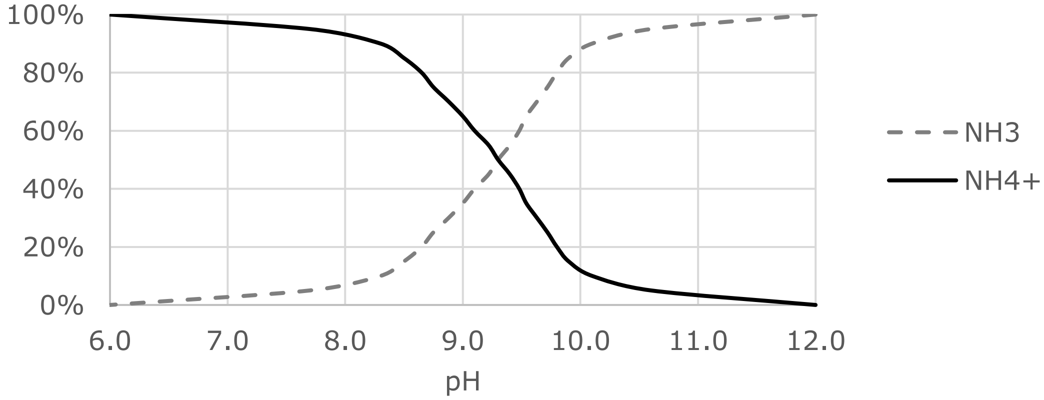

Figure 3 pH Speciation of Ammonium Ions and Ammonia in Water (Gerardi, 2002)

1.2 Anthropogenic Concerns and Benefits

Wastewater collection, treatment and related sanitary services are part of the foundations of the success of societies and can be considered a hallmark in the actual and potential for growth and improvement of the economic, education, health and well-being of societies, and quality of the environment. Quantifying the true value of wastewater treatment services to society and natural environment may not appear so easy (ASCE, 2020). However, the direct correlation between having adequate sanitary services and measurable improvement in the health and safety, education, economic, and social development, in societies, while reducing anxiety and the potential for disease transmission (WHO, 2020) has been measured.

The cost to sustain and expand wastewater collection and treatment services in the United States over the next two decades is projected to have a 45% shortfall in funding equating to $2.495 trillion. If that shortfall, and other funding shortfalls are not addressed, then a projected $1.8 trillion decrease in the United State economy and $10.3 trillion in gross domestic product (GDP) could occur by 2040 (ASCE, 2021), due to losses in service, productivity, health, and reliability. This is referred to as the infrastructure gap, where the cost to fund expansions and renewal of infrastructure is rising exponentially, while the funding available to meet those needs is only increasing linearly, as depicted in Figure 4 below.

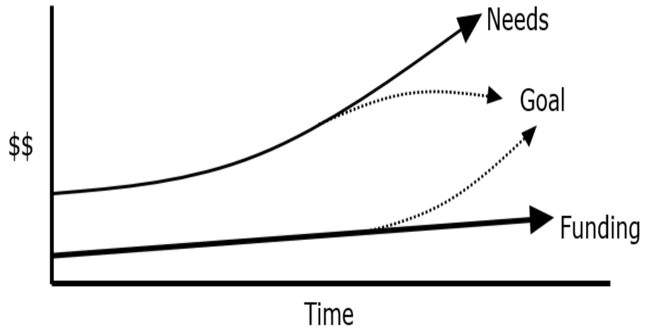

Figure 4 Infrastructure Gap (ASCE, 2017)

The solution to this gap is two-fold. The first is regarding the need to increase the funding to match the level of need, which is managed at a local, State, Regional, and National level through policy, law, tax, fee, and other programs. The cost to the individual community to sustain capacity, service, compliance, and to expand utilities as needed to support growth directly impacts livability. Entities responsible for such infrastructure are stewards of those investments, with the responsibility to protect the health, safety, and welfare of their citizens and environments. The potential of being able to increase funding to match the level of the needs is less feasible and relies on other individuals with less overall impact in the decision-making processes that define the magnitude of such long-term costs, than those delivering and managing that infrastructure.

The second is the need to significantly reduce the cost to plan, design, deliver, operate, maintain, administer, and manage infrastructure to meet the level of service required, and to match the level of funding available to sustain the benefit that wastewater collection and treatment provides. This includes optimizing the efficiency of biological wastewater treatment processes.

A critical concern for both solutions is timing. The faster the long-term cost to operate and renew infrastructure is reduced, the lower the long-term cost to operate will be. This can provide a longer life cycle with less re-investment in the future for renewal and replacement, and ultimately would not require the funding to increase as significantly.

Economics is only part of this concern. As more maintenance, renewal, or expansion of infrastructure is deferred, the greater the potential or actual decrease in the level of service and compliance, in addition to increase negative downstream impacts on the quality of life, societies, and the degradation of environments. If that is true for one of the wealthier societies, then the need could be even more perilous for citizens of less wealthy societies.

Efforts focused on implementing wide-spread sanitation services for benefits of waste reduction, environmental quality, or for the improvement of health have occurred at various times across the World. In China such efforts did not begin based on a goal of improving environmental quality, but the link between treating human wastes and animal excrement, and generating energy was made over 800 years ago. In 1776, the link between decaying organics and energy producing gas was established and further studied. Modern biogas anaerobic digesters were invented in the 1930’s and installed around Shanghai. After Japan invaded China in 1937, those were destroyed, and not re-visited until 1972, where over the subsequent five years, approximately two million digesters a year were installed at rural homes and communities, accounting for approximately 10 million new digesters representing a significant investment in wastewater treatment infrastructure. Those digesters were designed based on a capacity for 66 pounds of feces, and 13 gallons of urine and water daily to support the biological process and gas production of the digesters. However, by 1980, at least five million of those digesters were broken. Without the training, tools, and knowledge to operate and maintain that infrastructure, they fell by the wayside, and the practice was generally abandoned (George, 2009).

This practice was re-instituted across rural communities in China in 2001, where in the Guanxi Providence, 250,000 digesters were installed per year, equating to almost a million digesters, supporting approximately 15.4 million rural households, for the purpose of treating and managing the biological wastes from the people and livestock, and to create energy for cooking and heating (George, 2009).

Those examples demonstrate how large initial financial commitments and contributions by governing entities over a significant timeframe, provided communities with some of the initial infrastructure to meet sanitation needs, greatly reduce environmental impacts, and improve the health and capacity of those societies. Conversely, they also demonstrate how large investments can become burdens without the commitment to sustain, renew, and replace that infrastructure.

1.3 Waste and Nutrient Scale, Progression and Projection

The wastes generated by societies are vast in scale, composition, and variety. Wastes are also unique to the location, quality of life, access to and type of resources consumed and disposed, intensity of industrialization, climate, age, and the number and magnitude of generators. Biological treatment processes of such wastes are generally focused on certain subsets, arranged as follows (Evans, 2013); 1) direct animal origin, including feces, urine, manure, animal based food waste, and other abattoir wastes; 2) plant materials, including raw, cooked or processed foods, yard and landscape, and other putrescible waste; and 3) industrial waste, such as raw or processed inorganic materials, chemicals, by-products, backwash, leachate or supernatant from other partial or complete waste collection, reduction or treatment processes. Each subset of waste listed above has varying concentrations and characteristics of biodegradable organics, non-biodegradable organics, nutrients, microorganisms, metals, inorganic materials, or anthropogenic synthesized materials.

Biological wastewater treatment processes mainly focus on the management and reduction of impacts to natural nutrient cycles including carbon, nitrogen, and phosphorus. In addition to adequately addressing current needs to minimize such impacts, the potential scale of future conditions is daunting and is what sets the tone and pace of the need for improvements. The differences in the mass flow of carbon and nitrogen per capital has risen exponentially during modern history, and as documented through 1998, depicted in Table 1 below.

Constituent	Ancient	1988
Carbon	110	4,850
Nitrogen	11	88
Phosphorus	2	0.88

Table 1 Mass Flow per Capita, lb/capita/year, (Evans, 2013)

The projected potential population is depicted in Figure 5 and the related anthropogenic carbon, nitrogen, and phosphorus waste, in Figure 6 below.

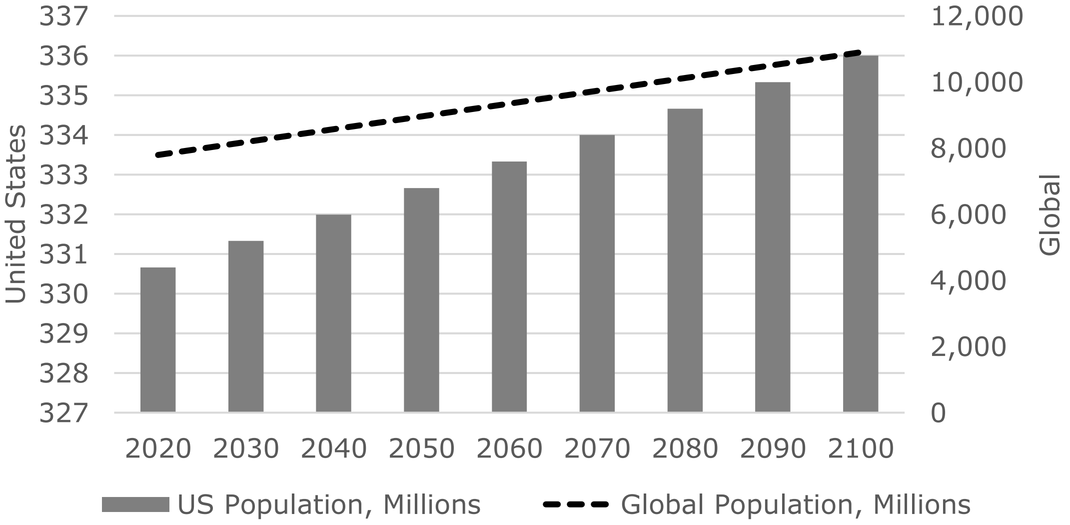

Figure 5 Population Projections (World, 2021)

The volumes produced, not accounting for variations in known and potential variations in growth, industrialization, consumption, and climate, predict a 40 percent increase in the mass of carbon, nitrogen, and phosphorus throughput, with an equal increase in nutrient loading in the anthropogenic waste streams as depicted in Figure 6 below, as developed from data from biowaste and biological waste treatment (Evans, 2013), and projections based on corresponding Global population growth projections as depicted in Figure 5 above.

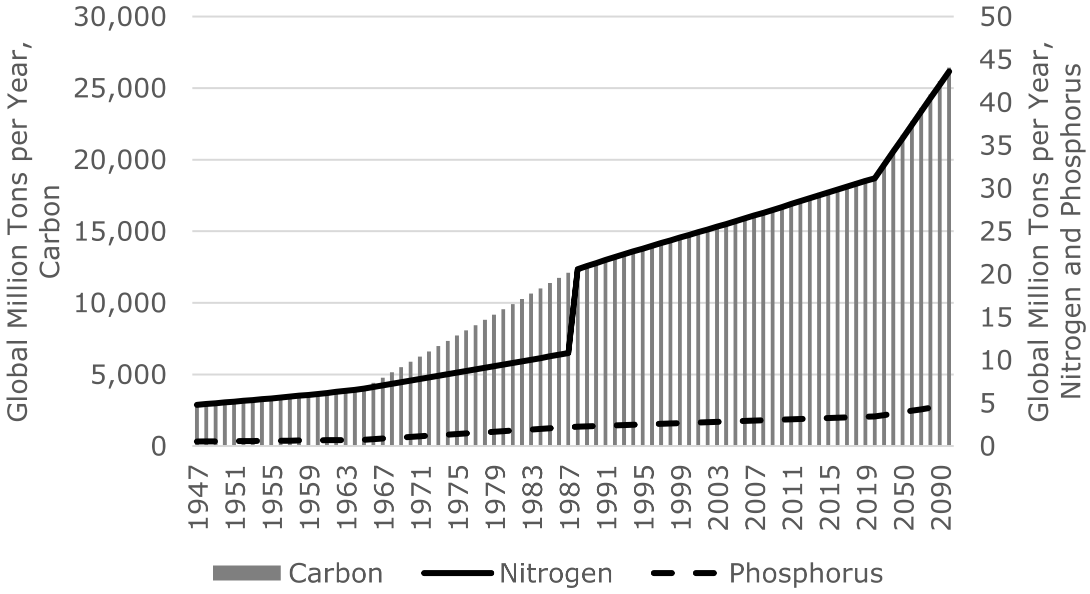

Figure 6 Nutrient Mass Flow Projections

Biological wastewater treatment processes have remained moderately unchanged over the past 100 years, with most of the improvements in biological process and efficiency occurring in the past 30-years.

A notable step change in the United States occurred in 1972, beginning with the passing of amendments to the Federal Water Pollution Control Act of 1948, becoming a law known as the Clean Water Act (CWA). Those amendments established the authority and regulations for limiting pollutant discharges to waters of the United States, and to address non-point source pollution. Additionally, the CWA established federal funding, required for such a significant increase in the quantity, performance, and construction of wastewater treatment plants and improvements. Prior 1972 in the United States, most wastewater treatment plants provided only primary treatment, and a minority providing secondary treatment, prior to discharge. After those new regulations and funding to support that change, the addition of secondary treatment became standard, and the inclusion of tertiary treatment followed. The results were a measurable improvement in the quality of natural environment by improved biodiversity and return of wildlife to aquatic environments, and in the health and prosperity of communities, as demonstrate by the notable increase in GDP and population growth since those changes.

However, 50-years later we now have the imbedded cost of renewing and replacing that additional treatment infrastructure and need to begin re-thinking the magnitude of investments in conventional infrastructure we commit to. In addition to continuing to focus on improving the knowledge of, and implementation of biological wastewater treatment processes towards reducing operating costs, improving performance, and ultimately improving the environment.

Significant improvements to the quality, diversity, potential, and progress of societies have had a foundational reliance on the ability to understand and manage biological and chemical wastes. Optimization of efficiencies in wastewater treatment processes, in addition to how such improvements and additions are planned, delivered, and managed, are required to achieve a more sustainable model to provide the wastewater treatment and disposal benefits to the health and prosperity of societies and the quality of the environment. As discussed above, the needs of infrastructure, including wastewater treatment infrastructure, is diverging from the ability to support, renew, and maintain it.

Biological treatment of wastewaters is already a cost-effective method when compared with chemical and physical methods, incineration, wet air oxidation or solidification techniques, noting that the duration of biological treatment process is typically longer than those other methods. The key attributes that contribute to efficiencies of wastewater treatment are those that reduce consumables such as energy, external carbon sources, other nutrients, and those that reduce or improve the quality of waste streams. Reduced treatment durations, increased effluent and solids quality, and maximizing microbiological processes are the benefits of those efficiencies.

Exploring such efficiencies in the processes that remove nitrogen from wastewaters has potential to find opportunities to reduce energy consumption, reduce sludge production, and to reduce carbon dioxide emissions, all equating to cost savings. Wastewater collection and treatment processes require energy to move wastewater, add nutrients and air, and to manage biosolids and waste streams. This is an intense energy demand, in addition to other consumables to develop and maintain vital treatment processes.

As academics and practitioners of wastewater treatment, and such infrastructure, it is a shared responsibility to promote and engage in proactive efforts to identify opportunities, invest time, and actions towards improvements in efficiencies that can help reduce the cost to operate, improve performance, and support healthier people and environments.

This thesis does not explore or discuss all those aspects but is focused on progressing the body of knowledge regarding the shortcut nitrogen removal processes in wastewater treatment that can return economic savings, increase environmental and societal quality, and help converge the infrastructure gap.

1.4 Nitrogen Cycle

The Nitrogen Cycle is the circulation of nitrogen and nitrogen compounds across and between the atmosphere, hydrosphere, lithosphere, and pedosphere as depicted in Figure 7 below. Nitrogen is the greatest percentage of elements in the atmosphere, accounting for 78 percent by volume, with the remainder comprising 21 percent oxygen, 0.9 percent argon, and the remaining 0.1 percent consisting of carbon dioxide, ozone, methane, nitrous oxides, and other trace gases.

Although nitrogen, nitrogenous compounds, and ions are essential to sustain biological life, plants and animals do not directly fix or use nitrogen (N_2). Instead, life relies on microbiological organisms to both transform nitrogen into compounds and ions, and to degrade biological material into usable food or energy sources. Only in those other states, can plants convert, consume, or fix nitrogen, which can then be consumed and fixed by animals. Later as living biology dies and decomposes, those microbiological organisms again transform biological material to various compounds, and ions of nitrogen, continuing the nitrogen cycle.

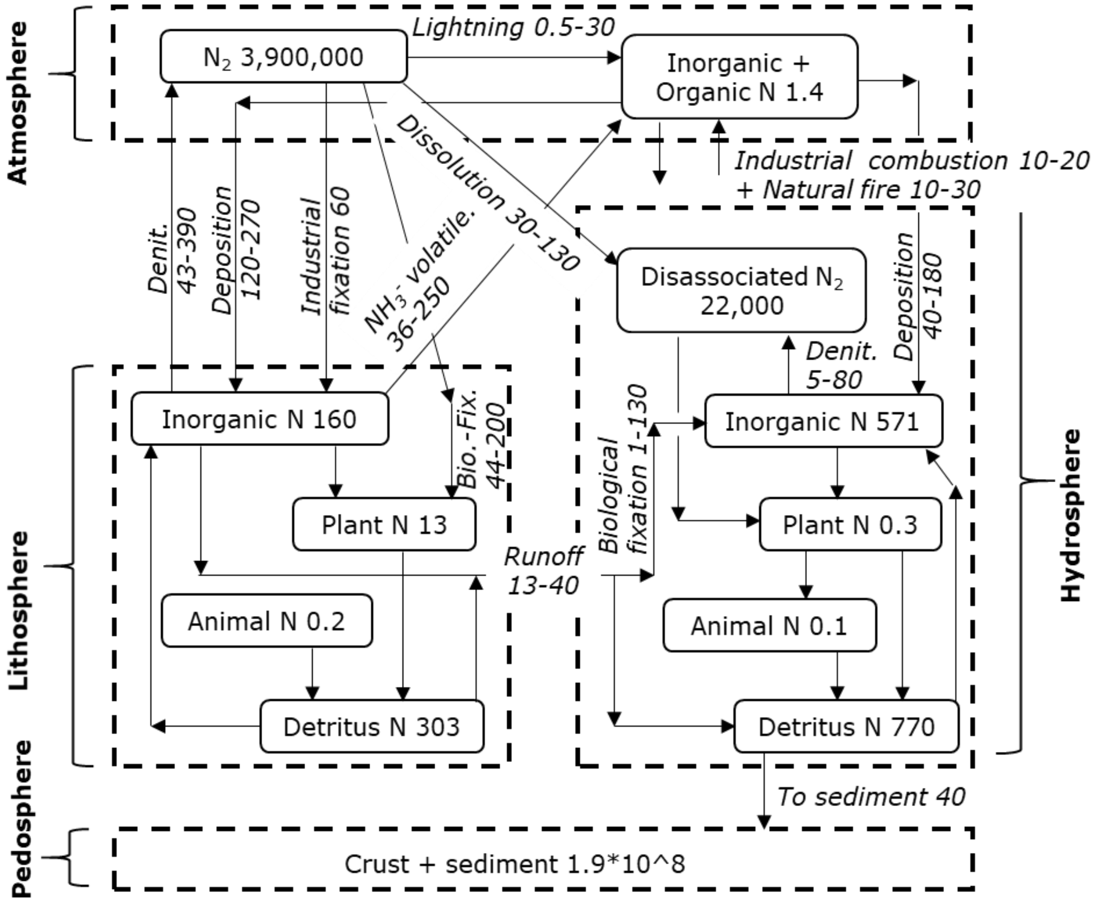

Figure 7 Global Nitrogen Cycle, Values inside boxes are in Pg N, fluxes in Tg N/year (Halling-Sørensen, Jørgensen, 1993)

There are five major classifications of nitrogen transformation as follows:

- Nitrogen fixation is the process where atmospheric nitrogen is transformed either by microbiological organisms, or other natural processes, including lightning and dissociation caused by the interaction of ultraviolet rays and nitric oxide, or industrial processes, to ammonia, nitrate, or nitrite.
- Nitrogen assimilation is the process where inorganic nitrogen is used to form compounds, or where plants, fungi and bacteria incorporate organic nitrogen compounds and ions as a food or energy source, as they cannot fix nitrogen directly.
- Ammonification is a metabolic process of microbiological organisms, fungi, and bacteria, that convert or oxidize nitrite to ammonium or ammonia.
- Nitrification is the process by autotrophic chemolithotrophs to metabolize ammonia to nitrite.
- Denitrification is the process where heterotrophs utilize inorganic sources for carbon, and consume other organic compounds, to reduce Nitrate-N to Nitrogen gas (N_2), where it is either released back to the atmosphere or the hydrosphere.

Through the processes described above and depicted in Figure 8 below, nitrogen, nitrogenous compounds, and ions, are cycled through the environment. The microbiological organisms, fungi, and bacteria that fix, assimilate, and transform nitrogen, take part in those processes, and are the foundation of nitrogen removal through the environment and wastewater treatment processes.

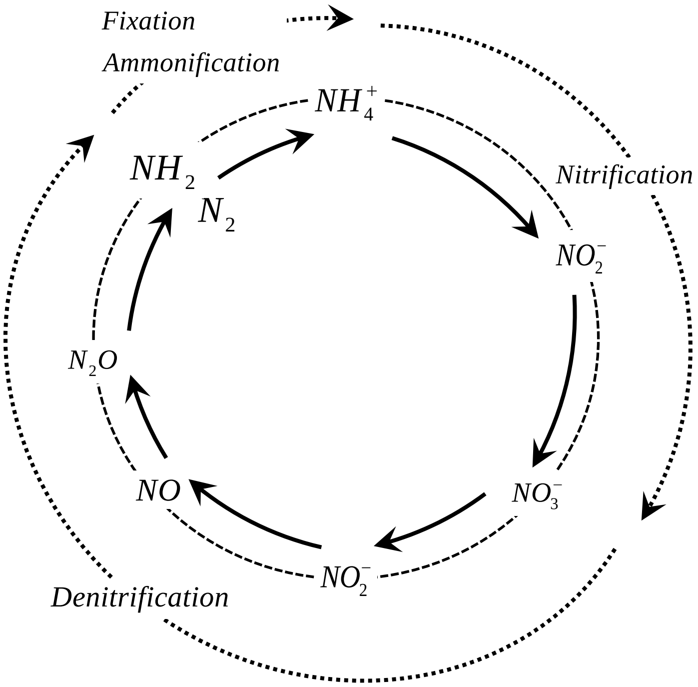

Figure 8 Nitrogen Transformation Cycle (WEF, 2015)

The depth of comprehension and control of those biological, chemical, and physical processes to optimize and maximize the performance and efficiency of anthropogenic waste treatment processes are further discussed and explored in the chapters below.

1.5 Purpose and goals

Nitrogen removal and energy reduction in wastewater treatment are shared goals. Approaches to achieve those goals include the techniques of shortcut nitrogen removal utilizing nitrite shunt, nitritation, deammonification, biocatalyst, and simultaneous nitrification-denitrification. The practice of those techniques is newer in the industry of wastewater treatment but continues to develop, along with the understanding of the biological and chemical activities that drive those processes. The kinetics and stoichiometry of traditional and shortcut nitrogen removal reactions are generally well understood to date. However, the thermodynamics of those processes are complex and deserve additional research to better understand the dominant factors that drive cell synthesis. Additionally, the implementation of nitrogen shortcut techniques can reduce the footprint of wastewater treatment processes that implement nitrogen removal at least 5 percent and can reduce operating costs by between 12 and 26 percent annually. Combined, nitrogen shortcut techniques can contribute to significant reduction in the long-term cost to operate, due to lower energy and consumable requirements, faster reaction times resulting in shorter solids retention times, and improvement efficiency in nitrogen removal from wastewater. This dissertation explores and defines the dominant factors that contribute to the success of efficiencies in traditional and shortcut nitrogen removal techniques, focusing on the natural microbiological processes. The culmination of these efforts was used to develop decision matrices to promote consideration of nitrogen shortcut techniques by practitioners during conceptual planning, design, and optimization of wastewater treatment facilities.

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CHAPTER 2

FUNDAMENTALS

2.1 Wastewater Treatment related Nitrogen Processes

Much like carbon, nitrogen follows a critical cycle that is vital to all living organisms. Wastewater treatment process takes part in the nitrogen cycle, and re-introduce nitrogen to the natural cycle, while minimizing negative environmental impacts. Toxic conditions in waterways and the environment can propagate when high levels of nitrogen are introduced that provide an increased nutrient source, resulting in algae growth, and later reduced dissolved oxygen concentrations. Each are detrimental to the health and quality of biological populations.

Nitrogen enters wastewaters mainly by urea, which otherwise would be directly discharged to the environment, if not collected and conveyed for treatment. Nitrogen also contributes to wastewater collection and treatment systems from animal-based food waste, processed food waste, household cleaning agents, industrial wastes, and other abattoir wastes.

Nitrogen is present in several forms in water and wastewaters as categorized by three main groups, including the following:

- Ammonia Nitrogen (Ammonia-N) is comprised of percentages of the gaseous ammonia molecule, and the Ammonium ion, also referred to as Ammonium.
- Nitrite-N molecule and the Nitrate-N molecule, both generally formed during natural and wastewater treatment process.
- Organic Nitrogen (Organic-N) consists of the minor percentage of the cell mass of the existing microbiology, and other aqueous organic compounds.

Each category of nitrogen in water and wastewater may be measured or evaluated individually, or as follows:

- Total nitrogen (TN), which is the sum of all categories of nitrogen as described above.
- Total Kjeldahl Nitrogen (TKN), which represents the sum of Ammonia-N and Organic-N.
- Total Inorganic Nitrogen (TIN) represent the sum of the Ammonia-N, Nitrite-N, and Nitrate-N molecules, or as the TN minus the Organic-N.

When nitrogen enters wastewaters as urea or other wastes, it is comingled with an additional water source, either associated with flushing, or in combining with existing wastewaters in the collection system and at wastewater treatment plants. At the time of introduction, the nitrogen is almost fully hydrolyzed into Ammonia-N. In water, the bulk of the gaseous ammonia portion of Ammonia-N is converted to Ammonium. The decomposition chain, from proteins and amino acids to nitrogenous compounds and ions, in relation to their respective energy is depicted in Figure 9 below.

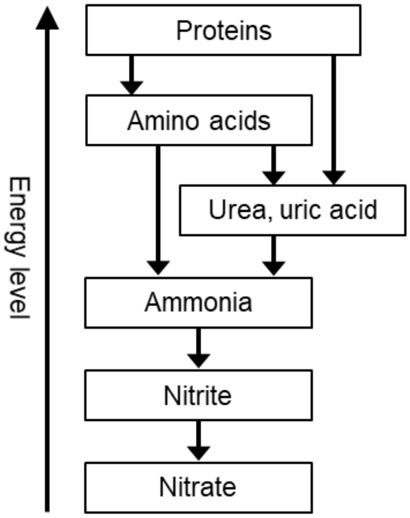

Figure 9 Decomposition Chain (Halling-Sørensen, Jørgensen, 1993)

As depicted in Figure 9 above, organic matter, proteins, urea, and uric acid decompose to ammonia, which can be used as a chemical energy source for microbiological organisms. This fundamental transformation cycle of energy and resources is part of the focus of biological waste, and wastewater treatment processes.

The techniques and processes selected to treat and/or manage a waste stream is generally based on the specific types and qualities of that waste. Treatment processes may be discrete or combined to achieve the desired or required performance, and ideally are matched to the specific waste stream to maximize efficiencies. Such treatment processes may utilize physical, mechanical, chemical, and biological treatment processes to separate, reduce and stabilize those wastes to varying degrees of quality, suitable for the specific disposal, discharge, or storage.

Each treatment process type is limited by the characteristics of the waste. Regarding biological treatment process, the available nutrients and microorganisms can become the limiting factors. Additionally, the quantity and quality of a community's waste stream can change over time and may cause a loss of efficiency and performance, when not adequately planned, designed, operated, or maintained to accommodate such changes.

Those wastes are generally classified as either solid waste, or wastewater. Each is based on how they are regulated, treated and/or disposed, or by the type and classifications of the facilities and utilities that collect and manage them. General types of wastewaters generated include the following: (Evans, 2013)

- Municipal wastewater
- Industrial wastewater
- Stormwater (direct and indirect)
- Septage (septic and vaulted wastes)

- Foods, Oils and Grease (F.O.G.)
- Digester supernatant
- Solids handling supernatant
- Filter wash
- Water treatment residuals
- Equipment cleaning (hydrocarbons)

The selection and implementation of wastewater treatment processes, including biological processes, need to consider the characterization, and quantity of wastewaters, as each will be unique in the types and quantities of food and energy available to support biological processes.

2.2 Microbiology

Microorganisms are living single-cell, or a colony of cells, only observable at a microscopic level. Microorganisms are classified in groups as either bacteria, protozoa, or metazoa. Viruses can also be discussed in terms of microorganisms. However, they are classified as non-living, as viruses cannot self-replicate, without a host living organism, and do not generally react to environmental changes.

Microbiology is the pillar of natural systems that degrade and recycle waste products and materials, and in manmade biological wastewater treatment systems they are responsible for degrading undesired concentrations of compounds that would otherwise be toxic if not for the service they provide.

To identify opportunities to optimize and gain efficiency in wastewater treatment systems, specifically in the context of nitrogen removal, one must first identify and quantify the fundamentals and variations in the microbiology capable of contributing to wastewater treatment.

The basis of the growth and reproduction of microbiology is the presence, and use of, carbon and energy sources referred to as substrate, present in wastewaters.

Key substrates include alkalinity, carbonaceous biochemical oxygen demand (cBOD), and nitrogenous biochemical oxygen demand (nBOD).

Generally, microorganisms are classified as either prokaryotes or eukaryotes. Prokaryotes are cells with no internal membrane bound organelles, does not have a distinct nucleus, but will include cytoplasm, ribosome to synthesize proteins, and a cell wall. Eukaryotes are cells containing membrane bound organelles, in addition to a nucleus, cytoplasm, Golgi apparatus to regulate metabolism, lysosome consisting of enzymes, and a cell membrane.

Prokaryotes include eubacteria and archaebacteria, which are the two most common and important bacteria in wastewater treatment (Gerardi, 2006).

The common and fundamental eukaryotes important to wastewater treatment include fungi, protozoa, rotifers, and nematodes. Each are free-living, commonly present in natural water and soil systems, and generally enter wastewater treatment systems through inflow or infiltration.

Protozoa are generally strict aerobes, but can also propagate in anaerobic conditions, including flagellates and amoebae. Protozoa are classified by their mean of movement, including amoebae flagellates, free-swimming ciliates, crawling ciliates, or stalked ciliates. Key traits that such microbiology contribute to wastewater treatment processes include consuming dispersed cells and particles, promoting the floc of particles, and recycling nutrients (Gerardi, 2006). Typically, protozoa feed along the perimeter of floc and particles.

Rotifers and nematodes are both metazoa that provide similar services as protozoa, but also can degrade substrate within floc which promotes additional degradation of nutrients by penetrating and introducing dissolved oxygen, nitrate, nutrients, and substrate.

Fungi are unique, and not always present in wastewater treatment processes, as they are strict aerobes, and are very tolerant in acidic environments, although they can propagate in conditions with a pH range of 2.0 to 9.0 su. However, fungi have been found to be most prevalent at a pH less the 6.5 su and are optimal at a pH of 5.6 su. Large concentrations of fungi, specifically filamentous fungi can contribute to performance issues in clarification wastewater treatment processes but are effective for treatment of some industrial wastes as they can tolerant low nutrient environments and degrade cellulose materials. As an aerobe, fungi degrade carbon dioxide (CO_2) and water (H_2O) by using free molecular oxygen (O_2) as an energy source. Also, as a facultative anaerobe, fungi can degrade sugars to ethanol (CH_2CH_2OH) in the absence of free molecular oxygen (Gerardi, 2006). The selection and dependence on fungi for nitrogen removal has not been a conventional focus. However, research has identified that denitrification can be facilitated by certain fungi at greater rates than bacteria, in addition to the capacity to reduce toxic chemicals (Guest, 2002).

Archaebacteria relevant to wastewater treatment include halophiles, which are organisms that require elevated concentrations of sodium ions (Na^2), which are required for cell wall integrity, potassium ions (K^+) to support required enzyme activity, produce gas vacuoles used to regulate cell buoyancy, and are commonly referred to as salt-loving bacteria (Gerardi, 2006). Also relevant are methanogens, which produce methane (CH_4) as a byproduct of stabilizing waste products. Further discuss on bacteria in wastewater treatment is discussed in section 2.4. Other archaebacteria not as relevant include thermoacidophiles, which propagate in high-temperature and acidic environments, and cyanobacteria and photosynthetic bacteria, which also produce gas vacuoles, but unlike halophiles, they can generate their own food source.

2.3 Ecology

The ecology in wastewater treatment processes is complex with different effects on relationships and habits as based on environmental conditions. The focus of ecology relating to wastewater treatment processes generally focuses on abiotic and biotic factors, relating to the growth and reproduction of microorganisms and bacteria. Those cycles and processes can be symbiotic or predatory, providing food and energy either as a web or in a chain, both depicted in Figure 10, which transfers energy and carbon through metabolic processes. Each biological wastewater treatment process can be considered an amplifier, designed, and maintained to accelerate and/or intensify those biological processes (Gerardi, 2006).

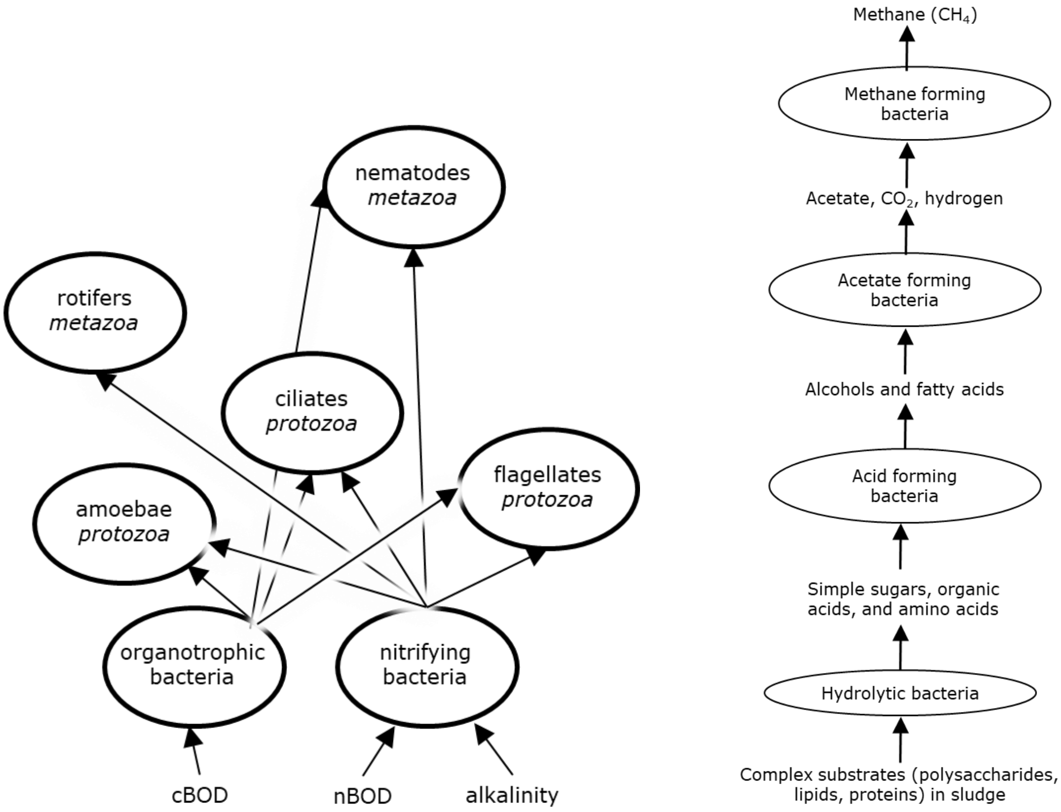

Figure 10 Carbon and Energy Web and Chain (Gerardi, 2006)

Within the ecology of these processes are non-living factors that control the environment, known as abiotic factors. Key abiotic factors in the activated sludge and biological treatment processes include alkalinity, pH, temperature, concentration of ammonium ions, concentrations of dissolved oxygen and inorganic matter, nutrients, substrates, and toxic wastes. Operational abiotic factors include the hydraulic retention time, rate of return activated sludge (RAS), and the rate of waste activated sludge (WAS) (Gerardi, 2006).

Conversely, biotic factors comprise the living components within the process, which can affect other organisms, and the abiotic factors. Key biotic factors include the abundance and type of bacteria and microorganisms, in addition to operational factors such as the mixed liquor volatile suspended solids (MLVSS) concentration and the mean cell residence time (MCRT) (Gerardi, 2006).

The abiotic and biotic factors and operational factors should be considered holistically, as each is part of either the food, energy, or transformation to move carbon and energy up to the chain or web with complex interactions that serve the demands of higher trophic levels.

As part of a predatory relationship, bacteria are then consumed by protozoa or metazoa. As that carbon and energy moves up through the interrelated processes to protozoa or metazoa, then to higher life forms such as rotifers and nematodes, greater carbon and energy is lost as heat or waste products for the synthesis of biomass, thus the cumulative weight of each higher life form decreases. This process is not always linear, as some substrates, carbon, and energy sources are fed upon by higher and lower trophic level microorganisms, each interdependent by their feeding habits, population, and abiotic factors.

The habitats for microorganisms, and the related interactions and movement of resources through the activated sludge process are unique to each microorganism and within each environmental operational condition. Where and when they inhabit, are important to understand throughout the activated sludge process. Several examples of the variety of habitats are described below (Gerardi, 2006).

- Aerobic bacteria will mainly propagate and inhabit where dissolved oxygen concentrations are greatest, such as along the exterior of floc particles, while oxidizing ammonia and nitrite.
- Floc-forming bacteria will propagate throughout floc particles, and in the bulk solution, prior to forming floc, while oxidizing soluble cBOD.
- Pseudomonads will propagate throughout floc particles, while oxidizing soluble cBOD, toxic phenol and phenolic compounds, and may have larger concentrations than other bacteria, due to their ability to degrade and consume a larger variety of substrate.
- Alcaligenes and Flavobacterium mainly degrade proteins and would normally only be present in waste streams that are high in protein but would be present at lower quantities or concentrations than other bacterium due to a limited ability to utilize varieties of substrate.
- Bacteria will have higher quantities than protozoa due to having a lager surface-to-volume ratio, and able to consume more soluble substrate, and with greater populations.

The examples listed above support the "competitive exclusion principal", in which the species that best utilize resources present in a habitat, will solely occupy that habitat at that time (Gerardi, 2006). Such competition is another biotic factor in the actual or potential success of microbiological organisms and biological processes.

Some microorganisms have larger surface areas, and thereby experience greater exposure to bulk solution, and can adsorb a greater quantity of nutrients. Additionally, if those microorganisms require less nutrients for propagation, then they are less inhibited than other bacteria in low nutrient environmental conditions. Like the proficiency towards lower nutrient conditions, other bacteria can proliferate in low dissolved oxygen conditions, due to a better affinity to utilizing available oxygen from a variety of substrates, such as *Sphaerotilus Natans* or *Haliscoemobacter hydrossis*. This is part of why nitrifying systems generally require a higher oxygen transfer efficiency (AOTE) of nine to 15 percent (Eckenfelder, 1992).

Similarly, sulfate-reducing bacteria will out complete methane-forming bacteria for the same resource, in a greater presence of sulfate (SO_4^{-2}). This is because the sulfate-reducing bacteria become more active, and wins the competition of that resource, then increases in population faster, continuing the trend of winning that competition. This is neither a symbiotic or predator-prey relationship, but an outcome of the abiotic and biotic factors present in the wastewater treatment processes.

Those examples describe some of the differences, variation, and relationships that comprise the ecology in wastewater treatment processes that must be understood to create and maintain the abiotic conditions and control the biotic factors to promote the ecology desired. To define that understanding, there are four main queries to be answered about the ecology to both explain current conditions, and to be able to predict outcomes from changes as follows: (Rittmann, McCarty, 2020)

1. Define the community structure(s).
    a. What is the different type of microorganisms and bacteria present?
    b. What is the concentration or abundance of each?
    c. What is the spatial relationship?
    d. Define the location, habitat, time, and duration.
2. Define the potential community function(s).

    a. What are the potential metabolic reactions?
    b. What are the anticipated symbiotic or predatory relationships?
3. Define the observed community function(s)
    a. What were the observed metabolic reactions?
    b. What were the observed feeding habits?
    c. What were the observed relationships?
4. Define the abiotic and biotic factors, and interactions between microbiology and the environment.

Understanding the ecology is key to identifying and creating niches to promote and sustain the right microbiology, conditions, and to remove targeted pollutants. This is known as microbial selection, as discussed in Chapter 4.

2.4 Bacteria

Bacteria are the simplest life forms, representing the largest group of living organisms, with the potentially largest variety of species, and comprise between 90 to 95 percent of the biomass in biological wastewater treatment processes. Bacteria are unicellular prokaryotes within the Monera kingdom, smaller than eukaryotic cells, and have greater growth rates due to greater surface-to-volume ratio.

The value of, and dependence on bacteria in the environment and wastewater treatment processes is the ability to recycle bound organic substrate and energy through metabolic reactions, transforming that material to inorganic compounds, making them available as food and energy by other microorganisms.

Bacteria are classified by their structure, nutrition, metabolism, and response to chemical stains. The primary common structures (shapes) include bacillus, which is rod shaped, coccus, which is generally spherical, and spirillum, which has a rigid spiral structure, and as depicted in Figure 11 below (Gerardi, 2006).

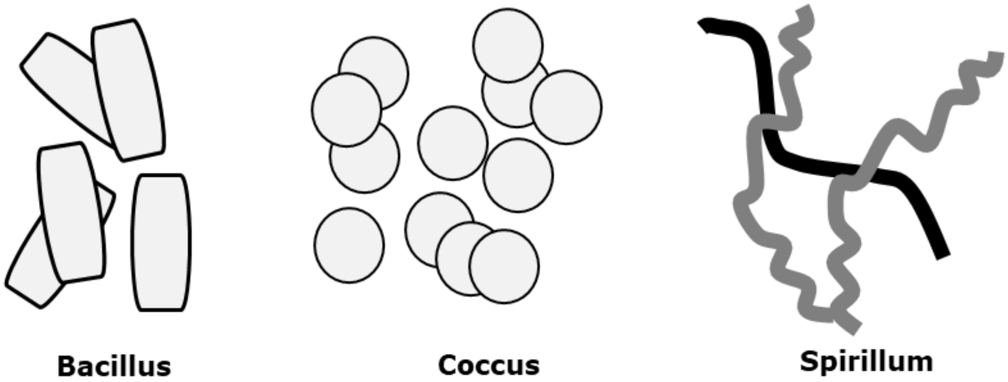

Figure 11 Common Bacteria Shapes

Additionally, there are seven uncommon shapes identified by their shape or movement, including Stalked, Sheathed, Actinomycetes, Budding, Blue-green Filamentous, Blue-green Coccoid, and Gliding bacteria as depicted in Figure 12 below.

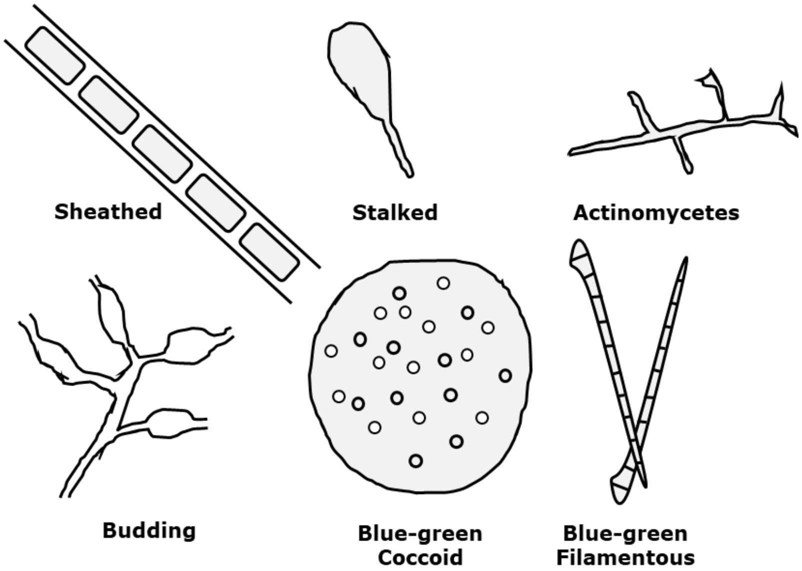

Figure 12 Uncommon Bacteria Shapes

The major elements of bacteria considered in wastewater treatment generally focus on the nutrition, consisting of the substrates used for food and energy, motility, being the means and methods of movement, or lack of, and factors that affect bacteria growth. The three major growth factors affecting bacteria growth are pH, temperature, and how bacteria respond to presence of free molecular oxygen (Schuyler, 2017).

Bacteria that use free oxygen as a final electron acceptor are known as aerobes. Bacteria that use combined oxygen substances, such as Nitrate-N, sulfate, and carbonate (CO_3^{-2}), when free oxygen is absent, are referred to as facultative anaerobes. Bacteria that cannot use free or combined oxygen are referred to as anaerobes, which use alcohols or organic acids as a final electron acceptor. Facultative anaerobes can be either aerotolerant, or oxygen intolerant (obligate). Aerotolerant facultative anaerobes have the most complex enzyme systems, where one system uses free molecular oxygen, and another for utilizing other molecules to degrade substrates when oxygen is not available (Schuyler, 2017).

The main nitrifying bacteria include *Nitrosomonas* and *Nitrobacter* are aerobic bacteria. Nitrification uses autotrophic chemolithotrophs to metabolize ammonia to nitrite. Nitrifying bacteria are slow growing and are inhibited by high concentrations of nutrients, such as BOD.

Chemoheterotrophs are dominant in ordinary biology conditions. However, *Nitrosomonas* and *Nitrobacter* can become dominant and successfully nitrify wastewater when the concentrations are suitable and there is sufficient solids retention time (SRT). Anoxic and anaerobic conditions are necessary for biological denitrification. Other denitrifying bacteria include *Pseudomonas*, *Alcaligenes*, *Bacillus*, *Spirillum*, and *Acinetobacter*, which convert Nitrate to nitrogen gas and nitrous oxide gases.

Bacteria response to free molecular oxygen is also critical in managing concerns of providing sufficient concentrations of oxygen in wastewater treatment processes for aerobe and facultative anaerobe activity, including concerns regarding endogenous respiration, floc formation, nitrification, and control of filamentous growth in low dissolved oxygen concentrations.

Endogenous respiration is the oxygen consumption by the bacteria and biomass to maintain cell growth and reproduction, in addition to the oxygen consumption required for degradation of substrates and toxins. Generally, minimum concentration of dissolved oxygen required in biological wastewater treatment plants to support and maintain endogenous respiration is 0.8 mg/L, but can be lower relating to nitrifying bacteria, where a minimum concentration of 0.4 mg/L may be sufficient, but generally 2.0 to 3.0 mg/L may be required. Minimum dissolved oxygens to support floc formation is generally 1.0 mg/L. The required dissolved oxygen concentration to prevent the growth of filamentous bacteria is relative to the chemical oxygen demand (COD) removed and must be individually measured and adjusted based on actual conditions (Gerardi, 2006).

In the activated sludge process, non-living substrates are used as energy and carbon sources, known as biochemical oxygen demand (BOD). BOD may be either carbonaceous BOD (cBOD) used by organotrophic bacteria, or nitrogenous BOD (nBOD), used by nitrifying bacteria.

Organotrophic bacteria use organic compounds as carbon and energy sources, such as Acetic Acid (CH_3COOH), Acetone (CH_3COCH_3), Ethyl Alcohol (CH_3CH_2OH), Glucose ($C_6H_{12}O_6$), Isopropyl Alcohol ($CH_3CHOHCH_3$), or Stearic Acid ($CH_3\left(CH_2\right)_{16}COOH$). As compounds are degraded or oxidized by bacteria in wastewater treatment processes, the concentration of carbonaceous waste is decreased.

Other bacteria utilize inorganic compounds or minerals for carbon and energy for cell synthesis, growth, and reproduction, are referred to as chemolithotrophs, which include iron bacteria, sulfur bacteria, and nitrifying bacteria.

As part of the microbial process, the chemical bonds of organic and inorganic compounds are broken as substrates, and degraded for energy, which releases electrons. That energy from the release of electrons is then stored in the bacteria adenosine triphosphate (ATP), as a phosphate bond, and then electrons are removed from the cell, through a process referred to as a final electron carrier molecule. That molecule may be in the form of free oxygen for aerobic bacteria and as part of nitrification, or in the form of nitrate or nitrite ions for anaerobic bacteria in the denitrification process and in an anoxic environment. The process of energy capture and transformation by bacteria is a key concept, depicted in Figure 13 below.

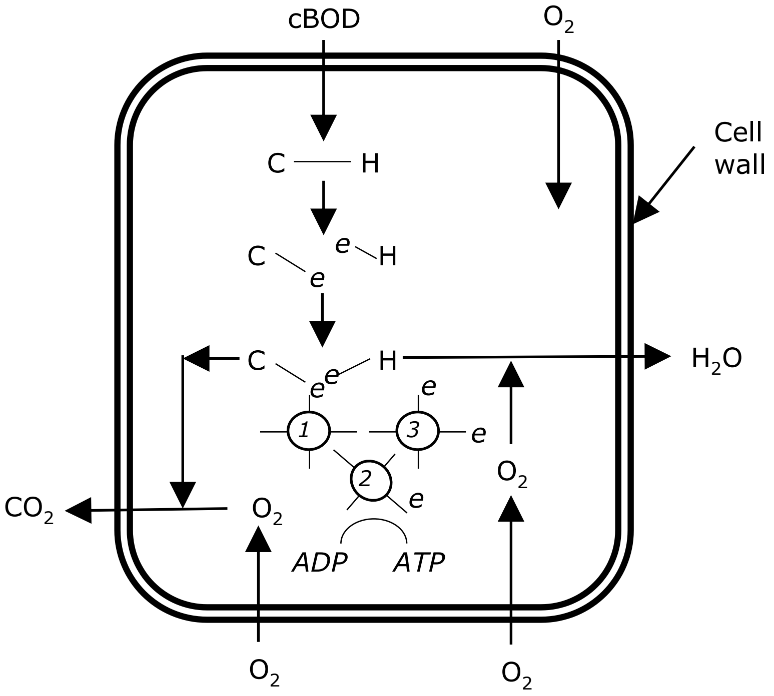

Figure 13 Electron Acceptors and Receivers (Gerardi, 2002)

As depicted in Figure 13 above, enzymes split the chemical bonds of the cBOD, and release energy, in the form of electrons from the carbon and hydrogen (e), which in return are captured by molecules 1, 2, and 3, and conveyed to the oxygen molecule, and then carried out of the cell as carbon dioxide and water. A portion of the energy from the electrons capture is used in the ADP/ATP process of creating high-energy phosphate bonds in the cell. This metabolism of the microbiology is further discussed in Chapter 6.

Specific bacteria focused nitrogen removal as discussed and referenced throughout the following chapters include the following.

- Nitrifying Bacteria – *Nitrosomonas*, *Nitrosospira*, *Nitrosococcus*, *Nitrosocystis*, *Nitrosolobus*, which oxidize the ammonia ion, and *Nitrobacter*, *Nitrospire*, and *Nitrospira*, which oxidize nitrite.
- Denitrifying Bacteria – *Alcaligenes*, *Bacillus* and *Pseudomonas*, which each use nitrate to degrade soluble cBOD to molecular Nitrogen and nitrous oxide (N_2O).

Examples of how certain nitrifying bacteria oxidize the minerals in such compounds is depicted in Equation 2 and Equation 3 below.

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$$

Equation 2 Nitrosomonas Bacteria, Utilizing Ammonium Ion Reaction (Gerardi, 2002)

$$NO_2^- + 0.5O_2 \rightarrow NO_3^-$$

Equation 3 Nitrobacter Bacteria, Utilizing Nitrite Ion Reaction (Gerardi, 2002)

Such bacteria utilize carbon dioxide the as a carbon source, which when carbon dioxide is dissolved in wastewater, Carbonic Acid (H_2CO_3) is formed per Equation 4 below (Gerardi, 2002).

$$CO_2 + H_2O \rightarrow H_2CO_3$$

Equation 4 Carbon Dioxide Dissolution into Wastewater Reaction

Once in the wastewater, a portion will disassociate into bicarbonate ions and hydrogen ions per Equation 5 below. Then the bicarbonate ion is utilized as the inorganic carbon source.

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

Equation 5 Carbon Dioxide Disassociation Reaction

2.5 Protozoa

Protozoa in general are key to a healthy ecology and performance of biological wastewater treatment processes. First, as an indicator of health and performance, and second by benefit of the removal of bacteria through predation, improving and promoting floc formation, and stimulating the degradation of organic and toxic waste by other bacteria.

Protozoa are generally classified based on cell structure, means of movement, and spore structure. Historically, there are four primary classification of protozoa including amoebae, ciliates, and flagellates as briefly discussed below, and sporoza. More recently there has been formal re-distribution or removal out of the kingdom of protozoa but are most referred to by their historical classification (WEF, 2015).

Amoebae are primarily characterized by a retractable pseudopodia used for feeding and for motility, with a wide variety of shapes. Amoebae found in biological wastewater treatment processes are generally Rhizopoda, phylum of the kingdom Protista, and are identified as either without a shell (naked) or with a shell (testate). Amoebae are generally not dominate in processes and are generally present in low quantities.

Ciliates are easily identifiable by various unique features including the presence of cilia or hair-like appurtenances, the presence of two types of nuclei, including a macronucleus and a micronucleus, and active conjugation where nuclear material is exchanges between ciliates during a brief amalgamation. The cilia will generally cover the structure, or at ends of the ciliates, with breaks at the mouth of the organism, although other specially formulated cilia may be present at the mouth, to promote the act of feeding. Most common ciliates observed in biological wastewater treatment processes include gymnostomes, cyrtophorids, nassulids, hymenostomes, hypotrichs, and peritrichs.

Flagellates are also easily recognizable by the presence of an appendage, typically longer than the body of the protozoa, and may taper or be hair-like, referred to as a flagella. The flagella provide the main source of locomotion and may incur current to promote feeding. Flagellates are present across four kingdoms, are generally either photoautotrophic, which can use sunlight and carbon dioxide to for food and energy synthesis, or heterotrophic relying on particles or dissolve sources for food and energy. Photoautotrophic flagellates are more prevalent in oxidation ditches, oxidation lagoons, and upper layers of trickling filters where sufficient interaction with sunlight is available. Heterotrophic flagellates are the most common overall (Curds, 1992).

Protozoa observed in biological wastewater treatment processes reproduce through and asexual process of either binary fission, which is an equal division of cell mass to new generations, or budding, which is an unequal fission, or multiple fission, which is the generation of daughter cells. However, the reproduction rates of protozoa very by type and the ecology of their environment. May ciliates can reproduce (or divide) every four to five hours, as compared to certain amoebae, in which their reproduction rate is generally measured in days.

Protozoa feed on a wide range of bacteria, including fecal coliforms and pathogenic bacteria, and have been observed to consume up to 500 bacteria per hour. Their observed presence has been documented in effluents with 95 percent or greater reduction in fecal coliform and other parthenogenic bacteria, and in their absence, approximately 50 percent of such target bacteria are removed through other processes.

The presence, quantity, and variety of protozoa in biological wastewater treatment processes can change as each achieve maturation and based on different environmental conditions. In one observed and recorded biological wastewater treatment process, the first observed protozoa dominance were flagellates, which was observed to decline consistently, while free-swimming ciliate populations increased to overtake dominance at a maximum of approximately 80 percent of the protozoa population, over 30-days. As the free-swimming ciliate population peaked near 60 percent dominance, the concentration of attached ciliates began to increase, achieving a dominance of approximately 90 percent over another 20 days of observation. Upon a quick decrease and loss of the attached ciliates population, the return of crawling ciliates was observed to near 100 percent dominance within another five days (WEF, 2015). The important of such observations is the ability to make informed decisions about process control discussed in Chapter 4. Additionally, at least two protozoa have been documented to enhance nitrification, including peritrich ciliates *Epistylis* and *Vorticella* (WEF, 2015).

2.6 Kinetics

A portion of these fundamentals is the various chemical reactions of molecules and ions of nitrogen compounds, substrate, and energy. But those reactions do not just occur in the presence of the right molecules or compounds with the right concentration of moles but rely on other processes to break the bonds of existing reactants, to form new compounds, molecules, or ions, and/or release energy that promotes the ability of microbiology to utilize available material for energy and food. The study of, and rates of, such reactions and processes are referred to as kinetics, or enzyme kinetics.

Prior to reviewing the activity and contributing factors relating to the kinetics of reactions, a review of the types of reactions and their general equation of kinetics is provided below. There are four major types of reactions, describe in terms of A, which represents the first molecule, compound, or ion (reactants) in the reaction. B represents a second molecule, compound, or ion in the reaction, C represents the product of the reaction, and z represents a catalyst in the reaction.

First Order reaction, $A \to C$, where the rate (kinetics) of the reaction is described as follows:

$$\frac{d[A]}{dt} = -K[A]$$

Equation 6 First Order Reaction

Second Order reaction, $A + B \to C$, where the rate of the reaction is described as follows:

$$\frac{d[A]}{dt} = -K[A][B]$$

Equation 7 Second Order Reaction

Third Order reaction, $2A + B \to C$, where the rate of the reaction is described as follows:

$$\frac{d[A]}{dt} = \frac{d[B]}{dt} = K[A]^2[B]$$

Equation 8 Third Order Reaction

Fourth Order reaction, where z catalyzes $A \to C$, where A is in abundance, and z is limited. The rate of the reaction is described as follows:

$$\frac{d[A]}{dt} = -K$$

Equation 9 Fourth Order Reaction

The reactions listed above are depicted as irreversible. However, many biological reactions are reversible, which is important relating to thermodynamics, as further discussed in Chapter 6.

Such reactions are dependent on the concentrations of the reactants as a function of time. As an example, the higher the order of reaction, the faster the reaction rate decreased as a function of time. Since reactants are consumed faster, the available reactants decrease faster, and therefore the kinetics decrease.

A key component of the consideration of kinetics in the context of this focus, is the change in energy in such reactions. The electrons present in initial compounds, ions, and molecules (reactants) require additional energy, for non-spontaneous reactions, to complete the transformation in the reaction.

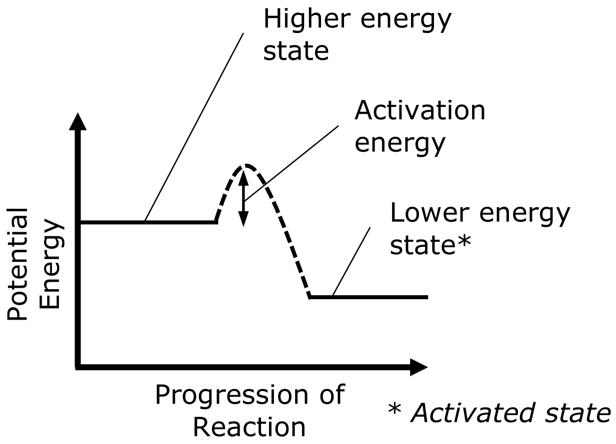

Figure 14 Energy States in Reaction Progression

This is referred to as the activated state, where the additional energy catalyzes the reaction, as depicted in Figure 14 above. The result of the additional energy allows the reaction to progress from a high potential energy state, to the result of the reaction, generally at a lower energy state.

These chemical reactions can occur spontaneously or can be assisted by a catalyst. Catalysis is present before, during, and after the reactions occurs, and does not change, and is not consumed as part of the reaction. The value added by the catalyst is it may either influence the rate of the reaction, or lower the energy required for the reaction to occur, as graphically depicted in Figure 15 below.

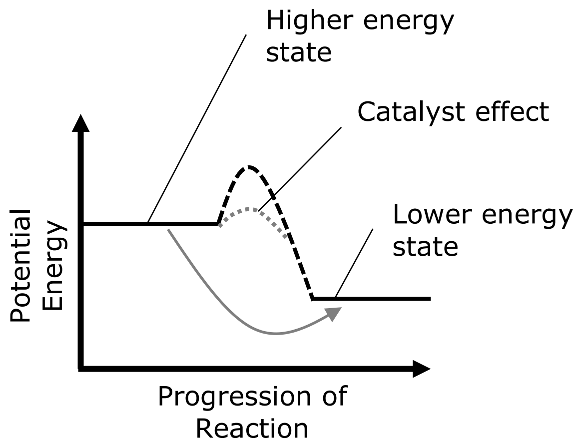

Figure 15 Catalyst Effect on Energy States in Reaction Progression

Both facilitate the progression and the rate of the reaction, as graphically depicted in Figure 15 above, and is a simplification of the effect of a catalyst. A catalyst could be another molecule or compound that may promote another transition state with less potential energy required, perhaps through requiring less concentration of the presence of reactants, or a lower temperature to achieve the activation state, and overall promote the reaction.

How kinetics occur includes both positive and negative catalysts, where the negative catalyst either increases the required activation energy, which can inhibit the occurrence of the reaction, or the rate that it occurs. A positive catalyst lowers the required activated energy, increasing the rate of the reaction.

Other contributors are the concentration of reactants present of the desired reaction. At higher concentrations, more reactions are available, which additionally increases the rate of the reactions, in addition to increasing the likelihood the reactions will occur. Also, the presence and ratio of molecules with higher kinetic energies, and when combined may provide the energy required to achieve an activation state. Noting that molecules have varying levels of kinetic energy, and the temperature generally only defined the average kinetic energy. Also, the bond strength, molecular shape, and availability of electrons contribute to the kinetics, in addition to the surface area of reactants. Finally, temperature may be the largest, or best contributor to increasing reaction rates, due to increasing the molecular action or activity. However, there are limitations to benefits of higher temperatures, which are variable based on which reactants are present, their individual and combined responses, speciation, or structure relating to temperature.

Kinetic parameters are known to vary among aerobic microbiology, based on species, growth rates, oxygen affinity, ammonia affinity, decay rates, pH, yield coefficients, and temperature. More specifically relating to the microbiology responsible for nitrogen removal, there are unique dependencies identified and quantified that influence the kinetics. Key kinetics for aerobic ammonia-oxidizing bacteria has been demonstrated and documented that the temperature dependency for *Nitrosomonas europaea* is as noted in Equation 10 below.

$$\mu_{\max} = 0.47 * 1.03^{T-15}$$

Equation 10 Temperature Dependance, *Nitrosomonas europaea*

However, it has also been demonstrated that the growth and reproduction of *Nitrosospira* favors lower temperatures than *Nitrosomonas* europaea and *Nitrosomonas eutropha* (Siripong and Rittman, 2007). Additional documented kinetics relating to aerobic bacteria is summarized in Table 2 below.

Growth rate (1/d)	Oxygen affinity $\frac{mgO_2}{L}$	Ammonia affinity $\frac{mgN}{L}$	Decay rate (1/d)	Yield coefficient (g biomass/g N)
0.65 – 2.05	0.03-0.6	0.14-2.4	0.048-0.19	0.147-0.21

Table 2 Range of Aerobic Ammonia-oxidizing Bacteria Kinetics, (WEF, 2015)

As part of the nitritation process, chemolithoautotrophic nitrite-oxidizing bacteria convert nitrite to nitrate, and can benefit from kinetics and temperature variables, that promote greater growth rates than aerobic bacteria, as *Nitrobacter* experience ideal growth rates between a temperature range of 30 degrees to 40 degrees Celsius, and *Nitrospira* at temperatures greater than 35 degrees Celsius (WEF, 2015). Such maximum temperature dependencies were demonstrated in 1965 as noted in Equation 11 below.

$$\mu_{\max} = 0.87 * 1.06^{T-15}$$

Equation 11 Temperature Dependance, *Nitrobacter*

Additional documented kinetics relating to nitrite-oxidizing bacteria is summarized in Table 3.

Growth rate (1/d)	Oxygen affinity $\frac{mgO_2}{L}$	Nitrite affinity $\frac{mgN}{L}$	Decay rate (1/d)	Yield coefficient (g biomass/g N)
0.31-2.0	0.13-5.3	0.25-0.39	0.048	0.147

Table 3 Range of Nitrite-oxidizing Bacteria Kinetics, (WEF, 2015)

Denitrification kinetics is a heterotrophic process, with different kinetics relating to nitrite-oxidizing bacteria as summarized in Table 4.

	Growth rate (1/d)	Oxygen affinity $\frac{mgO_2}{L}$	N affinity $\frac{mgN}{L}$	Decay rate (1/d)	Yield coefficient (g biomass/g N)
Denitrification with NO_3^-	2.6-4.8	0.2	0.14-0.5	0.1-0.62	0.4-0.67
Denitrification With NO_2^-	1.5	-	0.12	0.1	0.4

Table 4 Range of Heterotrophic Bacteria Kinetics, (WEF, 2015)

Understanding such kinetics is part of the foundation in developing and controlling biological wastewater treatment systems, where the relationship between catalyst and substrates can limit growth rates (Rittmann, McCarty, 2001). The definition of this relationship for kinetics, referred to as the Monod equation, is a mathematical representation of the primary electron donor (substrate) defined in Equation 12.

$$\mu = \left(\frac{1}{X_a} * \frac{dX_a}{dt} \right)_{syn} = \hat{\mu} \left(\frac{S}{S+K} \right)$$

Equation 12 Monod Equation (Rittmann, McCarty, 2001)

The use of that equation reveals how net specific growth rates, and synthesis are dependent on substrate concentrations, and where at lower substrate (S) concentrations, the kinetics have a first-order relationship. At higher concentrations of substrate, the kinetics have a zero-order relationship. This relationship is graphically depicted in Figure 16.

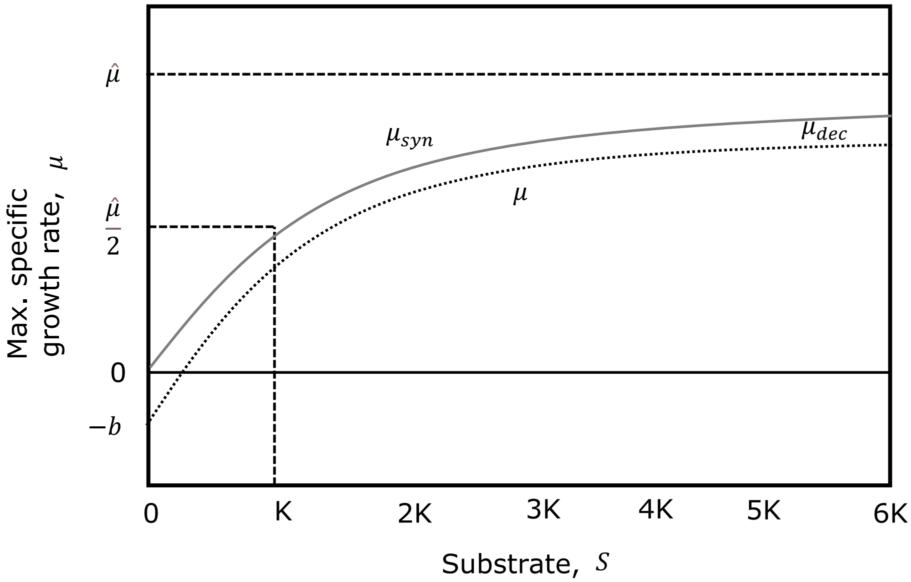

Figure 16 Saturation Function (Rittmann, McCarty, 2001)

Important from previous research and findings, is that biomass requires energy for maintenance, in addition to growth and reproduction, specifically energy for transport, motility, heat loss, and resynthesis, referred to as endogenous decay. The rate of endogenous decay is a first order reaction, noting that not all decay is related to cell maintenance, where some is for the conversion to inert biomass. The overall resulting net specific growth rate of biomass, including the decay for cell maintenance, is generally represented as follows:

$$\mu = \left(\frac{1}{X_a} * \frac{dX_a}{dt} \right) = \mu_{syn} + \mu_{des} = \hat{\mu} \left(\frac{S}{S+K} \right) - b$$

Equation 13 Biomass Net Growth (Rittmann, McCarty, 2001)

This results in the ability to focus on the rate of substrate utilization (r_{ut}), and the maximum specific rate that substrate can be utilized is simplified in Equation 14 below.

$$r_{ut} = -\left(\frac{\hat{q} S}{k+S} \right) X_a$$

Equation 14 Modified Monod Equation (Rittmann, McCarty, 2001)

These are the general fundamentals of kinetic rate equations used to demonstrate overall secondary biological wastewater treatment, and shortcut nitrogen removal.

2.7 Growth Rates and Inhibition

The growth rates of microbiology in wastewater treatment are a key focus of environmental engineers, in both the design, and operations of such facilities. There are four primary phases of growth, including Lag, Log, Endogenous, and Death or Decline. The Lag phase is when bacteria are actively synthesizing enzymes for substrate degradation, which provides energy and carbon for reproduction, but are not reproducing. This phase can be seen in the wastewater treatment process during initial start-up, and during recovery after shock loads and upsets, and represents the stage when biology is acclimating to new or changed environments. The duration of the Lag phase is affected by the conditions and variables relating to the environment, and which bacteria species are present.

The Log phase represents the active reproduction, which happens exponentially beginning with the uptake of substrate, followed by the rapid synthesis and growth of new cells, followed by a declining growth and reproduction rate.

The endogenous phase is when the maximum limit of biology that can be sustained with the available substrate is reached, and growth levels off. This is when there is a balance of growth and death, with no net increase of growth.

The final phase is cell death or decline, represented when the decline exceeds the growth, no more leveling off, and will vary in relation to the substrate loading. Each phase is represented graphically in Figure 17.

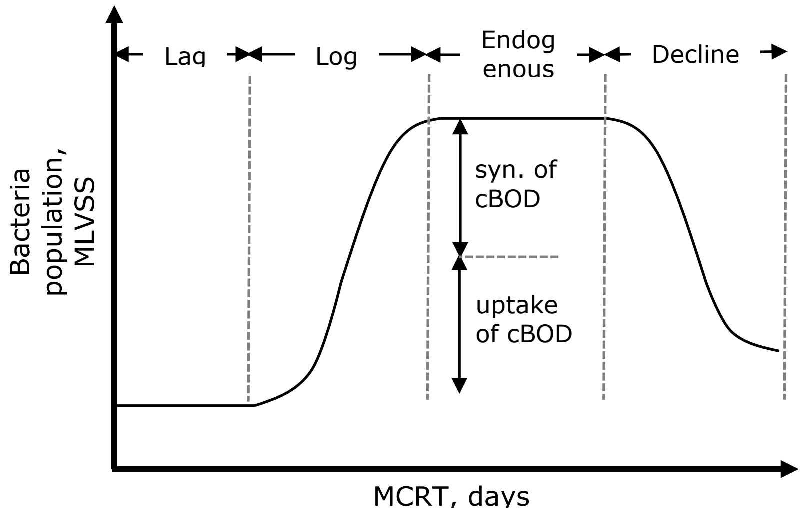

Figure 17 Biology Growth Curve (Gerardi, 2006)

There are two general types of growth, each based on how substrate is utilized. One type of growth is referred to as anabolism, in which bacteria cells are degraded to produce energy and/or carbon for cellular growth and molecular combination, forming larger molecules and greater sludge. The other is catabolism, in which those larger molecules get degrades to smaller ones for energy. Anabolism occurs prior to the endogenous phase, and catabolism beings with the onset of the endogenous phase. There are operational considerations for wastewater treatment processes relating to anabolism and catabolism discussed in Chapter 4.

There are demonstrated and documented values discussed below as part of the understanding of growth and inhibition. However, the ability to precisely determine the quantity of microbiological cells produced in the activated sludge wastewater treatment process is limited due to the following complexities (Gerardi, 2006).

- The wasting of activated sludge from wastewater treatment processes includes both young, old, and dead microbiology with varying ratios, concentrations, and growth rates not easily quantifiable.
- The variety of biology present in wastewater treatment processes have varying reproduction rates, such as organotrophs that can reproduce in 15 minutes, and nitrifying bacteria that required as much as 15-days.
- Lack of a medium for growth that can promote an enumeration of all bacteria present in any process.

Specific to nitrogen removal, it has been demonstrated that in general, nitrifying bacteria oxidize 30 molecules of ammonia ions for each one (1) molecule of carbon dioxide, or 100 molecules of nitrite ions, to oxidize one (1) molecule of carbon dioxide (Gerardi, 2002).

Regarding inhibition, there are a variety of competing and complimentary conditions specific to the bacteria and environmental conditions present in wastewater treatment processes. During the first stage of nitrification where ammonia is oxidized to a hydroxylamine compound (*amo*), which is an enzyme that is highly reactive, where some compounds become oxidized in an irreversible inhibition, as part of the catalytic cycle. Aerobic ammonia-oxidizing bacteria can also be inhibited by competitive compounds including methane, ethylene, and carbon dioxide. There is also non-competitive inhibition possible with the presence of ethane, chloroethane, thiourea, nitrapryrin or disphenyliodonium, and other mechanism-based inhibitions (WEF, 2015).

Regarding nitrite-oxidizing bacteria, inhibitions including limitations relating to the pH of the environment, where an optimal pH is 7.8 to 8.0, and as the pH increases, there is a transition in the equilibrium of the ammonium-ammonia ratio. This can result in inhibitions when concentrations from free ammonia reach 33 to 50 $mgNH_3 - {}^{N}\!/_{L}$. And can begin at concentrations less than 10. Free ammonia also inhibits aerobic bacteria but begins at concentrations greater than 10. Other inhibitors of nitrite-oxidizing bacteria include toxicity of hydroxylamine beginning at concentrations of 0.42 $mgNH_2OH - {}^{N}\!/_{L}$, with irreversible inhibition occurring with concentrations as low as 2.0 to 5.0 $mgNH_2OH - {}^{N}\!/_{L}$. Concentrations of free nitrous acid (HNO_2) also becomes inhibitory at concentrations of 0.2 to 2.8 mg/L, only at pH levels less than 7.5 su. Additionally, concentrations of Chlorate ($KClO_3$) greater than 1 mM can completely inhibit nitrite-oxidizing bacteria activity (WEF, 2015).

Regarding denitrification processes, unideal pH conditions include those below 7.0, or above 8.0 su, at 20 to 30 degrees Celsius, which can be inhibitory, in addition to the presence of undesired compounds. Those include phosphate concentrations starting at 15.5 $mg\,{}^{P}\!/_{L}$, and nitrous acid at concentrations of 0.13 mg/L (WEF, 2015). Finally, the concentration of dissolved oxygen is a primary inhibitor for the denitrification process, as oxygen is not a preferred electron acceptor. However, the carbon to nitrogen ratio (C:N), of 2:1 must be maintained for stable denitrification (WEF, 2015).

Other research determined approximate maximum growth rates ($\mu_{\max}$) for ammonia oxidizing bacteria as 0.032 $mol*L^{-1}h^{-1}$, and 0.032 $mol*L^{-1}h^{-1}$ for nitrite oxidizing bacteria, along with a half saturation affinity constant (K_S) for oxygen of ammonia oxidizing bacteria as $9.87*10^{-7}\ mol*L^{-1}$, K_S for nitrate/nitrite as $2.11*10^{-6}\ mol*L^{-1}$, and the decay rate of 0.01 h^{-1}. For ammonia oxidizing bacteria, a K_S for oxygen of $1.88*10^{-6}\ mol*L^{-1}$, K_S for nitrate/nitrite as $3.94*10^{-9}$, and the decay rate of 0.088 h^{-1} (Gogulancea, González-Cabaleiro, Taniguchi, Jayathilake, Chen, Curtis, 2019).

2.8 Stoichiometry

Describing the quantities and reactants involved in the nitrogen cycle and removal processes, partially discussed in the sections and chapters above, includes the quantities and reactants involved in the nitrogen cycle and removal processes, the overall stoichiometric reactions as stated in the equations below, and the thermodynamics of the energy and synthesis relationships, discussed in Chapter 6.

Beginning with the nitrification process, where ammonia ions are oxidized to nitrate, the first reaction is the conversion of ammonium to nitrite as follows:

$$NH_4^- + 1.5O_2 \rightarrow 2H^+ + H_2O + NO_2^-$$

Equation 15 Energy-yielding Reaction, *Nitrosomonas* (Elsevier, 1993)

There are two separate, but consecutive reactions within cells during this reaction, including the oxidation of ammonia to hydroxylamine, which is an anabolism reaction, to produce energy and/or carbon for cellular growth defined as follows:

$$NH_3 + O_2 + 2H^+ 2e^- \rightarrow NH_2OH + H_2O$$

Equation 16 Anabolism Reaction (WEF, 2015)

The second is a catabolism reaction that generates energy for the degradation of substrate, in the presence of nitrous acid, accounting for additional oxygen uptake defined as follows:

$$NH_2OH + H_2O \rightarrow HNO_2 + 4H^+ + 4e^-$$

Equation 17 Catabolism Reaction (WEF, 2015)

The next step of the nitrification process oxidizes nitrite to nitrate as follows:

$NO_2^- + 0.5O_2 \rightarrow NO_3^-$, or as:

Equation 18 Energy-yielding Reaction, *Nitrobacter* (Elsevier, 1993)

$$NO_2^- + 0.473O_2 + 0.005NH_4^+ + 0.020CO_2 + 0.005HCO_3^- + 0.005H_2O \rightarrow NO_3^- + 0.005C_5H_7NO_2$$

Equation 19 Energy-yielding reaction (WEF, 2015)

Measurement of the loss of energy in equation 18 is approximately 58 to 84 kcal per mole of ammonium, or 20.9 kcal per Equation 19. The measurement of the loss of energy in Equation 16 is approximately 1.4 to 20.9 kcal per mole of nitrite. That reveals that in the first step of oxidizing ammonium to nitrite generates greater energy, per mole of nitrogen, then in the reaction of oxidizing nitrite to nitrate. Adding Equation 15 and Equation 18 to derive the overall oxidation of ammonium ions to nitrate is as follows:

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

Equation 20 Energy-yielding Reaction, Ammonium to Nitrate (Elsevier, 1993)

Prior to describing the formation of biomass utilizing the equations above, it is important to note that most of the carbon utilized is in the form of soluble carbon dioxide, and that there is a unique equilibrium of the carbonic acid-bicarbonate system, based on the pH of the wastewater treatment process environment. During the oxidation, the ammonia ion is oxidized to nitrate per the following reaction:

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$$

Equation 21 Carbon Dioxide Reaction, Ammonium to Nitrite (Elsevier, 1993)

During the oxidation of nitrite to nitrate, the reaction is as follows:

$$CO_2 + H_2O \Leftrightarrow H^+ + HCO_3^-$$

Equation 22 Carbon Dioxide Reaction, Nitrite to Nitrate (Elsevier, 1993)

In both reactions above, the free acid produced during the oxidation reactions produces carbonic acid. Now utilizing the formula of ethyl cyanoacetate ($C_5H_7NO_2$) for biomass formation, the growth of *Nitrosomonas* as an example, supports the transformation of ammonium ions to nitrite, can be represented as follows:

$$15CO_2 + 13NH_4^- \rightarrow 10NO_2^- + 3C_5H_7NO_2 + 23H^+ + 4H_2O$$

Equation 23 Biomass Production, *Nitrosomonas* (Elsevier, 1993)

Likewise, for the transformation of nitrite to nitrate, utilizing *Nitrobacter* as an example, can be represented as follows:

$$5CO_2 + NH_4^- + 10NO_2^- + H_2O \rightarrow 10NO_3^- + C_5H_7NO_2 + H^+$$

Equation 24 Biomass Production, *Nitrobacter* (Elsevier, 1993)

Next, the equations above for biomass growth (synthesis) can be added to the oxidation reactions, represented as follows:

$$55NH_4^- + 76O_2 + 109HCO_3^- \rightarrow C_5H_7NO_2 + 54NO_2^- + 57H_2O + 104H_2CO_3$$

Equation 25 Synthesis Oxidation, *Nitrosomonas* (Elsevier, 1993)

$$400NO_2^- + NH_4^- + 4H_2CO_3 + HCO_3^- + 195O_2 \rightarrow C_5H_7NO_2 + 3H_2O + 400NO_3^-$$

Equation 26 Synthesis Oxidation, *Nitrobacter* (Elsevier, 1993)

Those equations reveal a biomass production of 14.6 mg of *Nitrosomonas* based on the oxidation of 100 mg of ammonium ions, and 2.0 mg of *Nitrobacter*.

Therefore, the overall nitrification process of converting 100 mg/L of Ammonia-N to Nitrate-N yields approximately 17 mg/L of nitrifying biomass, with an oxygen requirement of 4.32 mgO_2 per $mgNH_4^-$, based on 3.22 mgO_2 per $mgNH_4^-$ to oxidize ammonium to nitrite, and another 1.11 mgO_2 required to oxidize nitrite to nitrate, according to Equation 27.

$$NH_4^- + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7NO_2 + 1.041H_2O + 0.98NO_3^- + 1.88H_2CO_3$$

Equation 27 Ammonia-N to Nitrate-N Biomass Yield (Elsevier, 1993)

Regarding the denitrification process, nitrate is reduced over multiple steps, which may be performed by multiple bacteria than can complete all the following steps in Equation 28.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Equation 28 General Denitrification Pathway (WEF, 2015)

Such bacteria that perform some, or all the transformation of nitrate to molecular nitrogen are facultative anaerobes, that in anoxic environments, respirate by utilizing nitrate in place of oxygen. In general, such bacteria may use acetate as the carbon source, in the form of acetic acid, with adequate COD:N ratio in solution, in accordance with the follow reaction.

$$5CH_3COOH + 8NO_3^- \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^-$$

Equation 29 Denitrification Pathway, Acetate Carbon Source (WEF, 2015)

That reaction can also utilize the model compound of the wastewater, with adequate COD:N ratio, in accordance with Equation 30.

$$C_{10}H_{19}NO_3 + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^-$$

Equation 30 Denitrification Pathway, Model Compound Wastewater Carbon Source, (WEF, 2015)

2.9 Energy in Wastewater and Treatment Processes

The long-term costs to operate and maintain wastewater treatment plants over the life of the facility will generally far exceed capital costs to initially implement such systems. Therefore, reducing the cost to build and sustain wastewater treatment and disposal services, can be best served by reduction of the long-term cost to operate. There are two major items to consider in this query, including the quantity, and cost of energy and other consumables used to provide wastewater treatment services, and the potential energy in wastewater. The reduction of the first directly promotes the desired outcome. The latter provides a potential for offsetting the first.

Controlling and providing supplemental oxygen to biological wastewater treatment processes is critical to sustain the proper environments and to promote and control the growth of various microbiology. The total energy needs in wastewater treatment plants can be significant, but major consumers of that energy vary based on the location, climate, process types and quantities, flow, loading, treatment goals and regulations. Additional energy requirements vary based on the quantity and type of staff (operators, maintenance, management, laboratory and regulatory), whether solids are process on-site, hauled off, or discharged, the use of chemicals or polymers, and level and complexity of systems, and the magnitude of administrative buildings, warehouses, storage, and other support systems.

However, the energy required for wastewater treatment can be generalized, based on recent and historical surveys of wastewater treatment plants of similar scale and processes, regulation, and performance goals. Those results reveal that generally, most of the energy used at such facilities are treatment related and can be further divided into either energy consumption relating to the loading (BOD), or as energy consumption relating to the flow. The consumption, by major system/demand in a wastewater treatment plant is depicted in Figure 18.

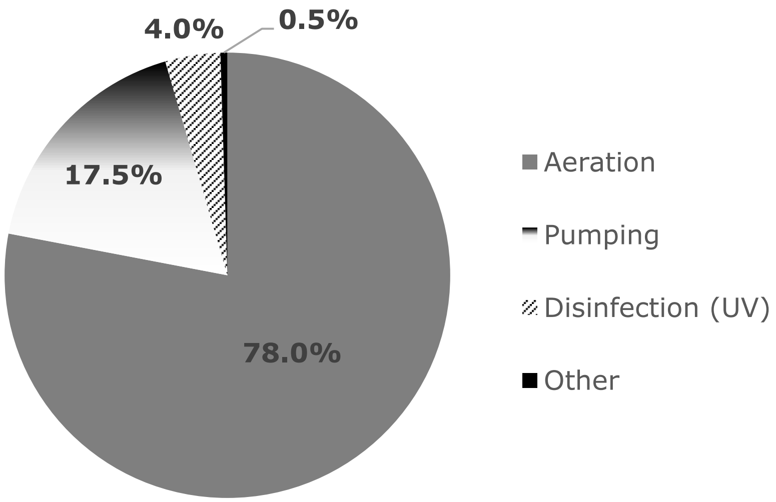

Figure 18 Energy use in WWTP (USDOE, 2017)

As represented in Figure 18 above, this data reveals that the energy required for aeration to promote biological processes can account for up to two-thirds, to over three-quarters of all energy consumption. Additionally, it is important to note that the efficiency of energy consumptions generally decreases as the quantity of flow, and/or loading decreases. Based upon the evaluation of 133 wastewater treatment plants, it was identified that small treatment facilities of less than 100,000 gallons per day (MGD) may be up to 10 times less efficient than facilities with greater than 50 MGD as depicted in Figure 19 below. This data was developed utilizing a normalized approach, in which the energy consumption efficiency was expressed as in units of hydraulic loading (Megawatt-hours per MGD or MWh/MG) and units of organic loading (kilowatt-hours per pound of treated BOD or kWh/lb BOD) and reported as a metric referred to as the Wastewater treatment plant Efficiency

Baseline (WEB), measured in units of megawatt per hour (MWh) per million gallons (mg) of flow, or kilowatt hour (kWh) per pound of BOD.

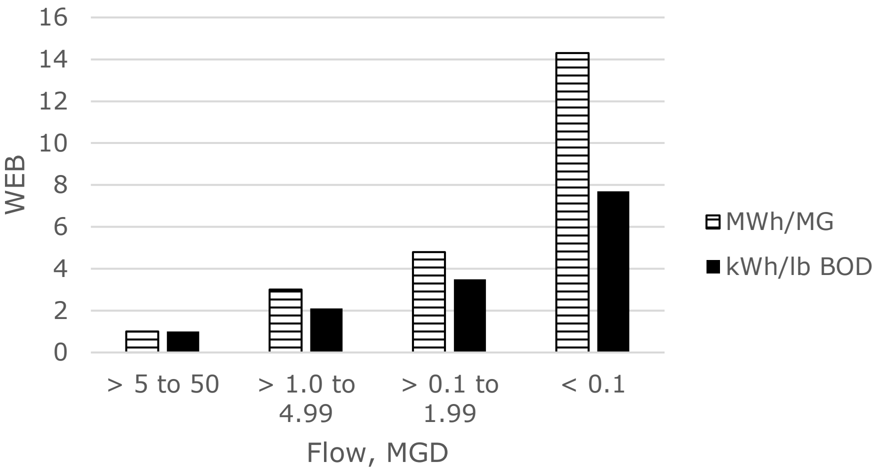

Figure 19 Energy Use versus Flow and Loading (PADOE, 2011)

Also, the type of unit processes will greatly influence the efficiency of energy use. This is represented in Figure 20, including conventional activated sludge (CAS), extended aeration (EA), oxidation ditch (OD), sequence batch reactor (SBR), trickling filter (TF) and membrane bioreactor (MBR).

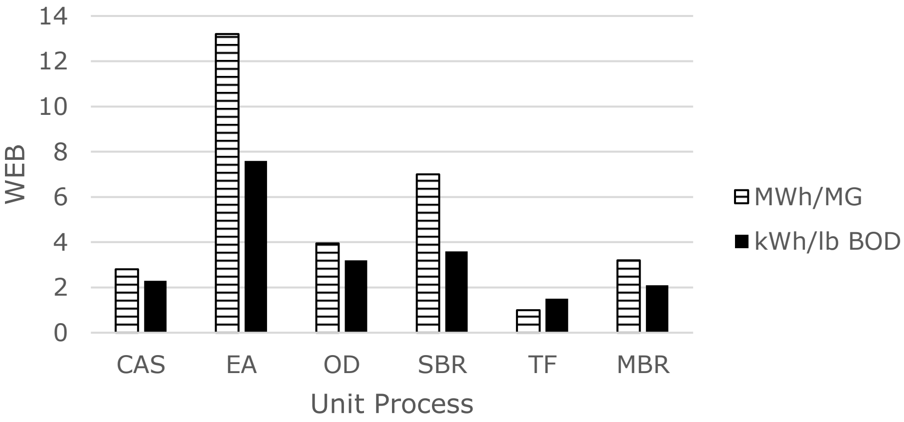

Figure 20 Energy Use by Major Unit Process (PADOE, 2011)

In general, approximately 1.2 lbs of oxygen is required for every 1 pound of BOD removal, and approximately 4.6 pounds of oxygen is required to reduce 1 pond of Ammonia-N to Nitrate-N (Gerardi, 2006). Therefore, the greater the loading, the greater the energy requirement to promote and sustain the biological activity, which is actually conducting most of the work of degrading nutrients and toxic constituents. This is critical enough to be re-stated. It is not the mechanical equipment, structures, or consumables that are providing the biological wastewater treatment services. Those components and systems are only creating and sustaining the proper environments for microbiology to thrive.

In whole, since aeration can account for up to two-thirds of all energy consumption at a wastewater treatment plant, a significant reduction in the energy requirement, either through optimized configuration, control of more efficient biological systems, or an increase in the efficiencies of adding oxygen to those processes will be required to meet the goal of reducing the long-term cost to operate.

The other relevant consideration is the potential energy in wastewater. There are four main forms of energy available in raw wastewater, including thermal, kinetic, potential, and chemical energy (WEF, 2015). To start, the average energy demand in a typical suspended activated sludge wastewater treatment plant, with anaerobic digestion is approximately 0.6 kWh per 158 gallons of raw wastewater, of which up to three-quarters is used for aeration processes. The chemical energy present in raw wastewater, typically measured as COD, is approximately 12 to 15 MJ/kg COD, and the organic fraction of the chemical energy is as much as 1.93 kWh per 158 gallons of raw wastewater. Therefore, there is more than twice the amount of chemical energy present, than energy required for typical wastewater treatment processes (WEF, 2015).

Other energy available is the potential conversion of hydraulic energy of the influent raw wastewater, intermediate hydraulic flows from pump stations, and/or at outfall or discharges, during or after treatment. Also, there is the potential for thermal energy recovery directly or indirectly. A direct source is from the raw wastewater, which can range from 68 degrees to 95 degrees Fahrenheit, which will vary based on the location and climate, collection system configuration and materials, and thermal qualities of discharges in the collection system.

As discussed in sections above, there are target ranges of temperatures for optimal conditions to promote and sustain biological treatment. Therefore, the benefit of recovering thermal energy directly from raw wastewater may not be conducive to help lower the overall performance, efficiency, and cost of wastewater treatment. However, indirect thermal recovery has been implemented and documented to provide real energy and cost savings. Specific examples include the recovery of heat from treatment processes are those that supplement heating of sludge for digestions, or for solids dewatering and drying. Additionally, the methane produced from anaerobic digestion, which in many cases is combusted at an on-site flare to reduce greenhouse gas emissions, can be recovered, and processed to either produce electricity, or natural gas. Both can be used to supplement, and offset other energy demands in the wastewater treatment plant and processes.

There are many other strategies to capture energy directly or indirectly from wastewater and wastewater treatment processes, including contributing factors relating to the various nitrogen removal shortcut techniques discussed in the next chapters.

CHAPTER 3

METHODS FOR AMMONIA AND NITROGEN MANAGEMENT

3.1 Nitrogen Removal Overview

As part of the biological wastewater treatment process of nitrification-denitrification (NdN), autotrophic bacteria (nitrifiers) convert the Ammonium ion (NH_4^+) to Nitrite-N (NO_2), and then to Nitrate-N (NO_3).

After dissolved oxygen concentrations in wastewater are reduced by the nitrifiers and other microbiology, heterotrophic bacteria (denitrifiers), use the oxygen attached to the Nitrate-N (NO_3) molecules for respiration. The main product from reactions produced by the denitrifiers is nitrogen gas (N_2), which is then safely released to the atmosphere, thus returning it to the nitrogen cycle, and reducing the concentration of TN in the discharge back to natural environments, waterways, or aquifers.

Those processes are based on the growth, reproduction, and decay of that microbiology. There are two methods in which that microbiology obtains carbon for growth, unique to autotrophs and heterotrophs as described below.

Autotrophs obtain the carbon required for growth from non-organic sources including alkaline bicarbonate (HCO_3) and carbon dioxide (CO_2), like how plants obtain carbon as an example. Conversely, heterotrophs require organic sources for carbon, and consume other organic compounds, much like how animals obtain carbon.

Heterotrophic BOD consuming denitrifiers have relatively fast reproduction and population growth rates in the wastewater treatment process as compared to autotrophic nitrifiers, which require greater time to mature and sustain their population. Therefore, it is the nitrifiers which command the solids retention time

(SRT) in wastewater treatment plants that aim to reduce nitrogen. The SRT is the duration of the presence of the solids in the process reactors. It is calculated as the quantity of solids retained divided by the quantity of solids out of the reactor, daily as expressed in Equation 31.

$$SRT = \frac{VX}{\left(Q - Q_w\right) X_e + Q_w X_R}$$

Equation 31 Solids Retention Time, (Gray, 1990)

Where V is the volume in the reactor(s), X is the concentration of biomass in the reactor(s), Q is the influent flow rate, Q_w is the waste sludge flow rate, X_e is the biomass concentration in the effluent, and X_R is the biomass concentration in the RAS returned to the reactor(s).

When insufficient SRT is provided in the wastewater treatment process, the nitrifiers will not have the time required to mature and develop a population sufficient for nitrification to occur.

The rate of growth of the autotrophic nitrifiers is most dependent upon the concentration of available dissolved oxygen and temperature, in addition to other variables. In warmer climates, and warmer temperatures of wastewater, and in the presences of higher dissolved oxygen concentrations, nitrifier growth rates are higher. Thereby, achieving sufficient maturity and populations within SRT's of approximately five to 10 days (Tchobanoglous, Burton, Stensel, 2014). In colder climates, and/or lower dissolved oxygen concentrations, nitrifiers have a slower growth rate, resulting in SRT's greater than approximately 15 to 20 days. The lack of dissolved oxygen in either temperature condition can also completely inactivate nitrifiers. Therefore, properly controlling the environments in, and SRT's of, wastewater treatment processes is required to promote adequate time and conditions for nitrifying microbiology.

Another environmental condition in wastewater treatment processes important to monitor to maintain and promote adequate populations of nitrifiers, is pH. Nitrifiers are most conducive within pH ranges of 6.8 to 7.5 su and can be completely absent in wastewaters outside that pH range.

The nitrification process can then begin once an adequate population of nitrifiers is present. This process begins with ammonia-oxidizing bacteria converting the ammonium ion (NH_4^+) and dissolved oxygen (O_2) present in the wastewater to acid (H^+), water (H_2O), Nitrite-N (NO_2), and energy, in accordance with Equation 32.

$$NH_4^+ + O_2 = H^+ + H_2O + NO_2 + Energy$$

Equation 32 Nitrification Step 1 Reaction (Reynolds, Richards, 1996)

As part of that reaction, the acid (H^+) produced has the potential to lower the pH in the wastewater, which may cause inhospitable conditions for nitrifiers and other microbiology, without sufficient alkalinity present to neutralize the additional acid. The energy in this reaction becomes available for microbiological growth and reproduction, and the Nitrite-N (NO_2) is then available for the second step in nitrification. Nitrite oxidizing bacteria (NOB) does the majority of converting the available Nitrite-N (NO_2) molecules and available dissolved oxygen to Nitrate-N (NO_3) and energy, in accordance with Equation 33 below. Thus, completing the nitrification process of converting Ammonia-N to Nitrite-N (NO_2), and then to Nitrate-N (NO_3) as depicted in Equation 34.

$$NO_2 + O_2 = NO_3 + Engery$$

Equation 33 Nitrification Step 2 Reaction

$$NH_4^+ + 1.682O_2 + 0.182CO_2 + 0.0455HCO_3^- \rightarrow 0.0455C_5H_7NO_2 + 0.955NO_3^- + 0.909H_2O + 1.909H^+$$

Equation 34 Nitrification Equation

The measure of the success of the nitrification process is generally deemed complete when the remaining Nitrite-N (NO_2) concentrations are less than 0.5 mg/L, and when Nitrate-N (NO_3) and Ammonia-N concentrations are less than 3 mg/L (Gerardi, 2006). At this point, nitrogen remains in the wastewater, both as Nitrate-N (NO_3) and as Organic-N. Although not as toxic as Ammonia-N to natural environments and biological life, Nitrate-N (NO_3) can still be a nutrient source for eutrophication and is toxic for human consumption in drinking water at concentrations above 0.10 mg/L according to the US EPA. Therefore, denitrification is an additional step required beyond nitrification, to reduce the Nitrate-N (NO_3) to Nitrogen gas (N_2), which is not toxic or harmful to the environment or biological life.

In contrast to the autotrophic nitrifier microbiology that conducts the nitrification processes, denitrification is completed by heterotrophic bacteria, which both consumes BOD and conducts the denitrification reactions. That heterotrophic bacteria consumes BOD as a food source by utilizing dissolved oxygen (O_2) to oxidize the BOD. However, if dissolved oxygen (O_2) is not present or available, the denitrifying bacteria will utilize other available sources of oxygen, such as Nitrate-N (NO_3). The environmental condition where dissolved oxygen (O_2) is not present is called anoxic, which is a required condition to promote denitrification.

The denitrification process begins with the conversion of BOD and Nitrate-N (NO_3) to Nitrogen gas (N2), base (OH-) and energy, in accordance with the equation below. As part of that reaction, the base (OH-) produced has the potential to buffer a portion of the acid (H^+) produced during nitrification, and finally, the desired result of this reaction is that the Nitrogen gas (N_2) is harmlessly released to the atmosphere and returned to the nitrogen cycle. Thus, completing the nitrification-denitrification process for removing nitrogen from wastewater.

$$BOD + NO_3 \rightarrow OH^- + N_2 + Energy$$

$$NO_3^- + 1.08CH_3OH + H^+ \rightarrow 0.47N_2 + 0.76CO_2 + 2.44H_2O$$

Equation 35 Denitrification Equations

Nitrification and denitrification processes are typically planned and designed to occur in wastewater treatment facilities, where specific aerobic and anoxic conditions are developed and maintained in dedicated basins, or reactors. Examples of such wastewater treatment facility process configurations include a conventional suspended activated sludge configuration, modified Ludzack-Ettinger, and the Bardenpho process, amongst others. There are other wastewater treatment process designs that provide the aerobic and anoxic conditions within the same basin or reactor, such as with the sequence batch reactor, or an oxidation ditch, in which there are dedicated aeration and anoxic zones.

It is important to that that nitrification-denitrification can also occur at unintended locations within a wastewater treatment process, that can have negative impacts on the intended performance those processes. This would include where clarification or settling is intended and where the Nitrogen gas released at the end of the nitrification-denitrification (NdN) process, that can float or shear flocculated particles and interrupt the clarification process. Similarly, NdN can intentionally, or unintentionally occur where micro-anoxic conditions develop throughout the wastewater treatment process, typically within bacterial flocs. This is referred to as simultaneous nitrification-denitrification (SNdN).

The discussions above provide the framework for the conventional nitrogen removal processes in wastewater treatment. The remainder of sections and chapters below, focus on actual and potential shortcut techniques in nitrogen removal, and expand on quantifying the limiting factors, kinetics, and thermodynamics of those shortcut techniques, and provide theoretical examples demonstrating those models.

3.2 Nitrite Shunt

Different from the conventional nitrification-denitrification processes as described above, the Nitrite Shunt process, depicted in Figure 21 below, uses a partial nitrification process to prevent the formation of nitrate and converting nitrite directly to nitrogen gas. This can be completed by inhibiting nitrite oxidizing bacteria and favoring ammonia-oxidizing bacteria. This requires variations in the control of these reactions inside the biological wastewater treatment process, focusing on providing higher temperatures, shorter solids retention times (SRT), and controlling dissolved oxygen concentrations by utilizing a variable mixing regime.

The main advantage of this shortcut is a reduction of approximately 25 percent of the dissolve oxygen requirement, where 75 percent of the oxygen required in the aerobic nitrification process is required to convert Ammonium ions to nitrite ions, and the remaining 25 percent of the oxygen demand is to convert nitrite ions to nitrate ions.

This also results in carbon savings, as 40 percent of the carbon required to convert nitrate ions to nitrite ions during the anoxic heterotrophic process is not required. The remaining 60 percent of the required carbon is then utilized for the conversion of nitrite ions to nitrogen gas and nitrous oxides. The Nitrite Shunt shortcut in the nitrogen cycle is depicted in Figure 21 below.

The savings in energy is in part because there is less nitrogen species to be oxidized, but also because the denitrification rate is approximately 1.5 to 2 times faster in this shortcut, as compared with the conventional nitrification-denitrification process. A faster denitrification rate also provides cost savings, as a smaller anoxic reactor is required. Key factors to be considered with the Nitrite Shunt process are bacteria growth rates, temperature, SRT, free ammonia, pH, and dissolved oxygen.

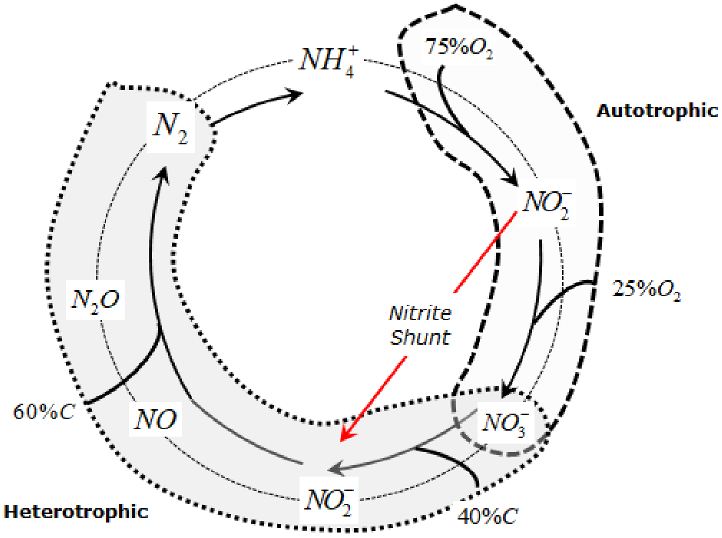

Figure 21 Nitrite Shunt Process (WEF, 2015)

First, this shortcut is possible as aerobic ammonia-oxidizing bacteria grow faster at temperatures higher than 59 degrees Fahrenheit (15° C) than nitrite-oxidizing bacteria, and the accumulation of sufficient oxidizing-bacteria, with the inhibition of nitrite-oxidizing bacteria as depicted in Figure 22 below.

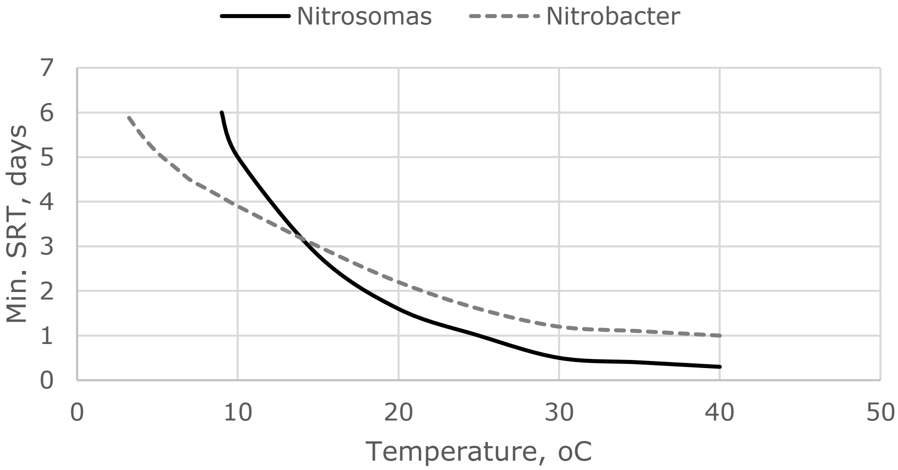

Figure 22 SRT Washout (WEF, 2015)

The control of dissolved oxygen concentrations in biological wastewater treatment processes does not directly inhibit nitrite-oxidizing bacteria. However, the oxygen saturation coefficients between those two types of bacteria are different, where aerobic ammonia-oxidizing bacteria have a coefficient between 0.74 and 0.99 mg/L, and nitrite-oxidizing bacteria have a coefficient between 1.4 to 1.75 mg/L (WEF, 2015). This means that in reactors where the dissolved oxygen concentrations are maintained under 1.5 mg/L, aerobic ammonia-oxidizing bacteria will grow faster, but may require a greater SRT as depicted in Figure 22 above.

Another consideration is pH and chemical equilibrium, where higher pH favors the ammonia (NH_3) species of ammonia-N, which is the preferred substrate for aerobic ammonia-oxidizing bacteria, versus the ammonium ion (NH_4^+). Recalling that in water, the bulk of the gaseous ammonia portion of Ammonia-N is converted to ammonium (NH_4^+). Therefore, specific focus on the control of pH, and the potential addition of acidic or caustic chemical may be required to promote an environment in the reactor(s) where the Nitrite Shunt process is planned, adding cost for consumables and additional infrastructure. Reported ideal pH range for optimal oxygen utilization in this shortcut process is between 6.5 and 8.0 su, and generally aims to control pH at 7.0 su or higher (WEF, 2015).

The limitation of available free ammonia in the wastewater can inhibit nitrite-oxidizing bacteria, such as *Nitrobacter*, is reported at ranges below 1.0 to 3.0 mg/L. Where aerobic ammonia-oxidizing bacteria, such as *Nitrosomonas*, become inhibited in free ammonia concentrations between 10 to 150 mg/L. Additionally, the presence of free nitrous acid (HNO_2) has been found to be more inhibitive to nitrite-oxidizing bacteria, especially during start-up of such reactors. Under such conditions, the compounding results of controlling the pH, temperature, dissolved oxygen, and SRT leads to nitrite accumulation and higher free nitrous acid concentrations, supporting a stabilized Nitrite Shunt process (WEF, 2015).

The Nitrite Shunt process is best fit for wastewaters with low carbon to nitrogen (C:N) ratios and higher temperatures, and are typically implemented as a side stream process, such as for the treatment of anaerobic digestion, thickening, and dewatering supernatant.

3.2.1 Sharon Shunt Process

There are patented technologies available that utilize the Nitrite Shunt process. One is known as the Single reactor system for High activity Ammonium Removal Over Nitrite (SHARON®) process. The SHARON® process provides ammonium conversion to nitrite only, while preventing further conversion to nitrate.

The SHARON® process was developed in the early 1990’s by Grontmij in collaboration with the Delft University of Technology and the Holandse Delta Water Board. The first full-scale plant was the Dokhaven wastewater treatment plant (WWTP) in Rotterdam, Netherlands, which began operation in 1998 (Van, L. M., 2001), and was combined with the Anammox process, discussed later.

The SHARON® system oxidizes ammonium in a single reactor system in an aerobic environment to nitrite, and then reduces the nitrite to nitrogen gas in an anoxic environment by adding an external carbon source and uses the pH of the wastewater to control the process, with no sludge retention. The general schematic of the single reactor process is depicted in Figure 23.

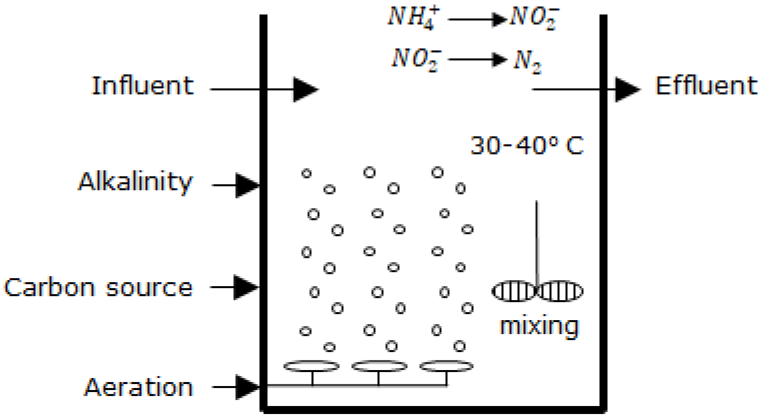

Figure 23 SHARON® Process Schematic (Grontmij, 2021)

This process utilized a high temperature and a short retention time, which promotes nitrite oxidizers to be washed out. Therefore, the formation of nitrite is isolated, which equates to a savings of up to 25% in oxygen requirement as compared with a conventional nitrification-denitrification. However, an external carbon source is required (Grontmij, 2021).

If nitrate is present in the wastewater, this can also be removed in the SHARON® process in an anoxic environment and successfully denitrified but requires 40% more carbon than for conversion of nitrite. The biochemical equation for the SHARON® process is depicted in Equation 36.

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$$

Equation 36 SHARON® General Equation

When autotrophic bacteria are present in the SHARON® process, nitrite is oxidized to nitrate for the nitrification process by the following:

$$NO_2 + 0.5O_2 \rightarrow NO_3$$

Equation 37 Nitrification in the SHARON® Process Equation

$$6NO_2^- + 3CH_3OH \rightarrow 3N_2 + 6HCO_3^- + 3H_2O$$

Equation 38 Denitrification of Nitrite Equation in the SHARON® Process

$$6NO_2^- + 5CH_3OH \rightarrow 3N_2 + 6HCO_3^- + 7H_2O$$

Equation 39 Denitrification of Nitrate Equation in the SHARON® Process

Nitrification creates an acid, and the ammonium ion oxidation process has been reported to stop when the pH of the wastewater stream being treated approaches 6.5 su. At that point the ammonium ion begins to oxidize and there will be insufficient free ammonium ions in the process for the growth of the *Nitrosomonas*. Based on actual implementation, the need for the addition of a base may be required to provide stable operation.

When this single reactor technique is implemented in either a side stream or mainstream process, there remains the need to waste activated sludge (WAS) from the reactor. Since the time to select and promote the growth of aerobic ammonia-oxidizing bacteria is longer, there is a need to recover that bacteria from WAS, which is typically configurated as a recycled activated sludge (RAS) stream, where such microbiology is captured and returned directly to the reactor.

As previously noted, wastewaters with higher ammonia concentrations, and higher temperatures, are well fit for the Nitrite Shunt shortcut. Evaluation of the use of the SHARON® process on digester supernatant found that when adding the mineral chabazite, at defined volumes, the rate of nitrification was increased by magnitudes, but noted that sodium is released from the chabazite, which can inhibit nitrification (Aponte-Morales, Ergas, 2015).

Practical considerations for implementing the SHARON® process exist beyond efficiencies in nitrogen removal. One example is when water reclamation plants (WRPs) are implemented within a wastewater collection system to generate reclaimed water for beneficial reuse, the centrate and waste streams from the WRPs are typically discharged back into the collection systems for treatment by a WWTP at the end of the collection system. Those waste streams typically have higher ammonium and ammonia concentrations. Also, the WRP’s are typically newer than downstream wastewater treatment plants, which may not be adequately equipped to manage hyper-nitrogen loading from the waste streams discharged from upstream WRPs or other wastewater treatment plants.

One implementation of the SHARON® process is at the Ward’s Island Water Pollution Control Plant (WPCP), located in Manhattan, NY. The plant has a capacity of 275 MGD and treats wastewater generation by approximately 1,000,000 people located in east Manhattan and the west Bronx and is responsible for the treating up to 1.85 MGD of anaerobically treated sludge centrate from other wastewater treatment plants, which are high in ammonia. The WPCP has been in operation since 1937, including numerous upgrades to reach the current capacity (Grontmij, 2021).

The SHARON® process was implemented in 2009 to improve nitrogen removal. This improvement directly benefited the quality of the effluent into the Long Island Sound and reduces the risk of algae blooms, which lead to low oxygen concentrations in the receiving waters and reduces the toxicity of the discharge.

The WPCP was the first implementation of the SHARON® process in North America and is the largest in the world. The cost of the upgrade was $237 Million. The success of this implementation of the SHARON® process has served as a catalyst for the New York Department Environmental Quality (DEP), along with other recent nitrogen removal upgrades at other wastewater treatment plants to commitment to invest an additional $1.1 Billon in stabilization and nitrogen removal measures, specifically at four other facilities. The implementation at the WPCP successfully reduced nitrogen concentrations discharged to the Long Island Sound by more 10,000 pounds per day. This demonstrates the implementation of the Nitrite Shunt process can have significant positive impacts, relating to both the cost to provide nitrogen removal services, and meet environmental quality concerns of communities. Other installations are in operation in Alexandria, VA, and more than 12 locations in Europe, providing additional nitrogen removal ranging from 70 to 3,500 kgN per day (WEF, 2015).

3.2.2 ANITA^{TM} Shunt Process

Another trademark technology available that utilizes the Nitrite Shunt process is the ANITA^{TM} process, which implements a traditional activated sludge and sequential Batch Reactor (SBR) configuration as part of the process to inhibit the transformation of nitrite to nitrate in the nitrification process, while reducing the quantity of sludge produced. Typically, the ANITA^{TM} process is aimed to be implemented for supernatant from anaerobic digestion systems, sludge composting, co-digestion, thermal drying, hydrothermal oxidation, and thermal hydrolysis systems. Mainly, the aim is for implementation of effluent with ammonium ion concentration greater than 100 mg/L (ANITA™ shunt, n.d.).

There are also multiple installations of the ANITA^{TM} as a side stream process, including at wastewater treatment plant in James River, VA (15 MGD), South Durham, NC (20 MGD), and Schaumburg, IL (50 MGD), providing additional nitrogen removal ranging from 240 to 940 kgN per day.

Both implemented technologies described above demonstrate they can be a valid alternative where sufficient nitrogen is present to warrant the Nitrite Shunt process. Implementation of both applications revealed an energy savings of approximately 25% due to lower oxygen requirements, and a 40% reduction in chemical addition costs (Xie, Li, Bai, Li, 2010).

Differences between the two technologies and process relate to mixing, equalization, and control strategies. The SHARON® process does not require equalization of side streams, as solids are not retained, but can be recycled, as part of the process. Conversely, the ANITA^{TM} does equalize nitrogen and side stream influent, as part of the SBR process. Both benefit from equalization as aeration equipment is typically designed to accommodate peak ammonia loading.

There are a variety of projected savings regarding long-term operations and maintenance expenditures, including less energy for aeration, faster denitrification reaction rates, and smaller footprints for anoxic reactors. However, there are other factors that can prevent the full actualization of theoretical projected savings in the nitrite shunt process. Such as the presence of sufficient carbon in the wastewater, where the potential carbon savings may be offset from any additional demand to oxidize the carbon as part of the overall wastewater treatment process. Additionally, the potential to operate at lower dissolved oxygen concentrations, and less aeration, may not be able to sustain adequate or require mixing to either support biological processes within the Nitrite Shunt reactor, or other processes.

Overall, there is a potential savings of approximately 25 percent aeration, 40 percent carbon savings, 40 percent less sludge production and 20 percent lower carbon dioxide emissions with the Nitrite Shunt process. The actual savings will be dependent on all the traditional wastewater treatment variables including location, climate, raw wastewater characteristics, process type and configuration, regulatory effluent goals, and available technical, managerial, and financial capability of the managers of that infrastructure. For a comparison of documented and demonstrated savings of upgrading existing wastewater treatment systems with a side stream Nitrite Shunt process, the following costs per kg/N removed is depicted in Figure 24.

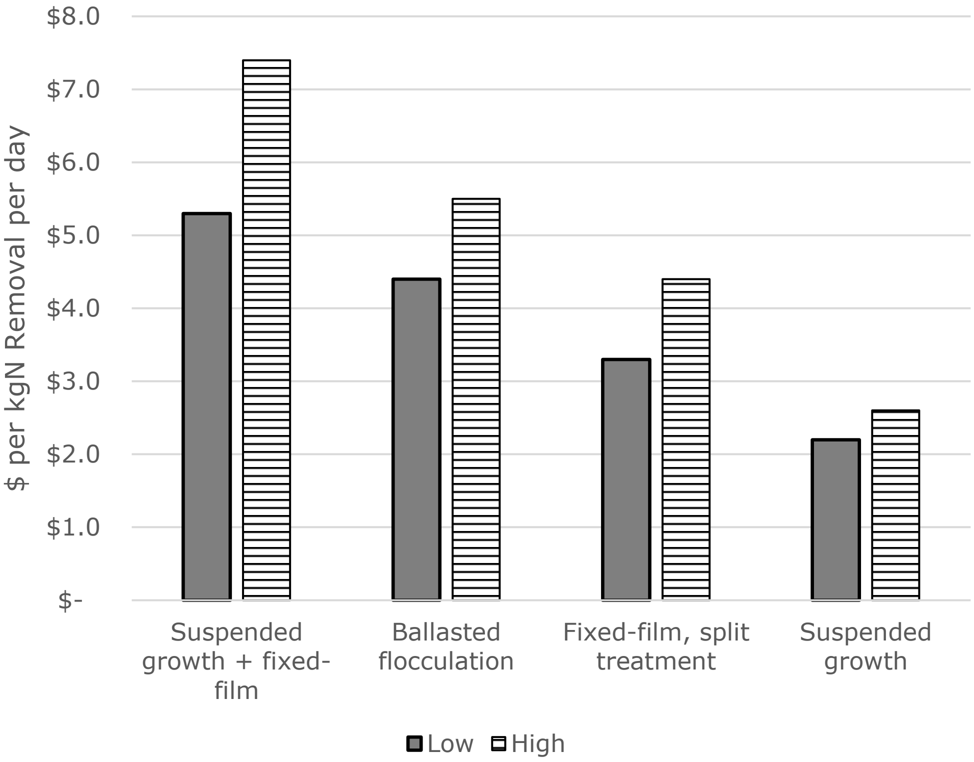

Figure 24 Nitrite Shunt Side Stream Upgrade Cost Comparison (WEF, 2015)

The magnitudes of the comparisons above, reveals less cost to pair with conventional activated sludge processes and increasing costs, and the range of potential costs increase, as the existing treatment type and configurations increase in complexity, or magnitude of reliance on a fixed film process.

A comparison of costs per kg/N removed of side stream physical/chemical nitrogen removal technologies versus the Nitrite Shunt process is depicted in Figure 25. This depicts at least a 50 percent savings as compared with a MBR process, and up to 80 percent savings as compared with stripping technologies.

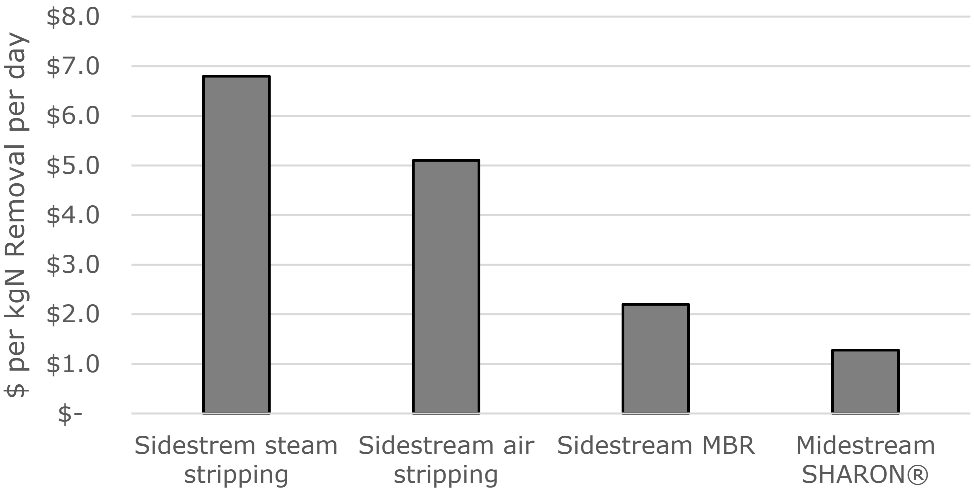

Figure 25 Side Stream Nitrogen Removal Cost Comparison (WEF, 2015)

The success of side stream Nitrite Shunt processes is also attractive to mainstream implementation. However, there are inherent conditions in mainstream applications, including lower nitrogen concentrations and lower temperatures, which are not ideal for the selection of nitrite-oxidizing bacteria. Key factors to improve the effectiveness of the Nitrite Shunt process for mainstream treatment are focused on controlling dissolved oxygen concentrations, residual ammonia, transient anoxia, and SRT.

The comprehension of the value in controlling dissolved oxygen concentrations is not definitive yet but has been identified as a critical factor required to control the Nitrite Shunt process on a mainstream application. Certain biological wastewater treatment processes promote the oxidation of ammonium ions followed by reduction to nitrogen gas, at low dissolved oxygen concentrations.

In review of literature, there is higher affinity for oxygen by aerobic ammonia-oxidizing bacteria than nitrite-oxidizing bacteria, and that low dissolved oxygen concentrations can suppress nitrite oxidation. However, the oxygen affinity coefficient (K_o) has considerable variability and is thought to be a result of oxygen mass transfer limitations, and therefore not an intrinsic biological characteristic. Additionally, in research at pilot and full-scale implementations it has been demonstrated that nitrite-oxidizing bacteria are not completely inhibited in low dissolved oxygen conditions (WEF, 2015). However, the opportunity to promote the growth and dominance of nitrite-oxidizing bacteria, at lower dissolved oxygen concentrations, in the presence of sufficient organic carbon, is theoretically feasible and key to the future success of the Nitrite Shunt process for mainstream applications.

Like the concerns regarding lower dissolved oxygen concentrations, is the same with free ammonia concentrations, which are generally too low for inhibition of nitrite-oxidizing bacteria. Alternating aerobic and anoxic conditions have been demonstrated to maintain residual ammonium ion concentrations, can be effective for nitrite-oxidizing bacteria selection, and has been demonstrated through modeling that the Nitrite Shunt reaction in continuous modes is a valid factor towards successful mainstream application.

Transient anoxia is a measured control approach for aerobic SRT, which introduces a process time lag for nitrite-oxidizing bacteria, between the anoxic to aerobic conditions. This technique has been demonstrated effective at high-strength wastewaters, and recently with low strength mainstream plug-flow type processes (WEF, 2015).

A final key process control factor is SRT, where the Nitrite Shunt process kinetics are managed with a relatively low SRT such that aerobic ammonia-oxidizing bacteria do not get washed out, but nitrite-oxidizing bacteria can get washed out. Additionally, the oxidation of ammonium ions is maximized based on the influent quality and C:N ratios by optimizing the SRT.

A final consideration for Nitrite Shunt implementation is that the effluent will contain nitrite, which can be a higher chemical demand when chlorine is used for disinfection. Conversely, the residual ammonium ions anticipated to be present from maintaining the nitrite-oxidizing bacteria reactions, are conducive for chloramination disinfection, and would reduce chlorine demand of nitrite. Residual nitrite will not negatively impact the performance of the application of ultra-violet light for disinfection (WEF, 2015).

3.3 Deammonification overview

The deammonification process is different from the denitrification process. Denitrification is the series of reactions to oxidize nitrite to nitrate, and then to nitrogen gas, after nitrification. Deammonification is a direct, or shortcut reaction, where ammonium ions are converted to nitrite and directly to nitrogen gas as depicted in Figure 26.

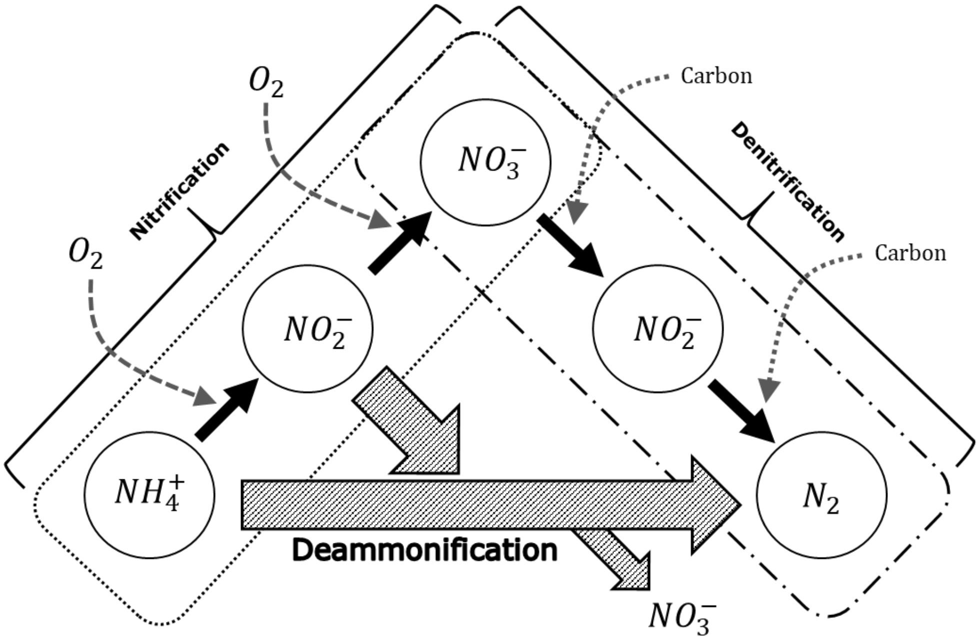

Figure 26 Deammonification Pathways (Feng, Lu, Al-Hazmi, Mąkinia, 2017)

As depicted in Figure 26, deammonification is a shortcut process that provides saving in oxygen/aeration reduction as the full nitrification pathway is not utilized plus carbon savings along the denitrification pathway. The stoichiometry of those reactions is presented in Equation 40.

$$2.34NH_4^{+}1.8O_2+2.66HCO_3^{-}\rightarrow 0.024C_5H_7NO_2+NH_4^{+}+1.32NO_2^{-}+2.54CO_2+3.94H_2O$$

Equation 40 Deammonification Nitritation Reaction

The nitritation reaction portion of the deammonification reaction assumes a biomass yield of $0.14g\, {}^{VSS}\!/_{g}\, NH_3-N$. The product of the reaction above produces water, carbon dioxide, ethyl cyanoacetate, and nitrite, with remaining ammonium. This portion of the reaction is facilitated by aerobic ammonia oxidizing bacteria, which are selectable by controlling key process parameters including the ammonia load, dissolved oxygen, pH, SRT and temperature. Then the deammonification reaction continues for anaerobic ammonia oxidation, which assumes a biomass yield of $0.7g\, {}^{protein}\!/_{g}\, NH_4-N$, as depicted in the Equation 41.

$$NH_4^{+}+0.066CO_2+0.066H^{+}+1.32NO_2^{-}\rightarrow 0.006CH_2O_{0.5}N_{0.15}+1.02N_2+0.26NO_3^{-}+1.96H_2O$$

Equation 41 Deammonification Ammonia Oxidation Reaction

This overall process, depicted in Figure 27, is facilitated by chemolithoautotrophic anaerobic ammonia oxidizing bacteria, which utilize nitrite.

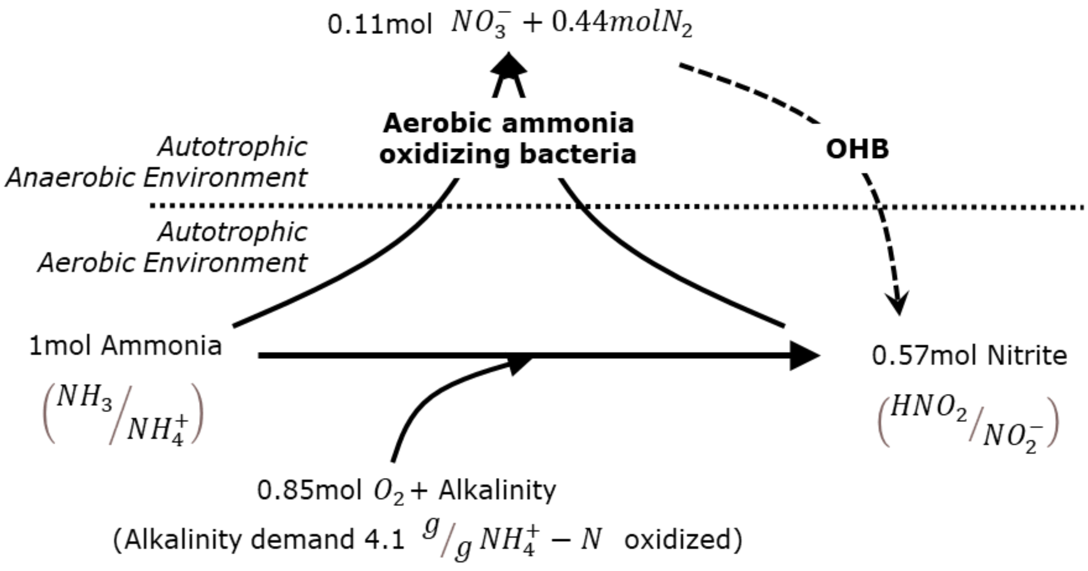

Figure 27 Deammonification Stoichiometry (Feng, Lu, Al-Hazmi, Mąkinia, 2017)

In whole, the deammonification approach can provide a combined potential savings of 60 percent as compared to traditional NdN oxygen demand, 40 percent less sludge production and up to 100 percent less organic electron donor.

Target wastewater characteristics are those with lower COD to total ammonia-N ratio, high ammonia-N concentration, but low fluctuation in that concentrations. Due to the affinity for effectiveness at higher ammonia-N concentrations, deammonification has been best demonstrated in side stream applications, with less dominance in mainstream application. Like the Nitrite Shunt process in a side stream implementation, the deammonification process has demonstrated average efficiencies of 75 percent total inorganic nitrogen removal and 80 percent ammonia reduction (WEF, 2015). Such side stream applications become beneficial for deammonification when the COD: NH_4^+ ratio is 2:1 or less.

Deammonification initially began as a focus on achieving sustainable nitrogen removal in high strength waste streams such as from dewatering, anaerobic digestion, and solids waste leachates. The first successful full-scale demonstrations were single-reactor techniques, which included partial nitritation with anaerobic ammonia oxidation all in the same process. Later, two-stage deammonification was implemented where those two processed are separated by reactor. It has been demonstrated in literature and in practice that single, and two stage deammonification reactor share ammonia oxidizing bacteria as the rate-limiting step (WEF, 2015).

The feasibility for mainstream deammonification has documented challenges to be further overcome including optimal operational control of competitive conditions and strategies between anaerobic ammonium-oxidizing bacteria and nitrite oxidizing bacteria, relating to promoting and maintaining sufficient populations of autotrophic and organotrophic bacteria diversity as part of the same reaction.

3.3.1 Deammonification – Anammox

Referred to as the Anammox process, this deammonification process utilizes an anaerobic environment to convert ammonium to nitrogen gas for release. This process focuses on planctomycete autotrophic bacteria and is also well suited for higher ammonia-N loading of wastewaters than conventional NdN processes.

This was initially hypothesized in 1977, through the study of using nitrate (III) or nitrate (V) as the electron acceptor for the oxidation of ammonium in anoxic conditions, and later confirmed in the 1990’s and named “anammox”, for the anaerobic ammonium oxidating bacteria that facilitate this reaction (Ziembińska-Buczyńska, 2019).

The Anammox process is noted to have a longer commissioning time due to the slow growth rate of the target bacteria. Different from the SHARON® process, no organic carbon source is required, as bicarbonate serves as the carbon source in anoxic conditions. The planctomycete bacteria utilize the ammonium in the wastewater as the electron donor for the conversion of nitrite to nitrogen gas, thereby skipping the conventional cycle to remove the nitrogen. Additionally, there is less sludge produced by this process as compared with conventional NdN. The biochemical equation for the Anammox process is presented in Equation 42 below.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2.03H_2O$$
$$+0.066CH_2O6NO_2^- + 5CH_3OH \rightarrow 3N_2 + 6HCO_3^- + 7H_2O_{0.5}N_{0.15}$$

Equation 42 Anammox Equation (Van, 2001)

Literature review of nitrogen removal with the Anammox process was found to generally focus on the biological kinetics, process control, practical applications, and efficiencies. All exploring the effects of nitrogen oxides on the bacteria promoted in the Anammox process. Findings within that research identified that within the natural nitrogen cycle, nitric oxide is toxic to many bacteria and can act as an intermediate and a cell signal transduction molecule. It was also noted that the bacteria need for the nitrification and denitrification processes, deploys proteins to detoxify the nitric oxide to nitrous oxide, which is less toxic but is still not a target form of nitrogen for discharge from the treatment process. Nitric oxide is known to inhibit the bacteria needed to promote the nitrogen cycle. When high nitric oxide loading was implemented in the study reactor, such toxicity and inhibition predicted was not observed, but decisive evidence that the success in treating the high concentrations could not be proved (Kartal, Tan, Biezen, Kampschreur, Loosdrecht, Jetten, 2010).

Other research explored the impacts and benefits of the parameters that drive the effectiveness of the anammox process. One study considered the effects of dissolved oxygen, in which concentrations near 0.5 mg/L were found to inhibit nitrite oxidation and full nitrification begins to be observed with DO concentrations near 6.0 mg/L. Other influencing factors evaluated included the concentration of salt in the wastewater, where concentrations near $12\,{}^{gNaCL}\!/_{L}$ promoted an increase in nitrite accumulation in reactors and stable partial nitrification at concentrations greater than $50\,{}^{gNaCL}\!/_{L}$.(Paredes, Kuschk, Mbwette, Stange, Müller, Köser, 2007).

Optimal growth rate of anammox bacteria is greater than 86 degrees Fahrenheit, which is warmer than most collected raw wastewater for treatment and may require additional heat in a mainstream application. The need for supplemental heat will offset some of the energy savings (Ziembińska-Buczyńska, 2019). However, studies and pilots have been completed and are ongoing in low temperature applications, which have revealed that pH can become the limiting factor, as at lower temperatures the anammox bacteria has greater sensitivity to pH. That research evaluated the addition of various oxides as a catalyst to overcome inhibition at low temperatures, and reduce the duration required to develop stable populations of the slower growing anammox bacteria. The best response was by developing ecologies with anammox dominate bacteria, then seeding that into the reactor (Ziembińska-Buczyńska, 2019). That application of a biocatalyst is discussed in the sections below.

There are several different commercially available technologies that provide deammonification treatment as described below.

3.3.2 Deammonification – ANAMMOX®

One available technology, provided by Paques, is the ANAMMOX® - Ammonia Removal technology, designed to deliver the anammox deammonification treatment for wastewaters and gases with high ammonia-N concentrations. Their technology was developed in collaboration with the Delft University of Technology and the University of Nijmegen. The initial full-scale implementation began in 2002, now with over 19-years of experience and over 40 installations.

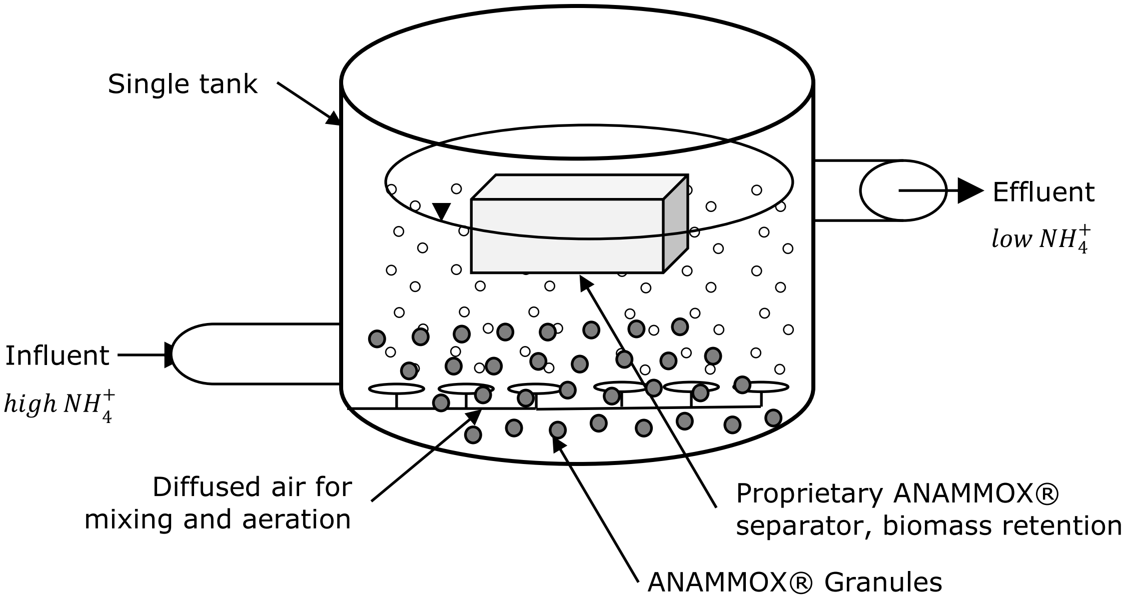

Figure 28 ANAMMOX® Reactor (Paques, 2020)

The ANAMMOX® technology is a single sludge process, where side stream, ammonia-rich influent is conveyed to a tank, where diffused air is used for mixing and aeration, proprietary granular biomass is used to promote the anammox reactions, and a proprietary selector is implemented to retain biomass, as depicted in Figure 28 above.

The operational strategy is continuously fed influent, along with continuous fine bubble aeration that promotes mixing of the wastewater with the granular biomass and consists of both anaerobic ammonia oxidizing bacteria and nitrifying bacteria. Effluent is collected at the top, through the selector, which recovers the granular biomass, and discharges the treated effluent at the top.

Paques, along with partners Hollandse Delta Water Board, and STOWA, is demonstrating a mainstream wastewater anammox treatment process, for low temperature (10 to 20 degrees C) conditions at the Dokhaven Wastewater Treatment Plant in Rotterdam, Netherlands. This project is referred to as the CENIRELTA (Cost-Effective NItrogen REmoval from wastewater by Low-Temperature Anammox®.

As a mainstream application, this process consists of the following major processes and configuration:

1. Influent is screened at 5 mm to prevent fouling of downstream processes.
2. "A-stage", which is an adsorption stage is where screened wastewater is aerated by diffused air and mixed with activated sludge. Dissolved oxygen concentration is maintained at a lower concentration.
    a. Ferric Chloride ($FeCl_3$) is then dosed in that same reactor for the purpose of binding phosphates.
    b. Due to the low dissolved oxygen concentrations, and short SRT and HRT, organic matter is adsorbed, rather than oxidized.
3. An intermediate clarifier is used to settle solids and separate the liquid stream. Some solids are returned to the "A-stage" and the remainder is conveyed to a solids treatment plant.
4. "B-stage", which the wastewater is re-aerated and mixed with activated sludge, and nitrification reactions are conventionally completed in this reactor.
    a. The CENIRELTA configuration, replaces this stage with the ANAMMOX® style reactor as described above. This promotes the total nitrogen removal in a single step.
5. Wastewater is then sent to the final clarifier to settle solids and separate the liquid stream. Some solids are returned to the "B-stage" and the remainder is conveyed to a solids treatment plant.
6. The solids treatment plant consists of anaerobic digestion, and biogas is captured for recover at a combined heat and power plant, for production of electricity and heat.

Early results from bench-scale and pilot plants at this location reveal potential savings in footprint, energy use for aeration, and full denitrification are feasible, and the project is aiming to move towards full scale demonstration.

3.3.3 Deammonification – Wold Water Works DEMON® Anammox Treatment Technology

Other deammonification technology available is offered by Wold Water Works, known as DEMON® Anammox Treatment Technology. DEMON is an acronym for DE-amMONification. This technology combines a pH aeration/anaerobic control philosophy, consisting of micro-cycles of feeding, aeration, and anoxic mixing conditions. This is effectively a time partitioning processes, within the same reactor as the conventional SBR process.

This technology has been demonstrated and studied in various process configurations including continuous and sequence batch mode, which has been implemented and researched in many locations. The sequencing batch reactor configuration is known as DEMON® SBR process. The DEMON® SBR can achieve full deammonification with SBR cycle times of six to 12 hours, dependent on influent ammonia-N concentrations and effluent goals (WEF, 2015).

The intent of the micro-cycles is to effectively optimize aerobic ammonia oxidizing bacteria and anaerobic ammonia oxidizing bacteria, allowing a portion of the ammonium to be oxidized to nitrite. The environmental conditions with the reactor are closely monitored for process control, with a specific focus on pH. As ammonium is oxidize, a slight pH decrease will occur ($\pm 0.02 su$), which is a trigger to begin the anoxic mixing and stop aeration. As more influent is introduced to the reactor, the pH will rise again, to another setpoint to stop mixing and re-start aeration. These cycles continue until the desired reactions are completed, then followed by a settle and decant cycle like a conventional SBR.

Unique to the DEMON® reactor, during aeration cycles, dissolved oxygen concentrations are kept low, near 0.3 mg/L, for brief periods to limit the amount of nitrite produced, as nitrite accumulation is inhibitory for nitrite oxidizing bacteria. Each cycle in succession provides a longer feeding duration for bacteria, which increases free ammonia concentrations for brief periods, to further suppress nitrite oxidizing bacteria. The final cycle is aeration at a lower pH that helps achieve the low ammonia effluent. Like the ANAMMOX® process, a micro-screen is utilized to separate granular sludge and maintain it within the reactor. Different from the ANAMOX® process, the DEMON® process includes a settling zone where heavier particles are retained and re-introduced. In whole, the DEMON® processes can complete deammonification with greater than 60 percent energy savings and with greater than 90 percent less sludge production than conventional NdN processes. No additional alkalinity or carbon is generally required, and the system has been noted to be able to be commissioned quickly by utilizing seed sludge, and with a short acclimation period.

There are many other side stream and mainstream deammonification technologies identified but not reviewed, including but not limited to, Cleargreen^{TM}, DeAmmon®, Anita^{TM}Mox, Terra-N®, ELAN®, and ESSDE®.

3.4 Simultaneous Nitrification - Denitrification (SNdN)

Simultaneous Nitrification – Denitrification (SNdN or SND) refers to the process when nitrification and denitrification reactions occur in either the same activated sludge floc, biofilm, or reactor. When this reaction occurs within a floc or biofilm, nitrification occurs in the aerobic outer layer, and denitrification occurs in the inner anoxic zone, due to the lack of dissolved oxygen and the presence of nitrate or nitrite, and as depicted in Figure 29.

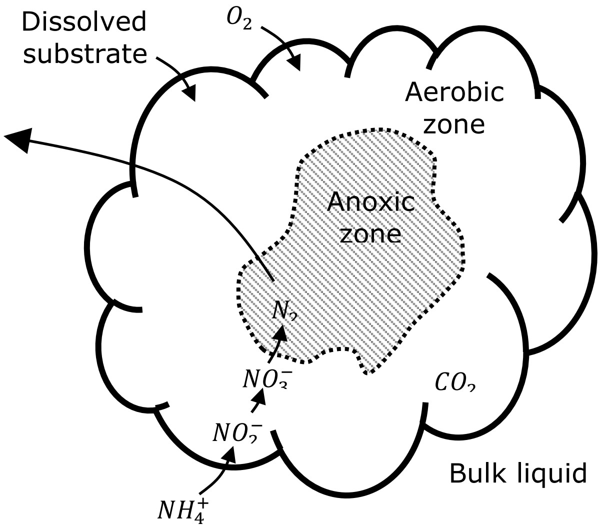

Figure 29 Activated Sludge Floc Zones (Tchobanoglous, Burton, Stensel, 2014)

Reaction rates in the SNdN process are generally less than optimal as compared with conventional nitrification and denitrification, as the available biomass and substrate is shared between those reactions. Denitrification rates are lower as substrate consumption in aerobic portions of the floc are lower, and nitrification rates are lower due to targeted lower dissolved oxygen concentrations, either to balance the denitrification reaction, in swing zone, or conditions between aerobic and anoxic. However, this can be overcome in processes with longer HRT's, such as oxidation ditches, where there is sufficient volume to overcome lower nitrogen removal reaction rates (Tchobanoglous, Burton, Stensel, 2014).

The rates that both reactions will occur are a function of the floc density, size, structure, kinetics, COD loading, SRT, and the dissolved oxygen concentration in the bulk liquid. The specific growth rate for nitrification is defined as follows:

$$\mu_n = \left(\frac{\mu_{nm} N}{K_N + N}\right)\left(\frac{DO}{K_o + DO}\right) - k_{dn}$$

Equation 43 Nitrification Specific Growth Rate (Tchobanoglous, Burton, Stensel, 2014).

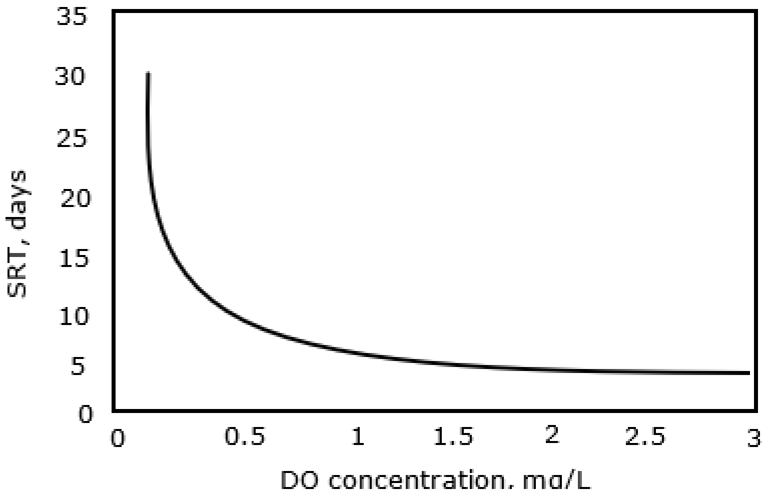

Figure 30 SRT Based on DO Concentrations for Effluent Ammonia-N of 1.0 mg/L (Tchobanoglous, Burton, Stensel, 2014)

The nitrification SRT is inversely proportional to the specific growth rate and based on ammonia-N concentration, as depicted in Figure 30 above, as compared with the dissolved oxygen concentration.

Similarly, in denitrification, the nitrate reduction rate in an anoxic reactor or floc, is inversely proportional to the specific growth rate and based on COD concentrations as defined in Equation 44 below and depicted in Figure 31.

$$r_{NO_x} = \left(\frac{1-1.42Y}{2.86}\right)\left(\frac{kXS}{K_s+S}\right)\left(\frac{NO_3}{K_{s,NO_x}+NO_3}\right)\left(\frac{K_0^{'}}{K_0^{'}+DO}\right)(\eta)$$
$$+\left(\frac{NO_3}{K_{s,NO_x}+NO_3}\right)\left(\frac{K_0^{'}}{K_0^{'}+DO}\right)\left(\frac{1.42}{2.86}\right)k_d X_\eta$$

Equation 44 Denitrification Nitrate Reduction Rate (Tchobanoglous, Burton, Stensel, 2014)

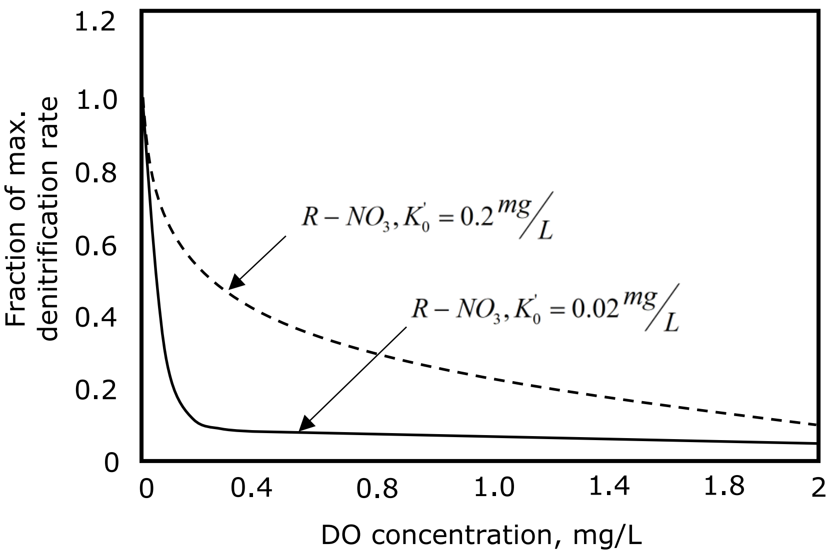

Figure 31 SRT Based on DO Concentrations for Max. Denitrification (Tchobanoglous, Burton, Stensel, 2014)

Again, the lower the dissolved oxygen concentration is maintained, the better efficiency in denitrification will occur, but longer SRT will be required.

This can be an unintended or planned reaction. Generally, the unintended reaction is when SNdN occurs in processes downstream of the intended reaction, such as in a secondary clarification process, where the conversion of nitrate to nitrogen gas can interfere with the planned settling process.

SNdN is considered a shortcut technique as both reactions are occurring simultaneously but not all the benefits of the other shortcut techniques may be fully actualized due to the need for longer SRT and greater volumes to achieve desired nitrogen removal efficiencies. Key factors that are identified as the largest impact include the following (WEF, 2015):

Bulk liquid oxygen concentration – This is the balance of dissolved oxygen concentrations that are required to allow both nitrification and denitrification to occur. Such conditions may vary throughout the reactor, and throughout the floc present, and therefore may not be easily controlled or predicted.

Availability of organic carbon – A limiting factor in successful denitrification is the availability of biodegradable organic-N, which is most readily used as an electron donor at low dissolved oxygen concentrations. Again, this is part of the balance with the bulk liquid oxygen concentration, which also needs to be adequate to support nitrification reactions.

Microenvironments – Gradients of dissolved oxygens concentrations will develop throughout reactors, within and across floc, due to preferential pathways for diffusion, variable fluid dynamics related to mixing and suspension, and based on rates of dissolved oxygen consumption. This means the environment will be nonhomogeneous and complete SNdN may not fully occur at all points, and the rate of those reactions will vary.

SNdN reactions in conventional nitrogen removal processes are generally understood to require a range of influent COD to nitrogen (COD:N) ratio of six to 10, and bulk liquid dissolved oxygen concentrations within the reactor of 0.3 to 0.7 mg/L (WEF, 2015). SNdN Nitrite Shunt process has an opportunity to take advantage of these reactions and conditions, where less COD is required, and existing wastewater treatment facilities that are deficient in COD could benefit from the implementation.

CHAPTER 4

MICROBIOLOGY SELECTION

4.1 Process Configuration Considerations

As discussed throughout the chapters above, there are a variety of environmental and biology specific limitations to the kinetics and thermodynamics of the growth and maturation that influence the selection of desired microbiology for wastewater treatment processes and desired outcomes. Additional considerations to be aware of, is the overall treatment process type or configuration, as each type requires different volumes, recycling and wasting rates, energy demands for mixing and aeration, HRT, and SRT as major process variables (Sedlak, 1991).

Regarding nitrogen removal there are two major configurations, including:

- Separate stage denitrification – where a separate unit process is implemented; and
- Single sludge process – where the carbon oxidation, nitrification and denitrification are combined.

Each of those major process configurations are also further categorized by the approach to nitrogen removal as depicted in Figure 32, 33, and 34 below.

Figure 32 Separate Stage, Carbon Oxidation, Nitrification, Denitrification (Sedlak, 2001)

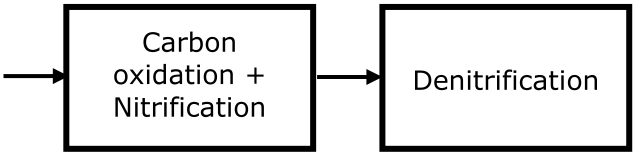

Figure 33 Combined Carbon Oxidation and Nitrification, Separate Stage denitrification (Sedlak, 2001)

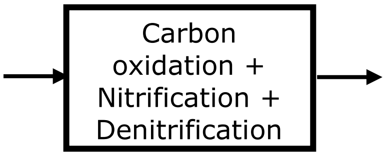

Figure 34 Combined Carbon Oxidation, Nitrification, and Denitrification (Sedlak, 2001)

Examples of single reactors include conventional suspended activated sludge (CAS) reactors, modified Ludzack Ettinger (MLE) reactors, multi-stage Bardenpho reactors, oxidation ditches (endless loop), sequence batch reactors (SBR), and others that implement membrane bioreactors (MBR) with CAS, MLE or Bardenpho.

Examples of separate stage denitrification processes include suspended growth reactors and attached growth reactors. Attached growth reactors include packed beds, deep bed granular filters, and fluidized beds.

The selection of the type of single sludge or separate stage denitrification each have unique benefits for performance, footprint, capital costs, and operational and maintenance considerations that will also vary based on the influent raw wastewater characteristics, and effluent regulatory requirements. Although each implementation must be uniquely evaluated, denitrification can become the limiting factor for process type selection. A high-level comparison of the general considerations regarding performance, cost, and operability between a single sludge (single-stage) and a separate stage process is depicted in Table 5 below. The factors that have a demonstrated benefit or savings is notated with a “+”. Those with a demonstrate disadvantage are noted with a “-“. Those with difference that are generally neutral or negligible are noted with a “0”.

Criteria	Factor	Single-sludge	Separate Stage
Performance	Nitrogen removal	+	+
	TSS control	0	+
Stoichiometry	Energy	+	-
	Alkalinity	+	-
	Supplemental carbon	n/a	Required
O&M	Control	+	+
	Operations	+	0
	Chemical	+	-
	Maintenance	0	0
Cost	Capital	+	-
	O&M	0	-

Table 5 Denitrification Process Type Matrix, (Sedlak, 2001)

Other non-biological based processes for nitrogen removal are available and well demonstrated but are not considered as nitrogen removal shortcut techniques. Such processes are physical and chemical techniques that are implemented when the characteristics of the influent raw wastewater are not economically suitable or feasible for biological treatment. Such instances occur when there are significant deficiencies in carbon, alkalinity, or other required substrate present in the influent, to make biological treatment processes attractive. Common physical/chemical nitrogen removal processes include air stripping, selective ion exchange, and breakpoint chlorination.

Air stripping for the removal of ammonia is effective when ammonia (NH_3) is the dominant species of ammonia-N in a waste stream versus ammonium ions

(NH_4^+), which occurs at higher pH values. This can be highly effective with high pH waste streams. Otherwise, the wastewater pH requires adjustment 10.5 to 11.5 su, followed by showering the wastewater, typically within a packed tower, while aeration is added in the reverse direction of the liquid stream, to physically strip the ammonia from the wastewater. The design of such air stripping towers, and process is based on Henry's Law for equilibrium of the gas/liquid reactions and ratios, and calculations for minimum aeration requirements, temperature, area, velocity, and time (Tchobanoglous, Burton, Stensel, 2014). Such process can achieve greater than 90 percent removal. However, there are performance, operational, and maintenance challenges including excessive buildup of calcium carbonate in the packed tower and on other appurtenances that can reduce efficiency and increase maintenance. Air quality concerns can exist in such towers, which have been noted to be mitigated by routing the exhaust through a sulfuric acid (H_2SO_4) solution, and then recycled (Sedlak, 1992).

Selective ion exchange is another demonstrated physical/chemical process for ammonia removal by utilizing a highly selective ion exchange media. Such media includes synthetic and natural zeolite clinoptilolite materials. This process promotes the exchange of the ammonium ions with either a calcium or sodium ion, present in the selected media, thus sequestering the ammonium at 90 to 97 percent efficiency. However, nitrite, nitrate, and organic nitrogen is not affected by this process. Such processes generally consist of a pre-filter to prevent fouling of the ion exchange media, and a backwash system to regenerate the media when, or prior to ammonium break through occurs. pH adjustment may also be implemented, as higher removal efficiencies are documented at higher pH, but may still be effective at a neutral pH.

Another physical/chemical process available is breakpoint chlorination. This process specifically adds sufficient chlorine to the wastewater stream to fully oxidize ammonia-N to nitrogen gas. This is a stepwise process generally described as follows:

$$NH_4^+ + HOCl \rightarrow NH_2Cl + H_2O + H^+$$

$$NH_4^+ + 0.5HOCl \rightarrow 0.5N_2 + 0.5H_2O + 1.5H^+ + 1.5Cl^-$$

Equation 45 Breakpoint Chlorination General reactions (Sedlak, 1992)

When the equations above are combined, the overall reaction can be expressed as follows: (Sedlak, 1992)

$$NH_4^+ + 1.5HOCl \rightarrow 0.5N_2 + 1.5H_2O + 2H^+ + 1.5Cl^-$$

Equation 46 Breakpoint Chlorination Overall Reaction (Sedlak, 1992)

This breakpoint reaction has a weight ratio requirement of Chlorine to ammonia-N of 7.6:1, also equivalent to a molar ratio of 1.5:1. Demonstrated implementation of this process has revealed actual breakpoint ratio of Chlorine to ammonia-N of 8:1 to 10:1. This process can provide 95 percent or greater removal efficiency, with the remaining ammonia converted to nitrate and nitrogen trichloride (NCl_3). Other operational and process configuration considerations include a near neutral pH of the influent raw wastewater, and potential increase in the total dissolved solids (TDS) in the effluent, due to the creation of chloride ions. Also, alkalinity addition may be required, as 14.3 mg/L of alkalinity is required for every 1.0 mg/L of ammonia-N.

4.2 Measurement and Control of Oxidation Rates

Measurement and monitoring of the BOD in a biological wastewater treatment process is critical to understanding the various demands and activity of microbiology, the ecology, presence or lack of certain bacteria, and effectiveness of the overall process. The primary method for the examination of wastewater is the measurement of biochemical oxygen demand (BOD). Through sampling and laboratory analysis, the rate that BOD is oxidized in the reactor can be measured and modeled. The most common test is 5-day test (BOD_5), in which a sample of wastewater is initially measured for dissolved oxygen concentration, then incubated for 5-days, and the concentration re-measured. The result is the rate of BOD oxidation, over 5-days, which can then be extrapolated. Another test is for the ultimate BOD oxidation, ($BOD_{ultimate}$), where the test is repeated until the concentration of dissolved oxygen is no longer reduced. That test may provide greater value when making design or optimization decisions.

Key to understanding nitrogen removal potential is the difference or increase in additional oxygen demand when nitrifying bacteria are present. Where the carbonaceous BOD is the demand by non-nitrifying bacteria and other organics, utilizing carbon (cBOD) as the food and energy source. As nitrifying bacteria typical take longer to propagate, and in the presence of BOD, such bacteria may not occur for 5 to 10 days, where nitrogenous BOD (nBOD) and alkalinity is utilized as a food and energy source and is measured as additional BOD in the sample. This is depicted in Figure 35 below.

However, if nitrifying bacteria is present in the initial sample, the total BOD may appear higher, which can lead to misrepresentation of sample measurement results, and incorrect assumptions about either the performance of the wastewater treatment process, or in quantification of the cBOD concentrations and oxidation rates. Therefore, one must comprehend the limitations of such examination techniques, including the potential longer duration to obtain results, whether for the BOD_5 or $BOD_{ultimate}$ tests, where there is a lack of stoichiometric validity once soluble organic matter has been reduced, only the biodegradable organics are quantified. Therefore, the effects or presence of nitrifying bacteria may skew the results or indicate that higher concentrations of active bacteria are present.

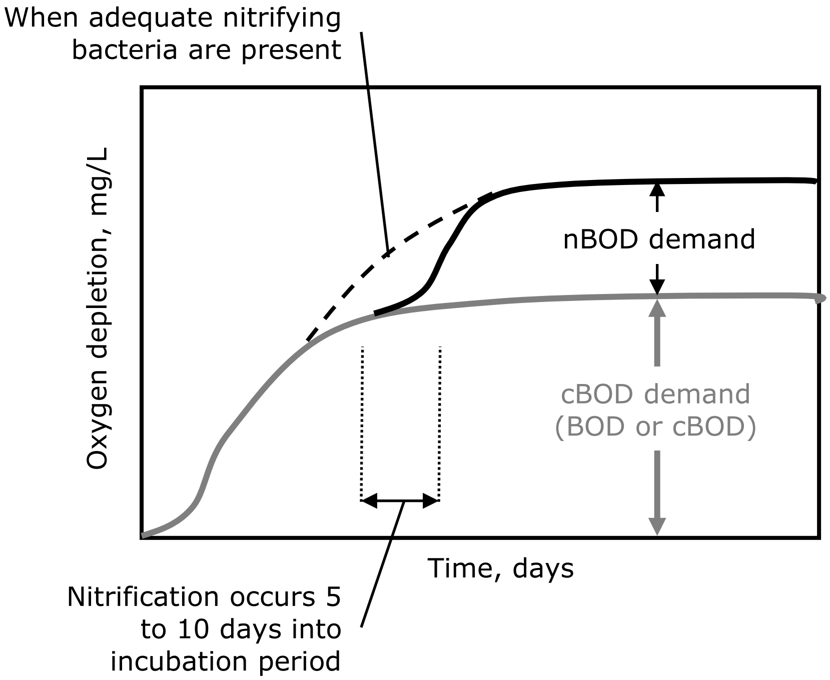

Figure 35 BOD Oxidation (Tchobanoglous, Burton, Stensel, 2014)

4.3 Process Operational Considerations

There are many raw wastewater characteristics and ecological factors that influence the biological wastewater treatment processes. A concise comprehensive outline is not easily feasible. However, key factors that should be noted in relation to success in nitrogen removal have been summarized by those that most influence protozoa presence and population, process configuration/duration, and process control, as discussed below.

Protozoa presence and population are influenced by the type, or configuration of the biological wastewater treatment process, such as plug flow or completely stirred reactors. Additionally, the success of maturation of protozoa are highly influenced by the following:

- Dissolved oxygen – sufficient oxygen is required to meet cBOD or nBOD demands
- pH – higher pH can lower protozoa populations
- Carbon dioxide – higher concentrations can be toxic, so if pH adjustment is implemented, careful consideration of downstream effects should be quantified.
- Sunlight – most protozoa require some sunlight, which means their presence and concentration will be greater in upper zones.
- Flow rate – protozoa are higher order microbiology, which require more time to mature and develop stable populations. Sufficient MCRT or SRT is required to promote and sustain such populations. Therefore, depending on process configuration, significant recycling may be required, and careful attention to wasting rates of activated sludge (WAS) should be monitored to prevent excessive washout.
- Toxic waste – Elevated concentrations of toxic constituents such as heavy metals, pesticides, polyfluorinated compounds, and others can inhibit or cause die-off of microbiology, leading the process upset or failure.
- Predation – The population and concentration of predatory biology can contribute to reduced populations of desired protozoa.
- Food/energy source – A lack of sufficient bacteria and substrate, and of the right type, can inhibit the thermodynamics and kinetics of protozoa ability to reproduce and provide adequate cell maintenance.

The process configuration and duration also influence each of the factors above. In a plug flow configuration, the ecology changes throughout the reactor, which can limit the potential and actual growth of desired bacteria or protozoa.

Therefore, significant recycling of activated sludge (RAS) is typically required to provide sufficient time for higher order microbiology to mature. Additionally, excessive wasting and insufficient MCRT or SRT can lead to washout, and loss of protozoa population. In a completely stirred reactor (CSTR), the ecology may remain more consistent, with lower recycling rates than in a plug flow reactor, but adequate MCRT, SRT, and proper wasting rates are still required.

When clarification or equalization basins are utilized in process configurations, retention time is also critical for the maturation of protozoa populations. Higher concentrations or volume of solids can lead to low dissolved oxygen concentrations, which may inhibit the growth of desired nitrifiers and protozoa. Lower dissolved oxygen can also promote the growth of free-swimming ciliates. Their feeding habits favor higher oxygen concentrations, in which they will generally swim to upper zones, making them more prone to washout.

Another aspect of process control is the monitoring, measurement, and recording of the types of bacteria and protozoa present in biological wastewater treatment processes. This is performed through microscopy, utilizing varying magnification, light conditions, and staining techniques to make a visual verification of the quantity of bacteria and protozoa, dominance, and feeding habits.

Observations of protozoa is considered one the most reliable holistic indicators of a healthy ecology and performance in biological wastewater treatment processes (Curds, 1992). The variation of different species of protozoa is not as critical as the presence of, relating to the overall health and performance. Such as, being able to visually confirm if, or which protozoa is dominant, can serve as an instant confirmation of the loading conditions and performance. For example, *Carchesium Polypinum* are immediate indicators of a biological wastewater treatment process providing high-quality effluent, where *Flagellated Protozoa* are more indicative of treatment processes yielding lower quality effluent.

Other key considerations in monitoring the microbiology for process control indicators including the following (Schuyler, 2017):

- Protozoa, particularly the peritrich ciliates, such as the *Vorticella*, and the *Epistylis* have been documented to enhance nitrification
- *Rotifers* and *Tardigrades* are usually not observed in high quantities are typically limited to less than four species in a single biological treatment system.
- The presence of *Rotifers* can generally be tied to low turbidity in secondary effluent as they will also consume non-floc forming bacteria.
- The presence of Rotifers may not be observed in Nitrite Shunt processes, as they are strict aerobes, generally requiring dissolved oxygen concentration of at least 3 mg/L.
- Nematodes are predators of *Rotifers*, *Tardigrades*, and ammelios, and are generally observed in larger quantities in colder conditions.
- *Digonota* can dominate fixed growth and activated sludge processes.
- A low sludge volume index (SVI) is ideal.
- When ammonia-N in the effluent increases above 2 mg/L, likely culprits include:
    - Aeration may be insufficient due to additional demand from the nitrification process
    - SRT may be too short, as nitrifying bacteria are slow growing and may not have sufficient time to mature.
    - Nitrifiers can be inhibited by pH that is out of range and may need to be adjusted.

    - Toxic loading may be present.
    - Waste less, increase MLSS, increase aeration.
- When Nitrate-N in the effluent is increasing, likely culprits include:
    - A lack of BOD in the influent to support nitrifiers.
    - Sufficient carbon may not be present to support nitrifiers.
    - A lack of sufficient time in anoxic zones.
    - Lack of BOD in influent.
    - Aeration may excessive, preventing full denitrification.

4.4 Biocatalyst

A biocatalyst technique can be considered as a nitrogen shortcut technique along with those described above but is discussed separately as this approach is focused on microbiology selection by the application of material science to enhance and expand the margins of benefit or efficiency of nitrogen shortcut techniques.

Traditional wastewater treatment and nutrient removal is focused on replicating natural conditions in a controlled environment and optimizing those man-made and controlled biological processes. However, as discussed above, the fastest growing microbiology is not generally the best fit, metabolically active, or desired biology for nitrogen removal. This technique aims to sequesters the desired microbiology that takes longer to reproduce and develop dominate populations, inside a biocatalyst that can then be introduced to biological wastewater treatment processes to achieve desired nitrogen or phosphorus reduction, in less time, with less energy.

This generally consists of the use of a polymer composite, or porous “beads”, generally made of food grade, biodegradable microplastics that are hydrophilic, have a higher density than water, resistant to biofilm formation, and can be manufactured at 3 to 10 mm outer diameter. This bead irreversibly retains single species microbiology and promotes rapid mass transfer of specific contaminants and nutrients. The pour size within each bead is such that water, contaminants, and nutrients can pass into, and out of each bead, but the selected microbiology is retained within.

The seeding of the biocatalyst begins with identifying the ideal microorganisms for the intended treatment and outcome. These could include yeast, fungi, archaea, microalgae, gram positive, or gram-negative bacteria, filamentous, thermophiles, consortia, or others. Then the specific ecology and environment that will promote the growth and reproduction of the selected microbiology is developed, and a specific biocatalyst is selected. The type of bead can be produced with a unique internal pore shape, size, and configuration to best retain the selected microbiology. After an incubation period where the beads are seeded with the microbiology, a stable population is formed, which varies in duration dependent on the biology. Then the combined biocatalyst is deployed as a packed bed, fluidized bed, or suspended system for wastewater treatment.

This technique provides an intensification of bacteria density, generally 10 times greater than concentrations in traditional conventional activated sludge systems, which overall reduces the quantity of consumables (energy and carbon) required and reduces the retention time, overall providing a variation on a shortcut for nitrogen removal. Additionally, when implemented in a nitritation (shunt) or anammox configuration, this technique can return similar savings in shortcutting the nitrogen pathways, but with additional savings.

4.4.1 Microvi Biocatalyst

One manufacturer identified with demonstrated pilots and successful installations is Microvi. They developed the advanced materials and techniques to implement the biocatalyst processes described above and offer a MicroNiche

Engineering (MNE) platform that creates natural habitats, or microniches, for the express purpose of optimizing the performance of organisms in industrial bioprocesses.

The MNE process generally consists of a reactor with up to 30 percent biocatalyst, operated as a completely mixed system in a rectangular or circular reactor, with mixing energy added to prevent hydraulic shortcutting and to suspend the biocatalyst, adequate retention time for biological reactions, the addition of dissolved oxygen to promote aerobic reaction and suspension, and a biocatalyst retention system to retain the beads in the process, which typically consists of either aerated sieves or a quiescent zone. Additionally, carbon may be added in anoxic applications.

Microvi offers biocatalyst process for nitrate and phosphorus reduction in water treatment, and nitrogen removal for wastewater treatment. There are four process configurations offered for nitrogen reduction in wastewater treatment:

- Tertiary – configured as a single pass reactor without any solids removal. This consists of soluble BOD and COD removal, then nitrate removal and ammonia removal, depicted in Figure 36.
- Secondary – configured as a single pass reactor, also consisting of soluble BOD and COD removal, then nitrate removal and ammonia removal. This configuration is depicted in Figure 37.
- Secondary Hybrid –integrated configuration with activated sludge or fixed film reactors, with BOD, COD, and ammonia removal, then total nitrogen removal. This configuration is depicted in Figure 38.
- Side stream – configured as a single pass reactor without any solids removal. This consists of ammonia removal by nitritation followed by total nitrogen removal by denitritation and nitritation.

The general configurations of those applications are depicted below.

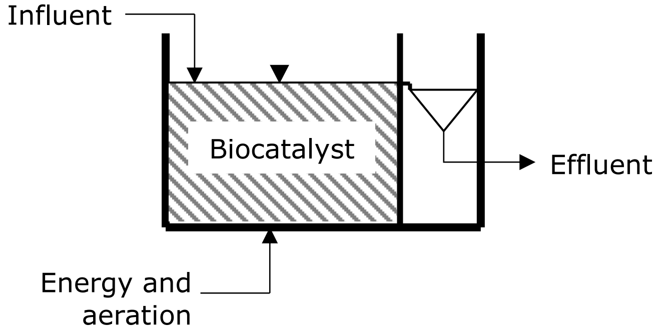

Figure 36 Single Pass Reactor for Tertiary Treatment

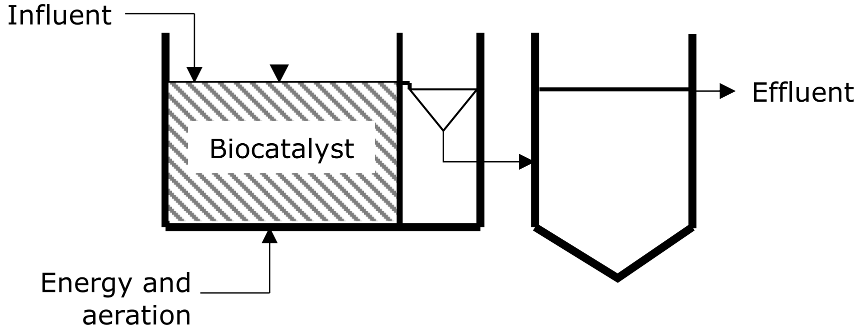

Figure 37 Single Pass Reactor with Clarification for High-Rate Applications

Treatment

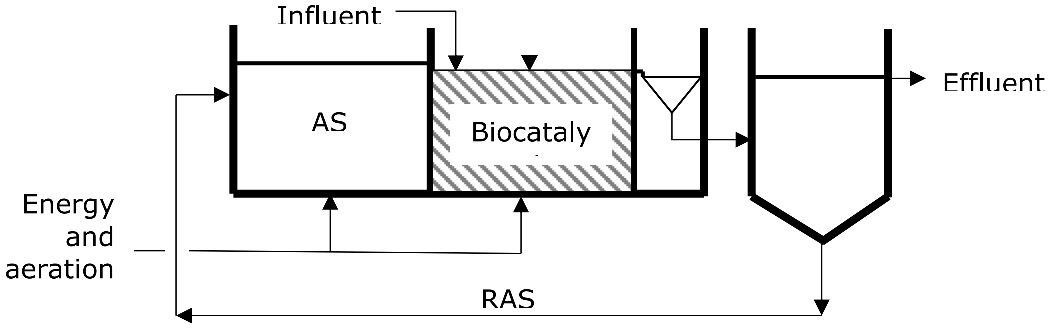

Figure 38 Activated Sludge Integrated Configuration

Common microbiology selected for nitrogen removal are *Nitrosomas* and *Nitrobacter*. Major concerns typically associated with tradition biological wastewater treatment systems, that can be partially or fully mitigated by these techniques include the following:

- Rapid spikes in loading from diurnal or seasonal changes.
- Pass through of spikes in BOD or TSS from upstream processes.
- Generation of suspended solids from the conversion of ammonia and carbon to cell mass.
- Rapid flow conditions that can promote ammonia breakthrough.
- Extended periods of low loading, where sufficient carbon and energy sources are not available, followed by a rapid increase in loading while biological populations are at their lowest.

Key operational benefits demonstrated by these techniques, and the intensification of selected microbiology, when integrated in a traditional modified Ludzack-Ettinger (MLE) suspended activated sludge process revealed the following:

- Anoxic Basin
  - RAS denitrification with improved oxygen and alkalinity recovery.
- Aerobic Basin
  - Lower endogenous and internal ammonia recycling oxygen demand.
  - Lower dissolved oxygen operating condition (1.0 mg/L) including denitrification.
- Microvi MNE integrated reactor
  - Higher alpha factors in the MNE reactor, due to reduced COD fouling at the end of the reaction.
  - Protected and independent nitrification population, with no ammonia washout.

- Overall
    - Complete nitrification at sludge age reduced from 14-days, to 3-days.
    - Higher volatile solids in waste stream, with increased calorific content biosolids for increase biogas production during anaerobic digestion.
    - 50% reduction in infrastructure footprint.
    - 30% less aeration energy required.
    - Simultaneous denitrification for alkalinity and oxygen recovery.
    - Higher nitrification resilience to flow and loading variations.
    - 50% increase in biogas production.

Those benefits identified above were found to be similar across multiple pilots and full-scale installations as part of the literature review, with effective ammonia removal in as little as two to four hours retention times.

Implementation of the Microvi single pass reactor for tertiary treatment, with or without clarification did not substantial improve BOD or TSS removal, with an average of 50 percent removal of BOD and TSS, but with consistent ammonia reduction of greater than 90 percent. Therefore, in that configuration, this technique may be best for polishing ammonia concentrations as a tertiary process, in addition to other wastewater treatment processes.

Implementation of the Microvi MNE process as integrated with an activated sludge process, was observed to be effective, reducing BOD and TSS concentrations by greater than 90 percent, and reducing ammonia concentrations by 90 to 98 percent, and demonstrates effectiveness for high efficiency secondary treatment.

Other documented benefits of the use of a biocatalyst includes protection from predation, and freedom from unwanted biofilm development on the biocatalyst.

The understanding of how biofilm is prevented, is due to the hydrophobic properties of material of the polymer, which does not promote the ability for biofilm growth on or in the synthetic sphere, and due to the pore size, in which the living target bacteria colonies are retained, but allows for dead bacteria and substrate to be washed out.

The longevity of the biocatalyst is no different that of a non-contained colony, where, if there is the right ecology and substrate, the target bacteria will continue to propagate and sustain. However, it is understood that in an event of a die-off event due to shock loading, unfavorable environmental conditions, or lack of the right substrate, bacteria population will decline, but not be replaced inside the synthetic sphere by other bacteria. When favorable conditions, and required substrate returns, that target bacteria population can resume. No significant additional maintenance requirements were identified relating to the biocatalyst.

Potential limitations to be considered with the use of a biocatalyst is the availability and capability to produce and sustain large quantities of seeded biocatalyst beads, for initial start-up, potential re-seeding after significant process upsets, or in high demand markets.

CHAPTER 5

CARBON SUPPLY

A primary factor that influences the success of biological processes, and nitrogen removal as discussed above is the availability of carbon. Most of the carbon available and used in biological wastewater treatment processes comes from the influent raw wastewater. The concentration of carbon to nitrogen ratio (C:N) varies across all wastewater systems based on the types and quantities of dischargers. However, it is that C:N ratio that is critical to supporting nitrogen removal efficiencies in biological wastewater treatment processes and understanding when supplemental carbon addition may be required. The general relationships of C:N relating to nitrogen removal efficiency are listed in Table 6.

C:N $g\,{}^{COD}\!/_{g}\,N$	Resulting nitrogen removal efficiency
> 9	correlates to high efficiency
7 to 9	correlates to good efficiency
5 to 7	correlates to moderate efficiency
< 5	correlates to poor efficiency

Table 6 C:N Efficiencies, (WEF, 2015)

The types of nitrogen shortcut techniques discussed above include both side stream and mainstream configurations, each with varying carbon availability. Side stream configurations tend to be more carbon limited, but mainstream applications may also suffer from limited carbon. Conventional biological wastewater treatment processes generally require a C:N of six to 10 $g\,{}^{COD}\!/_{g}\,N$, where shortcut techniques can be successful with C:N ratios as low as 2.9 $g\,{}^{COD}\!/_{g}\,N$ (WEF, 2015).

This is partially due to nitrifying bacteria utilizing nitrogenous BOD and alkalinity for a food and energy source for reproduction, instead of carbonaceous BOD as required by organotrophic bacteria. Additionally, the deammonification shortcut process can provide full nitrogen removal without any carbon. This introduces another benefit of alkalinity recover. However, alkalinity recovery from ammonia removal is limited, as up to half of the required alkalinity (WEF, 2015). Therefore, the available alkalinity may also be a limiting factor, and may need to be supplemented based on influent alkalinity concentrations.

Carbon provides an electron donor for energy used in microbiological growth and maintenance, and success of the supplemental carbon source may be based on the type, yield, and ability for microbiology to utilize that carbon provided electron. Commonly used and known supplemental carbon sources used in biological wastewater treatment process, including the C:N ratio, yield, and specific denitrification rates are summarized in Table 7.

Electron Donor	C:N	Yield	Denitrification rate, $mg\,{}^{N}\!/_{g}VSS * hr$
Methanol	4 to 5	0.2 to 0.4	3 to 30
Ethanol	6	0.2 to 0.5	5 to 6
Acetic acid	9	0.3 to 0.6	2 to 76
Waste alcohols	4 to 6	0.2 to 0.5	3 to 30
Glycerin	4 to 5	ND	2 to 7
Fatty acids	3.5 to 6	0.3 to 0.65	3 to 12
Dairy waste	3.6 to 4.7	0.2 to 0.4	3 to 8
Elemental sulfur	2.5	0.1	Non detect (ND)

Table 7 Supplemental Carbon Sources (WEF, 2015)

Each type of supplemental carbon that may be selected has other treatment and non-treatment related concerns to be aware of in the decision process. The non-treatment related concern is the health and safety of wastewater treatment plant operators, where Methanol is explosive, and requires careful handling and storage. This causes an increase in the overall risk of use and implementation, and should be carefully considered, as this will elevate capital costs for developing proper transfer, storage, and handling facilities, and increase long-term operational and maintenance costs due to additional safety training, precautions, and requirements.

Treatment related concerns include the specific biological populations that could be present in the specific process, and that could develop undesired conditions. This has been documented regarding the use of glycerol-based products as a supplemental carbon source with a nitrogen shortcut process. In that specific case, it was revealed that there was a propagation of glycerol acclimated biomass (GAB) that gravitates to conduct denitrification, leading to nitrite buildup that is converted back to nitrate prior to full denitrification. Such nitrite buildup was identified in anoxic areas of the process and is referred to as nitrite lock.

Other treatment concerns related to the viscosity of the supplemental carbon sources, in which successful pumping and transfer can become limited, and requires adequate planning and engineering for temporal fluctuations. Additionally, the viscosity will vary based on the concentration of the supplemental carbon source, and designers should specifically consider what concentrations are available, or may become available to design the storage and delivery systems accordingly (WEF, 2015).

CHAPTER 6

THERMODYNAMICS

6.1 Thermodynamics Overview

Thermodynamics is the study of, and physics, of the various types of energy, including mechanical, electrical, and chemical, and the relationships between energy, work, temperature, and heat, specifically relating to the transformation of energy from one form, or place, to another. Thermodynamics cannot provide insight about kinetics, only about the affinity or feasibility for certain reactions to occur. Therefore, it is the thermodynamics that promote, activate, or inhibit the likeliness of reactions to occur, but the kinetics that control the rate of those reactions.

Important to this study, and thermodynamics in general, is the accounting of energy throughout those transformations. A classical approach in describing such energy transformations is the identification and quantification of changes in thermal conditions, or the heat. Additionally, the total energy in a system or process is not generally the focus of the study or discussion of energy, but more so the change in that energy, referred to as Internal Energy (U), which is a state function.

A state function is defined as the thermodynamic equilibrium of variables of a system and is not described by the path to achieve at the current state. Other examples of a state function include enthalpy (H) and entropy (S), both described by their equilibrium condition.

Entropy is a key concept in the discussion of thermodynamics, that represents, or quantifies the amount of thermal energy, not available for conversion or contribution to work, as measured in thermal energy, per unit of temperature. This is also referred to as the degree of disorder of a system. Entropy is defined as follows:

$$S \equiv k_B \ln \Omega$$

Equation 47 Entropy (Moran, 2003)

Where,

S = Entropy

k_B = Boltzmann constant, equal to $1.38065 * 10^{-23}$ J/K

Ω = the number of microstates

Entropy is how we discuss the number of different possible states or conditions, and in the focus of biological processes and chemical reactions, it is important to note that the total entropy of a system and surroundings always increases for spontaneous processes.

Enthalpy is key concept of the thermodynamics, applied to the stoichiometry in biological reactions, and is defined or measured as the energy stored within bonds. The change in enthalpy is the differences of bond energy between the products and reactants and measured as either heat released or absorbed per reaction. Enthalpy is part of the accounting of thermodynamics and can describe the amount of heat. Enthalpy is defined as follows:

$$H \equiv U + PV$$

Equation 48 Enthalpy (Moran, 2003)

Where,

H = Enthalpy

P = Pressure

V = Volume

These are important in discussing and quantifying the two major components of thermodynamics, including heat and work. Heat is defined and calculated by the temperature and entropy and involves random particle or molecule movement.

Work, mechanical, or chemical, defined by the change in physical place or volume, or in the chemical changes, both involve non-random movement of particles or molecules.

Internal Energy is a summation of the energy related to the movement or motion of molecules, including place translation, rotation, or vibration, referred to as the kinetic energy. The energy related to the electrical energy of atoms is referred to as the potential energy. Internal Energy may not be able to be measured directly. However, the change in the Internal Energy can be measured directly.

In terms of biological thermodynamics, the change in Internal Energy is defined by the following equations:

$$dU = \delta Q - \delta W \text{ , or}$$

$$dU = TdS - PdV + \sum_i \mu_i dN_i \text{ , or}$$

$$dU = TdS + YdX$$

Equation 49 General Biological Thermodynamics (Moran, 2003)

Where,

dU = the change in Internal Energy

δQ = change in heat and entropy

δW = change in work

T = temperature (heat)

dS = change in entropy

P = pressure

dV = change in system volume

μ_i = ions

dN_i = change in the number of moles

YdX = conjugate of the different work processes occurring

Each of the approaches above for evaluating energy is a way of defining and configuring the components and factors of energy in terms of heat. As an example, enthalpy is commonly used to eliminate other various work terms and equate energy to heat.

Free Energy is another energy term in biological thermodynamics, which is the extra energy available for different processes, referred to the Gibbs Free Energy. The Gibbs Free Energy equation includes both enthalpy (H), and heat (temperature (T) and entropy (S), defined as follows:

$$G \equiv U + PV - TS$$

$$G \equiv H - TS$$

Equation 50 Gibbs Free Energy (Moran, 2003)

The change in Gibbs Free Energy, as a state function, by transforming the independent and dependent variables through a Legendre transformation, is defined as follows:

$$dG = -SdT + VdP + \sum_i \mu_i dN_i$$

Equation 51 Gibbs Free Energy Legendre Transformation (Moran, 2003)

This is a useful technique in describing and calculating energy, as the entropy is not easy to maintain as a constant in describing the chemical work effects of the energy. Note that while the Internal Energy (U) is dependent on the entropy (S), volume (V), and the number of moles (N_i). Enthalpy allows the transformation, and sign, to be dependent on the entropy (S), pressure (P), and the number of moles (N_i). The Gibbs Free Energy equation allow the evaluation of energy with temperature (T) and pressure (P) held constant. This promotes the ability to evaluate a chemical or biological reaction in isothermal or isobaric conditions to evaluate and quantify how the chemical work uniquely effects the energy. This is most used in biological thermodynamics in an isothermal condition, where the temperature is held constant, and the change in energy can then be considered as an enthalpy and an entropy term, to describe the energy change of a reaction and how likely the spontaneous reaction is to occur. This is described in the equation as follows:

$$\Delta G = \Delta H - T\Delta S$$

Equation 52 Change in Gibbs Free Energy (Moran, 2003)

The change in the free energy is the most common variable used in accounting for energy use through microbiological and chemical reactions associated with wastewater treatment and nitrogen removal.

6.2 Microbiological Thermodynamic Fundamentals

In the previous chapters, the biological processes related to wastewater treatment and nitrogen removal have been described by the kinetics, stoichiometry, or physical/mechanical processes. However, when viewed macroscopically, almost all biological processes can be viewed in terms of the thermodynamics. Specific to the focus of this study is the ability to utilize thermodynamics to evaluate the change of the concentration of electrons in relation to chemical energy and processes, the related work completed, and as measured by the heat.

Focused on the microscopic level for biological wastewater treatment processes, the thermodynamic terms defined above can be used to provide insights of two key activities occurring as part of those reactions, and important to better understanding how to promote and sustain the desired biological reactions and propagation that provide wastewater treatment, and nutrient removal services.

These thermodynamic terms and techniques can be used to determine whether a spontaneous reaction will, or the likeliness that it will occur. For example, if the Gibbs Free Energy (ΔG) is negative, then the conditions are thermodynamically favored for the spontaneous reaction to occur, and the energy will decrease over the entire reaction. If ΔG is at or near zero (0), then the reaction is reversible at equilibrium. If ΔG is positive, then the conditions are thermodynamically unfavorable for the spontaneous reaction to occur, and the reverse reaction is favored. This compromise between entropy and enthalpy in thermodynamics is graphically depicted in Figure 39.

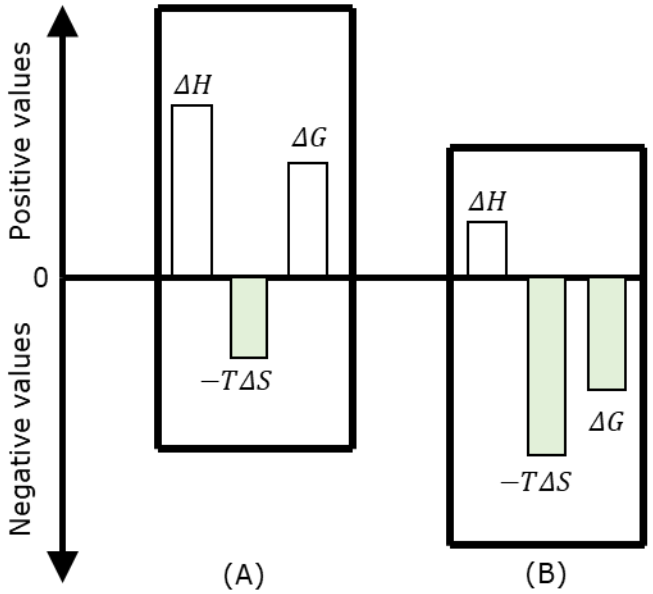

Figure 39 Gibbs Free Energy result for Likeliness of Reaction to Occur

When the magnitude of temperature and entropy ($-T\Delta S$)is greater than the enthalpy (ΔH), then the reaction is referred to as entropically driven. Conversely, when the enthalpy is greater in magnitude than the temperature and entropy, the reaction is referred to as entropically driven. Such functions are often attributed to the microbiology, which are responsible for these reactions. This is also where enthalpically favored or dominant thermodynamics can promote the ability for larger and more complex structure formation. This would not generally be possibly or would be inhibited in entropically dominated conditions, noting that in extremely high temperatures, entropy dominates thermodynamics.

An example of the use of thermodynamics to describe the biological processes and reactions is in the solvation of urea. Solvation is reaction in which bonding, bond formation, and van der Waals forces occur and reorganize solvent and solute molecules into more complex bonds. This reaction is driven by increasing entropy, that is greater than the enthalpy, even if the enthalpy is increasing. The result is a lower temperature, as greater energy is required to break bonds, than energy released from the product, and is therefore an endothermic reaction.

Another similar example of solvation by water is referred to as hydration. Other examples where the mixing of reactants makes the surrounding environment warmer, is a result of a greater decreasing enthalpy, than the decreasing entropy, and is an example of an exothermic reaction (Jaganade, Chattopadhyay, Raghunathan, Priyakumar, 2020).

The various reactions described in the chapters above relating to ammonia and nitrogen removal, including nitrification, denitrification, nitritation, and deammonification, and other individual biological processes that oxidize nitrite, ammonia, and that utilize a variety of carbon, nitrogen, alkalinity, and other substrates for energy and food production. Each can be evaluated in terms of the thermodynamics and how likely those reaction will occur. Those reactions rely upon the ability to break bonds in existing molecules and compounds, and the ability to utilize free electrons for energy, and to make new bonds and bind together. These activities are dependent on the concentrations of the molecules and compounds, the equilibrium conditions, and the Gibbs Free Energy.

Specifically, we can use the components of, and Gibbs Free Energy, to better understand the thermodynamic limitations and preferences of these reactions. As an example, enthalpy can be used to identify insights about the changes in the bonds, and bonding within reactions, electron charge interactions, hydrogen bonds, and van de Walls forces in the biology and the system. Entropy can be used to identify insights about the arrangement and configurations, and the magnitude of disorder of molecules and compounds in the system. A useful approach is to calculate the equilibrium constant for a reaction, based upon the Gibbs Free Energy, utilizing the following equations:

$$\Delta G = -RT \ln K_{eq}$$

Equation 53 Gibbs Free Energy and Equilibrium (Moran, 2003)

Where,

ΔG = Gibbs Free Energy

R = $8.314 Jmol^{-1}K^{-1}$, or $0.008314 kJmol^{-1}K^{-1}$

T = temperature

K_{eq} = equilibrium constant

This equation can be rearranged as follows to solve for the equilibrium constant (K_{eq}):

$$\ln K_{eq} = -\frac{\Delta G^o}{RT}\text{, or}$$

$$K_{eq} = e^{-\Delta G^o / RT}$$

Equation 54 Equilibrium Constant Based on Gibbs Free Energy (Moran, 2003)

Eucaryotes energy production is generally by aerobic respiration, photosynthesis, alcohol fermentation, or acid fermentation. Procaryotes use those and additional means and pathways for energy production, including the acetyl (CODH) pathway, reverse TCA cycle, light-driven non-photosynthetic, methanogenesis, anoxygenic photosynthesis, lithotrophy, anaerobic respiration, and other fermentation pathways (Todar, 2020). In whole, these are the summation of biochemical reactions for energy generation through catabolic reactions, and cell synthesis through anabolic reactions. This relationship is outlined in Figure 40.

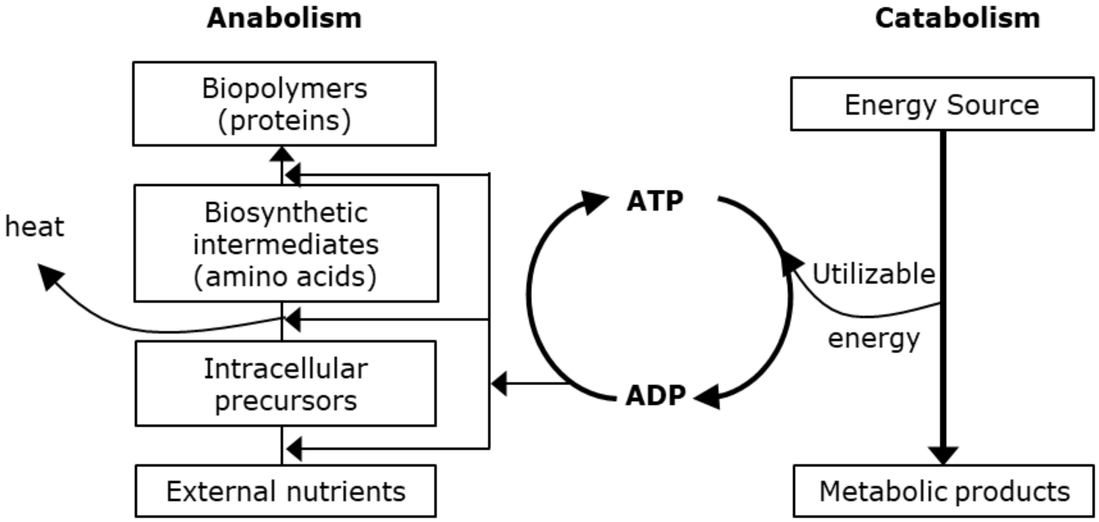

Figure 40 Catabolism and Anabolism Relationship (Todar, 2020)

Figure 40 above depicts the transformation of energy as aligned with the laws of thermodynamics. Some energy is lost as heat, the remainder is divided among the biosynthetic metabolic demands of the anabolism reactions. Additionally, regardless of the substrate or compound used, converted energy is briefly stored as adenosine triphosphate (ATP), which is a high energy bond requiring approximately $8\, {}^{kcal}\!/_{mole}$ to convert from Adenosine di-phosphate (ADP) to ATP. Later when hydrolyzed from ATP to ADP releases the same (Todar, 2020).

A method used to quantify the potential, or likeliness, of chemical compounds to receive or donate electrons in transformation processes is by the Oxidation-Reduction Potential (ORP). ORP (ε) is a commonly used measurement of water, typically measured in millivolts (mV), or volts (V), which quantifies the intrinsic reduction potential, as is dependent on both the concentration of dissolved oxygen (O_2), and other compounds and substances that function like oxygen. The scale of ORP generally ranges from -800 to +400 mV.

Reduction is when electrons are gained or accepted, and oxidation is when electrons are donated. The greater, or more positive the ORP, the more likely reduction will occur. The more negative the ORP, the less likely reduction will occur, and the more likely oxidation will occur. These are both measured by the standard reduction value, as based on the standard hydrogen electrode as the reference value that has an ORP value of zero (0) mV based on the half-reaction as follows:

$$2H^{+}_{(aq)} + 2e^{'} \rightarrow H_{2(g)}$$

Equation 55 Standard Hydrogen Reduction Potential Half Reaction

Higher ORP values indicate higher concentrations of oxygen is present and is typically measured in addition to the measurement of O_2, as ORP can provide additional insights to the ecology and quality of the aqueous environment. This is because ORP is measuring the transfer of electrons, not the absolute concentration, but can theoretically be equivalent. Aerobic bacteria activity is higher in parallel with higher positive ORP values, and anaerobic bacteria activity is higher in parallel with negative, and lower ORP values. However, there are facultative anaerobic bacteria that can be active in both ranges of positive and negative ORP values, where oxygen can be used from nitrate and other inorganic compounds.

Standard ORP values for healthy natural waterways and aquatic environments can range from 300 to 500 mV. In the practice and operations of wastewater treatment processes, ORP is a valuable metric of potential biological reactions that are either oxidation or reduction processes that support cBOD reduction, nitrification, and denitrification. Where positive ORP values indicate increasing concentrations of O_2, and negative ORP values can indicate higher concentrations of cBOD and substrate, required for desired biological reactions.

ORP measurements throughout biological wastewater treatment processes is a useful tool for operational decisions, and for indications of proper performance, and can be used in combination with other typical parameters monitored, including O_2, hydraulic retention time, and pH.

Nitrogen shortcut techniques can facilitate nitrification and denitrification with lower potential or actual concentrations, which is part of the savings of those techniques. Multiple studies indicate that a ORP value of zero (0) was common in nitritation and deammonification processes. Additionally, utilizing a combination of ORP and pH as unit process parameter controls, promoted frequent success in limiting events where over aeration inhibits the reduction of nitrite to nitrate, and was key in maintain nitrogen shortcut techniques (Sadowski, 2015).

6.3 Microbial Thermodynamic Literature Review

Application of these techniques to evaluate and identify opportunities to improve nitrogen removal efficiencies in biological wastewater treatment process are discussed in research, and as summarized below.

One such research demonstrated through testing of *Nitrosomonas* in varying concentrations of ammonia, the nitrite oxidation rate was linearly interrelated with free energy changes in ΔG, and that a thermodynamic model, based on the Monod kinetic equation, the rate of ammonia conversion was twice the nitrite conversion rate (Yantarasri, Garcia, Brune, 1992). That study further predicted that the use of a thermodynamic model could be used to optimize reactor operations to minimize increase nitrite concentrations in the effluent during peak loading events.

Other research aimed to unify biological, physical, chemical, and mechanical models and parameters to be an effective tool to extend the application of Individual based Models (IbM) beyond research (Gogulancea, González-Cabaleiro, Taniguchi, Jayathilake, Chen, Curtis, 2019).

One outcome of those efforts was the identification that pH and chemical speciation is key to effectively connecting thermodynamics to the biological models but was not able to identify or predict specific uptake rates. A key principle of pH relation to microbiological growth is the determinant speciation of compounds utilized as food and energy sources. For example, ammonia oxidizing bacteria utilize ammonia, but not ammonium. Therefore, the ammonia-N specification, as depicted in Figure 3, is directly linked to the buffering ability of the wastewater, and on which, and how much substrate is available for growth of microbiology. Models that do not account for the pH, or the speciation of chemicals, will not be adequate for maximizing the use of thermodynamic techniques to predict growth rates. Additionally, sufficient run time of models and those that consider three dimensional (3D) computational dynamics may be required to simulate realistic conditions. Such attempted models utilized the approach to thermodynamic yield estimation by assuming maximum growth yield for the biomass (Y_{XS}), with respect to the electron donor, by the anabolic free energy pathway (ΔG_{ana}), catabolic free energy pathway available (ΔG_{cat}), and the energy required for cell maintenance (ΔG_{dis}), noting that ΔG_{dis} is computed by a correlation to ΔG_{ana} , and ΔG_{cat} , and as follows:

$$Y_{XS} = \frac{\Delta G_{cat}}{\Delta G_{ana} + \Delta G_{dis}}$$

Equation 56 Biomass Thermodynamic Growth Yield (Gogulancea, González-Cabaleiro, Taniguchi, Jayathilake, Chen, Curtis, 2019)

That study developed the specific growth rate per bacterial cell (μ), as follows:

$$\mu = q_{\max} * Y_{XS} * \Pi_i \left(\frac{C_{si}}{K_{Si} C_{Si}} \right) - m_{bac}$$

Equation 57 Bacteria Growth Yield, Monod-type Expression (Gogulancea, González-Cabaleiro, Taniguchi, Jayathilake, Chen, Curtis, 2019)

Where:

$q_{\max}$ is the maximum uptake of the substrate

Y_{XS} is the growth yield

K_{Si} is the health saturation affinity constant

C_{Si} is the growth limiting concentration

m_{bac} is the cell maintenance requirement

On those basis, there are three possible growth scenarios, where either 1) there is an increasing biomass, 2) the biomass remains constant as growth and decay are essentially equal, or 3) the decay is greater than the growth and maintenance, based on a first order reaction. The pH calculation portion of the model was built on a thermodynamic framework, including hydration reactions and deprotonations, assumed occurring instantaneously and modeled in equilibrium. This results in a non-linear charge balances, where the proton concentration is solved at each point in the domain with a Newton Raphson algorithm, modified for the application (Gogulancea, González-Cabaleiro, Taniguchi, Jayathilake, Chen, Curtis, 2019). The mass transfer portion of the model assumes bacteria only use one form of substrate, and then products of cellular decay are limited to carbon and nitrogen sources per anabolic reaction. Specific thermodynamic parameters used in the model for formation, dissipation, and yield are depicted in Table 8 below.

Group	ΔG formation (kJ/Cmole-x)	ΔG dissipation (kJ/Cmole-x)	Calculated Yield (C-mole-X/mole-eDonor
Ammonia oxidizing bacteria	-67	-3,500	0.155
Nitrite oxidizing bacteria	-67	-3,500	0.077

Table 8 Model Thermodynamic Parameters, (Gogulancea, González-Cabaleiro, Taniguchi, Jayathilake, Chen, Curtis, 2019)

Several valuable takeaways from that research are that such an IbM, with coupling of the pH and chemical speciation, can be used to quantify allowable or controlled substrate to promote or inhibit desired microbiological growth, and the implementation of thermodynamics in the model would allow for application of any microbial system. However, their conclusion was that insight to actual, and predictable uptake rates was not able to be gleamed from the model, and further genomics research and understanding is required (Gogulancea, González-Cabaleiro, Taniguchi, Jayathilake, Chen, Curtis, 2019).

Other research focused on nitrifying bacteria, as a sole microbiology that could complete the nitrification process alone and would have a metabolic advantage in biofilms. That research focused on how nitrifiers and ammonia oxidizing bacteria compete, and quantified the differences of growth rate and yield, which concluded that difference is not significant and does not identify an advantage (González-Cabaleiro, Curtis, Ofiţeru, 2019).

Other research has focused on the possible thermodynamic pathways relating to nitrification production of N_2O. That approach was based on distinguishing energy between the cell synthesis and the overall biological reaction. Focused on free energy transfer efficiencies, that research identified between 50 and 70 percent efficiency for autotrophic conditions, and that the conversion of ammonia to nitrate by nitrite was the dominant process and confirmed that ΔG values were negative when energy was released because of the reaction and was positive when energy was required to convert carbon to energy.

Following the known half reactions for the nitrification processes, as describe earlier, it was determined that the calculated free energy was negative in each case, and in terms of thermodynamics, each reaction can occur spontaneously.

However, there were three intermediates in the nitrification process, including nitric oxide (NO), hydroxylamine (NH_2OH), and nitrous oxide (N_2O) that each have an impact as an electron donor and acceptor that influenced the efficiency of energy transfer and maximum cell synthesis and growth. The various pathways to nitrous oxide production, through the electron transport process is depicted in Figure 41.

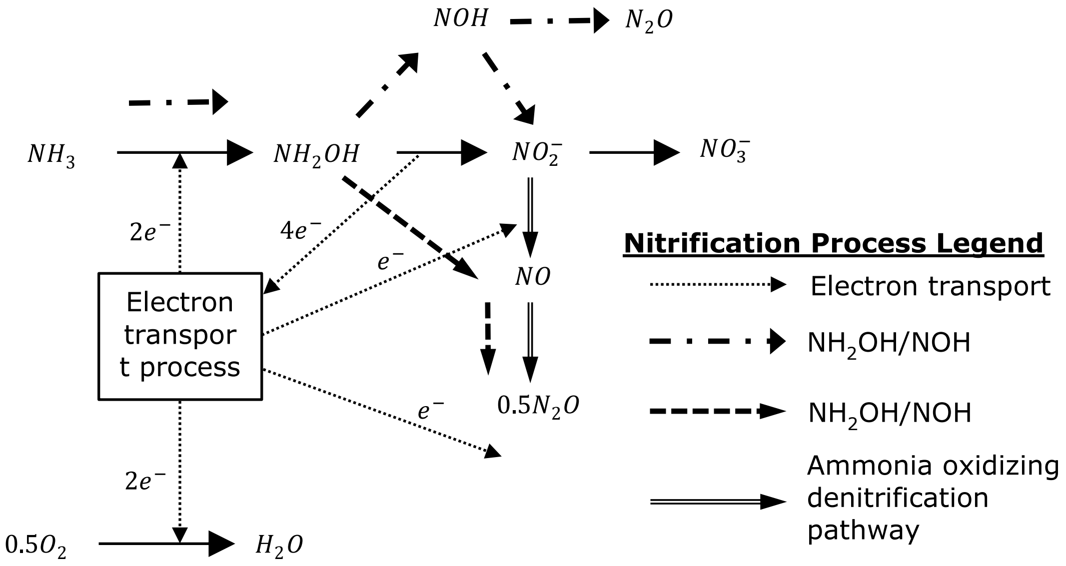

Figure 41 Thermodynamic Pathways of Nitrification, (Hu, Tian, Zhao, Wu, Yang, Chen, 2017)

When hydroxylamine was the electron donor, and nitric oxide the acceptor, cell synthesis was maximized. When ammonium was the electron donor, and nitrite was the acceptor, cell synthesis was at the minimum, and noting the valence of nitrogen in nitrous oxide (-3), is lower than nitrite (+3). Evaluation results confirmed that it was the conversion of ammonia to nitrite that produced nitrous oxide. Therefore, the ammonia oxidizing bacteria is responsible for nitrous oxide production, not nitrite oxidizing bacteria. Additionally, nitrous oxide was determined to be a byproduct of the oxidation of hydroxylamine to nitric oxide and is an energy generating reaction that benefits ammonia oxidizing bacteria, in addition to other sources, and that the dominant pathway was ammonia, to nitrite, to nitrate. The ammonia oxidizing bacteria portions of the nitrification reactions were calculated to have net positive energy. However, most of that energy was determined to be used for cell maintenance with very small energy used for cell synthesis. The conclusion was that the theoretical yield coefficient for autotrophic production of nitrous oxide ranged from 0.097 to 0.194 $^{gcell}\!/_{gN}$ for ammonia oxidizing bacteria (Hu, Tian, Zhao, Wu, Yang, Chen, 2017).

As an ongoing development of the body of knowledge relating to the microbial thermodynamics there is substantial efforts, specifically relating to efficiencies in nitrogen and ammonia removal, that are developing and testing techniques to predict theoretical yields, identifying inhibitors and limitations, and quantifying the amount and percentage of energy used for microbial cell synthesis and maintenance. However, there generally appears to be an ongoing challenge with the following:

1. A lack of ability to identify or predict specific uptake rates.
2. Validate ecological conditions and variability on the actual ATP and ADP efficiencies.

3. Develop interconnected models that fully accommodate the 3D considerations for the ability and feasibility for microbiology to find substrate, over a sufficient duration and depth to improve the precision of the results.

In conclusion, tracking the free energy in metabolic reactions can be utilized to estimate the likeliness that such reactions occur. However, further genomics research and improved precision in the quantification of, and ability to track energy across various electron transport pathways is needed. Additionally, utilizing pH and chemical speciation is key in quantifying allowable or controlled substrate for microbiological selection approach to optimizing wastewater treatment, and nitrogen removal processes.

CHAPTER 7

DECISION MATRICES AND MODEL

7.1 Key Nitrogen Removal Factors

The need and potential for gained efficiencies in wastewater treatment has been demonstrated in the chapters above, both in performance and economics. Additionally, improved nutrient reduction and improved efficiency will be paramount to meet current needs and the projected increasing anthropogenic contribution to nitrogen discharges within communities and the environment.

The capacity to collect and treat wastewaters requires an ongoing commitment that must be increased and accelerated to meet those challenges successfully. The optimization of those processes, including those for shortcut nitrogen removal from wastewaters, provide an opportunity to make an important contribution to those efforts, and potentially reduce the magnitude of current and future commitments.

The information described and detailed in the chapters above are first briefly summarized to identify patterns and opportunities in optimizing nitrogen removal techniques. The results of that summary can them be used to develop decision matrices for implementing or optimizing nitrogen shortcut removal techniques.

A summary of the key factors that contribute to the success of efficiencies in traditional and shortcut nitrogen removal techniques, were organized by general, ecological, microbiological, kinetics, thermodynamics, and carbon categories and outlined below.

7.1.1 Key Ecological Factors

1. Ecological focuses on abiotic and biotic factors, relating to the growth and reproduction of microorganisms and bacteria.

    a. Growth and reproduction cycles can be symbiotic or predatory, providing food and energy either as a web or in a chain that transfers energy and carbon.

2. Key abiotic factors in the activated sludge processes include:
    a. Alkalinity, pH, temperature, and concentration of ammonium ions, dissolved oxygen, inorganic matter, nutrients, substrates, and toxic waste.
    b. Hydraulic retention time, rate of return activated sludge (RAS), rate of waste activated sludge (WAS) are key operational factors.
3. Biotic factors comprise the living components that are most influenced by the following key factors:
    a. The abundance and type of bacteria and microorganisms.
    b. Mixed liquor volatile suspended solids (MLVSS) concentration.
    c. Mean cell residence time (MCRT).
    d. Bacteria will have higher quantities than protozoa due to having a lager surface-to-volume ratio, and able to consume more soluble substrate, when greater propagation.
        i. This is referred to as the “competitive exclusion principal”, in which the species that best utilize resources present in a habitat, will solely occupy that habitat at that time.
4. Microbiological habitat ecological factors:
    a. Aerobic bacteria propagate where dissolved oxygen concentrations are greatest while oxidizing ammonia and nitrite.

    - b. Floc-forming bacteria propagate throughout floc particles, and in the bulk solution, prior to forming floc, while oxidizing soluble cBOD.
    - c. Pseudomonads propagate throughout floc particles, while oxidizing soluble cBOD, toxic phenol and phenolic compounds.
    - d. Alcaligenes and Flavobacterium mainly degrade proteins and would normally only be present in waste streams that are high in protein but would be present at lower quantities or concentrations than other bacterium, due to a limited ability to utilize varieties of substrate.

7.1.2 Key Microbiological Factors

1. Ammonia is used as a chemical energy source for microbiological organisms.
2. Prokaryotes, including eubacteria and archaebacteria, are the most common and important bacteria in wastewater treatment.
3. Eukaryotes are also important to wastewater treatment, and include fungi, protozoa, rotifers, and nematodes.
    - a. Protozoa are generally strict aerobes but can also propagate in anaerobic conditions.
    - b. Rotifers and nematodes (metazoa) provide similar services as protozoa, but also can degrade substrate within floc which promotes additional degradation of nutrients by penetrating and introducing dissolved oxygen, nitrate, nutrients, and substrate.

4. Microorganisms that have larger surface areas, have experience greater exposure to bulk solution, and can adsorb a greater quantity of nutrients.
    a. Microorganisms that require less nutrients for propagation are less inhibited in low nutrient environmental conditions.
    b. Bacteria that have a better affinity to utilizing available oxygen from a variety of substrates, such as Sphaerotilus natans or Haliscoemobacter hydrossis, can proliferate in low dissolved oxygen conditions.
    c. This is part of why nitrifying systems generally require a higher actual oxygen transfer efficiency (AOTE) of nine to 15 percent.
5. Key factors for bacteria in wastewater treatment include:
    a. Nutrition, consisting of the substrates used for food and energy.
    b. Motility.
6. The three major growth factors affecting bacteria growth are:
    a. pH, temperature, and how bacteria respond to presence of free molecular oxygen.
7. Bacteria that use free oxygen as a final electron acceptor are known as aerobes.
    a. Nitrosomonas, and Nitrobacter are the main nitrifying, aerobic bacteria.
        i. Nitrification uses autotrophic chemolithotrophs to metabolize ammonia to nitrite.
        ii. Nitrifying bacteria are slow growing and are inhibited by high concentrations of nutrients, such as BOD.

        iii. Chemoheterotrophs are dominant in ordinary biology conditions.
        iv. Nitrosomonas and Nitrobacter can become dominant and successfully nitrify wastewater when the concentrations are suitable and there is sufficient solids retention time.

8. Bacteria that use combined oxygen substances, such as Nitrate-N, sulfate, and carbonate, when free oxygen is absent are referred to as facultative anaerobes.
    a. Facultative anaerobes can be either aerotolerant, or oxygen intolerant.
        i. Aerotolerant facultative anaerobes have the most complex enzyme systems, where one system uses free molecular oxygen, and another for utilizing other molecules for to degrade substrates when oxygen is not available.
        ii. Bacteria that cannot use free or combined oxygen are referred to as anaerobes, which use alcohols or organic acids as a final electron acceptor.
9. Anoxic and anaerobic conditions are necessary for biological denitrification.
    a. Other denitrifying bacteria include Pseudomonas, Alcaligenes, Bacillus, Spirillum, and Acinetobacter, convert Nitrate to nitrogen gas and nitrous oxide gases.
10. Bacteria response to free molecular oxygen is critical in managing concerns of providing sufficient concentrations of oxygen in

wastewater treatment processes for aerobe and facultative anaerobe activity.

   a. This includes concerns regarding endogenous respiration, floc formation, nitrification, and control of filamentous growth in low dissolved oxygen concentrations.

11. Non-living substrates are used as energy and carbon sources
    a. cBOD is used by organotrophic bacteria.
    b. nBOD is used by nitrifying bacteria.
12. Organotrophic bacteria use organic compounds as carbon and energy sources, including Acetic Acid, Acetone, Ethyl Alcohol, Glucose, Isopropyl alcohol, or stearic acid.
13. Chemolithotrophs utilize inorganic compounds or minerals for carbon and energy, including iron bacteria, sulfur bacterial, and nitrifying bacteria.
14. Chemical bonds of organic and inorganic compounds are broken as substrates are degraded for energy, which releases electrons.
    a. That energy from the release of electrons is then stored in the bacteria adenosine triphosphate (ATP), as a phosphate bond, and then electrons are removed from the cell, through a process referred to as a final electron carrier molecule.
    b. That molecule may be in the form of free oxygen for aerobic bacteria and as part of the nitrification, or in the form of nitrate or nitrite ions for anaerobic bacteria in the denitrification process and in an anoxic environment.

15. Nitrifying Bacteria
    a. Nitrosomonas, Nitrosospira, Nitrosococcus, Nitrosocystis, Nitrosolobus oxidize the ammonia ion.
    b. Nitrobacter, Nitrospire, and Nitrospira oxidize nitrite.
16. Denitrifying Bacteria
    a. Alcaligenes, Bacillus and Pseudomonas use nitrate to degrade soluble cBOD to molecular Nitrogen and nitrous oxide.
17. At least two protozoa have been documented to enhance nitrification including peritrich ciliates Epistylis and Vorticella.
18. Predation
    a. Bacteria are consumed by protozoa or metazoa.
    b. Protozoa or metazoa are consumed by rotifers and nematodes.
        i. Greater carbon and energy are lost as heat or waste products for the synthesis of biomass.
        ii. The cumulative weight of each higher life form decreases.
        iii. This process is not always linear, as some substrates, carbon, and energy sources are fed upon by higher and lower trophic level microorganisms, each interdependent by their feeding habits, population, and abiotic factors.

7.1.3 Key Kinetic Factors

1. The rate of reactions is dependent on the concentrations of the reactants as a function of time.
    a. The higher the order of reaction, the faster the reaction rate decrease as a function of time.

            i. Since reactants are consumed faster, the available reactants decrease faster, and therefore the kinetics decrease.

2. Electrons present in initial compounds require additional energy for non-spontaneous reactions to complete the transformation.
3. Activated state:
    a. Additional energy allows the reaction to progress from a high potential energy state, to the result of the reaction, generally at a lower energy state.
    b. Catalyst may either influence the rate of the reaction, or lower the energy required for the reaction to occur.
4. At higher concentrations, more reactions are available, which additionally increases the rate of the reactions, in addition to increasing the likelihood the reactions will occur.
    a. The presence and ratio of molecules with higher kinetic energies, and when combined may provide the energy required to achieve an activation state.
5. Temperature may be the largest, or best contributor to increasing reaction rates, due to increasing the molecular action or activity.
    a. However, temperature generally only defined the average kinetic energy.
    b. There are limitations to benefits of higher temperatures, which are variable based on which reactants are present, their individual and combined responses, speciation, or structure relating to temperature.

6. The bond strength, molecular shape, and availability of electrons contribute to the kinetics, in addition to the surface area of reactants.
7. Key kinetic parameters include:
    a. growth rates, oxygen affinity, ammonia affinity, decay rates, pH, yield coefficients, and temperature.
8. Key kinetics for aerobic ammonia-oxidizing bacteria, Nitrosomonas eutropha, is temperature dependency.
9. Growth and reproduction of Nitrosospira favors lower temperatures than Nitrosomonas europaea and Nitrosomonas eutropha
10. In the Nitritation process, chemolithoautotrophic nitrite-oxidizing bacteria convert nitrite to nitrate, and can benefit from kinetics to temperature variables, that promote greater growth rates than aerobic bacteria.
11. Nitrobacter experience ideal growth rates between a temperature range of 30 degrees to 40 degrees Celsius.
12. Nitrospira at temperatures greater than 35 degrees Celsius.
13. Net specific growth rates, and synthesis are dependent on substrate concentrations.
    a. At lower substrate concentrations, the kinetics have a first-order relationship.
    b. At higher concentrations of substrate, the kinetics have a zero-order relationship.
14. Biomass requires energy for maintenance in addition to growth and reproduction, specifically energy for transport, motility, heat loss, and resynthesis, referred to as endogenous decay.

    a. The rate of endogenous decay is a first order reaction, noting that not all decay is related to cell maintenance, where some is in the conversion to inert biomass.

15. Growth Rates and inhibition.
    a. There are two general types of growth each based on how substrate is utilized.
        i. Anabolism, in which bacteria cells are degraded to produce energy and/or carbon for cellular growth and molecules combine, forming larger molecules and greater sludge.
            1. Anabolism occurs prior to the endogenous phase.
        ii. Catabolism, in which those larger molecules get degrades to smaller ones for energy.
            1. catabolism beings with the onset of the endogenous phase.
    b. Biology have varying reproduction rates:
        i. Organotrophs that can reproduce in 15 minutes.
        ii. Nitrifying bacteria that required as much as 15-days.
    c. Specific to nitrogen removal, nitrifying bacteria oxidize 30 molecules of ammonia ions for each one (1) molecule of carbon dioxide, or 100 molecules of nitrite ions, to oxidize (1) molecule of carbon dioxide.
    d. During the first stage of nitrification where ammonia is oxidized to a hydroxylamine compound, some compounds become oxidized in an irreversible inhibition as part of the catalytic cycle.

e. Aerobic ammonia-oxidizing bacteria can be inhibited by competitive compounds including methane, ethylene, and carbon dioxide.

f. There is non-competitive inhibition possible with the presence of ethane, chloroethane, thiourea, nitrapryrin or disphenyliodonium, and other mechanism-based inhibitions.

g. Inhibitions of nitrite-oxidizing bacteria include limitations regarding the pH of the environment, where an optimal pH is 7.8 to 8.0.

    i. As the pH increases, there is a transition in the equilibrium of the ammonium-ammonia ratio.

    ii. This can result in inhibitions when concentrations from free ammonia reach 33 to 50 and can begin at concentrations less than 10.

    iii. Free ammonia also inhibits aerobic bacteria but begins at concentrations greater than 10.

h. Other inhibitors of nitrite-oxidizing bacteria include toxicity of hydroxylamine beginning at concentrations of 0.42, with irreversible inhibition occurring with concentrations as low as 2.0 to 5.0.

i. Concentrations of free nitrous acid becomes inhibitory at concentrations of 0.2 to 2.8 mg/L, only at pH levels less than 7.5 su.

j. Concentrations of Chlorate greater than 1 mM can completely inhibit nitrite-oxidizing bacteria activity.

16. Unideal pH conditions for Denitrification processes are those below 7.0, or above 8.0 su, at 20 to 30 degrees Celsius.
    a. Including phosphate concentrations starting at 15.5, and nitrous acid at concentrations of 0.13 mg/L.
17. The concentration of dissolved oxygen is a primary inhibitor for the denitrification process, as nitrate is the preferred electron acceptor for denitrification, where oxygen is the preferred electron acceptor for nitrification.
18. The carbon to nitrogen ratio (C:N), of 2:1 must be maintained for stable denitrification.
19. Maximum growth rates:
    a. 0.032 for ammonia oxidizing bacteria.
    b. 0.032 for nitrite oxidizing bacteria.
20. Biomass production of 14.6 mg of Nitrosomonas based on the oxidation of 100 mg of ammonium ions, and 2.0 mg of Nitrobacter.
21. The overall nitrification process of converting 100 mg/L of Ammonia-N to Nitrate-N yields approximately 17 mg/L of nitrifying biomass, with an oxygen requirement of 4.32, based on 3.22 per to oxidize ammonium to nitrite, and another 1.11 required to oxidize nitrite to nitrate.
22. Bacteria that perform some, or all the transformation of nitrate to molecular nitrogen are facultative anaerobes.
    a. In anoxic environments, facultative anaerobes respirate by utilizing nitrate in place of oxygen, and can utilize acetate as the carbon source, in the form of acetic acid, with adequate COD:N ratio.

23. In general, conventional NdN:
    a. 1.2 lbs of oxygen is required for every 1 pound of BOD removal.
    b. 4.6 pounds of oxygen is required to reduce 1 pond of Ammonia-N to Nitrate-N.
24. The greater the loading, the greater the energy requirement.
    a. Aeration can account for more than two-thirds of all energy consumption at a wastewater treatment plant.
    b. There are four main forms of energy available in raw wastewater, including thermal, kinetic, potential, and chemical energy.
    c. The average energy demand in a typical suspended activated sludge wastewater treatment plant, with anaerobic digestion, is approximately 0.6 KWh per 158 gallons of raw wastewater treated.
    d. The chemical energy present in raw wastewater, typically measured as COD, is approximately 12 to 15 MJ/kg COD, and the organic fraction of the chemical energy is as much as 1.93 kWh per 158 gallons of raw wastewater.
        i. Therefore, there is more than twice the amount of chemical energy present, than energy required for typical wastewater treatment processes, if it could be harnessed.

7.1.4 Key Thermodynamic Factors

1. Entropy quantifies the amount of thermal energy, not available for conversion or contribution to work, as measured in thermal energy, per unit of temperature.
    a. This is also referred to as the degree of disorder of a system.
    b. Entropy is how we discuss the number of different possibility of states or conditions, and in the focus of biological processes and chemical reactions.
        i. The total entropy of a system and surroundings always increases for spontaneous processes.
    c. Entropy can be used to identify insights about the arrangement, configurations, and the magnitude of disorder of molecules and compounds in the system.
2. Enthalpy is the energy stored within bonds.
    a. Change in enthalpy is the differences of bond energy between the products and reactants and measured as either heat released or absorbed per reaction.
    b. Enthalpy is part of the accounting of thermodynamics and can describe the amount of heat.
    c. Enthalpy can be used to identify insights about the changes in the bonds, and bonding within reactions, electron charge interactions, hydrogen bonds, and van de Walls forces in the biology and the system.
3. There are two major components of thermodynamics, including heat and work.

    a. Heat is defined and calculated by the temperature and entropy and involves random particle or molecule movement.
    b. Work, mechanical or chemical, is defined by the change in physical place or volume, or in the chemical changes, both involve non-random movement of particles or molecules.
4. Internal Energy is a summation of the energy related to the movement or motion of molecules, including place translation, rotation, or vibration, referred to as the kinetic energy, and the energy related to the electrical energy of atoms, referred to as the potential energy.
    a. Internal Energy may not be able to be measured directly. However, the change in the Internal Energy can be measured directly.
5. Enthalpy is commonly used to eliminate other various work terms and equate energy to heat.
6. Free Energy is the extra energy available for different processes, referred to the Gibbs Free Energy.
    a. The Gibbs Free Energy equation includes both enthalpy, and heat (temperature and entropy).
    b. The change in the free energy is the most common variable used in accounting for energy use through microbiological and chemical reactions associated with wastewater treatment and nitrogen removal.
7. Eucaryotes energy production is generally by aerobic respiration, photosynthesis, alcohol fermentation, or acid fermentation.
8. Procaryotes use those and additional means and pathways for energy production, including the acetyl (CODH) pathway, reverse TCA cycle,

light-driven non-photosynthetic, methanogenesis, anoxygenic photosynthesis, lithotrophy, Anaerobic respiration, and other fermentation pathways.

9. Oxygen reduction potential (ORP)
    a. Oxidation-reduction potential (ORP) is a commonly used measurement which quantifies the intrinsic reduction potential.
        i. ORP is a valuable metric of potential biological reactions that are either oxidation or reduction processes that support cBOD reduction, nitrification, and denitrification.
    b. The greater, or more positive the ORP the more likely reduction will occur.
        i. Higher ORP values indicate higher concentrations of oxygen is present and is typically measured in addition to the measurement of dissolved oxygen.
        ii. Positive ORP values indicate increasing concentrations of dissolved oxygen.
        iii. ORP can provide additional insights to the ecology and quality of the aqueous environment.
            1. This is because ORP is measuring the transfer of electrons, not the absolute concentration, but can theoretically be equivalent.
        iv. Aerobic bacteria activity is higher in parallel with higher positive ORP values.
    c. The more negative the ORP, the less likely reduction will occur, and the more likely oxidation will occur.

        i. Anaerobic bacteria activity is higher in parallel with negative, and lower ORP values.
        ii. Negative ORP values can indicate higher concentrations of cBOD and substrate, required for desired biological reactions.
    d. Facultative anaerobic bacteria that can be active in both ranges of positive and negative ORP values, where oxygen can be used from nitrate and other inorganic compounds.
    e. ORP measurements can be used on combination with other typical parameters monitored including, hydraulic retention time, and pH.

10. The nitrite oxidation rate is linearly interrelated with free energy changes for Nitrosomonas in varying concentrations of ammonia.
11. Thermodynamic modeling results, based on the Monod kinetic model, reveal the rate of ammonia conversion is twice nitrite conversion.
    a. Thermodynamic models can be used to optimize reactor operations to minimize increase nitrite concentrations in the effluent during peak loading events.
    b. Models that do not account for the pH, or the speciation of chemicals, will not be adequate for maximizing the use of thermodynamic techniques to predict growth rates.
    c. Sufficient run time of models and those that consider three dimensional (3D) computational dynamics are required to simulate realistic conditions.
    d. Free energy transfer efficiencies are between 50 and 70 percent efficiency for autotrophic conditions.

12. pH and chemical speciation are key to effectively connecting thermodynamics to the biological modeling and process understanding.
13. A key principle of pH related to microbiological growth is the determinant speciation of compounds utilized as a food and energy source.
14. Ammonia oxidizing bacteria utilize ammonia, but not ammonium.
15. Ammonia-N specification is directly linked to the buffering ability of the wastewater, and how much substrate is available for growth of microbiology.
16. Conversion of ammonia to nitrate by nitrite is the dominant process, and free energy will be negative when energy is released because of the reaction and was positive when energy was required to convert carbon to energy.
17. There are three intermediates in the nitrification process, including nitric oxide, hydroxylamine, and nitrous oxide that each have an impact as an electron donor and acceptor that influenced the efficiency of energy transfer and maximum cell synthesis and growth.
18. The various pathways to nitrous oxide production, through the electron transport process.
    a. When hydroxylamine is the electron donor, and nitric oxide the acceptor, cell synthesis is maximized.
    b. When ammonium is the electron donor, and nitrite was the acceptor, cell synthesis was at the minimum, and noting the valence of nitrogen in nitrous oxide (-3), is lower than nitrite (+3).

    c. It is the conversion of ammonia to nitrite that produced nitrous oxide, and therefore the ammonia oxidizing bacteria responsible for nitrous oxide production, not nitrite oxidizing bacteria.
    d. Nitrous oxide is a byproduct of the oxidation of hydroxylamine to nitric oxide and is an energy generating reaction that benefits ammonia oxidizing bacteria in addition to other sources, and that the dominant pathway was ammonia, to nitrite, to nitrate.
    e. Ammonia oxidizing bacteria portions of the nitrification reactions have net positive energy.
        i. Most of that energy is used for cell maintenance with very small energy used for cell synthesis.
    f. The theoretical yield coefficient for autotrophic production of nitrous oxide ranged from 0.097 to 0.194 for ammonia oxidizing bacteria.

19. Fully utilizing microbial thermodynamics to quantify efficiencies in nitrogen and ammonia removal, have ongoing challenge with the following:
    a. A lack of ability to identify or predict specific uptake rates.
    b. Ability to validate ecological conditions and variability on the actual ATP and ADP efficiencies.
    c. Ability to develop interconnected models that fully accommodate the 3D considerations for the ability and feasibility for microbiology to find substrate, over a sufficient duration and depth to improve the precision of the results.

7.1.5 Key Carbon Factors

1. C:N ratio is critical to supporting nitrogen removal efficiency in biological wastewater.
2. It is critical to understand when supplemental carbon addition may be required.
3. The general relationships of C:N relating to nitrogen removal efficiency are as follows:
    a. C:N > 9 correlates to high efficiency.
    b. C:N 7 to 9 correlates to good efficiency.
    c. C:N 5 to 7 correlates to moderate efficiency.
    d. C:N < 5 correlates to poor efficiency.
4. Side stream nitrogen shortcut configurations tend to be more carbon limited.
5. Mainstream nitrogen shortcut applications may also suffer from limited carbon.
6. Conventional biological wastewater treatment processes generally require a C:N of 6 to 10.
7. Nitrogen shortcut techniques can be successful with C:N ratios as low as 2.9.
8. This is partially due to the following:
    a. Nitrifying bacteria utilizing nitrogenous BOD and alkalinity for a food and energy source for reproduction, instead of carbonaceous BOD required by organotrophic bacteria.
    b. Deammonification shortcut process can provide full nitrogen removal without any carbon.

        i. This introduces another benefit of alkalinity recovery. However, alkalinity recover from ammonia removal is limited to up to half of the required alkalinity.
        ii. Therefore, the available alkalinity may also be a limiting factor, and may need to be supplemented based on influent alkalinity concentrations.

9. Carbon provides an electron donor for energy used in the microbiological growth and process, and success of the supplemental carbon source may be based on the type, yield, and ability for microbiology to utilize that carbon provided electron.
10. Certain supplemental carbon sources have non-treatment related concerns specifically relating to the health and safety of wastewater treatment plant operators.
11. Treatment related concerns can also arise, as documented with glycerol-based products when used with nitrogen shortcut process.
    a. This can lead to a propagation of glycerol acclimated biomass (GAB) that gravitates to conduct denitrification leading to nitrite buildup that is converted back to nitrate prior to full denitrification.

7.1.6 Key Traditional Nitrogen Removal Factors

1. There are two methods in which that microbiology obtains carbon for growth.
    a. Autotrophs obtain the carbon required for growth from non-organic sources including alkaline bicarbonate, and carbon dioxide, like plants.
    b. Heterotrophs require organic sources for carbon, and consume other organic compounds, like animals.

2. Nitrification
    - a. Autotrophic nitrifiers growth is most dependent upon the concentration of available dissolved oxygen and temperature.
        - i. In warmer climates, and warmer temperatures of the wastewater, and in the presences of higher dissolved oxygen concentrations, nitrifier growth rates are higher.
            1. Thereby, achieving sufficient maturity and populations within SRT’s of approximately five to 10 days.
        - ii. In colder climates, and/or lower dissolved oxygen concentrations, nitrifiers have a slower growth rate, resulting in SRT’s greater than approximately 15 to 20 days.
        - iii. The lack of dissolved oxygen in either temperature condition can also completely inactivate nitrifiers.
    - b. Nitrifiers will command the solids retention time.
        - i. When insufficient SRT is provided, nitrifiers will not have adequate time required to mature and develop a population sufficient for nitrification to occur.
    - c. pH is a critical abiotic to promote and sustain adequate populations of nitrifiers, is pH.
        - i. Nitrifiers are most conducive within pH ranges of 6.8 to 7.5 su and can be completely absent in wastewaters outside that pH range.
        - ii. Nitrification process can begin once an adequate population of nitrifiers is present.

        iii. As part of that reaction, the acid produced has the potential to lower the pH in the wastewater, which may cause inhospitable conditions for nitrifiers and other microbiology without sufficient alkalinity present to neutralize the additional acid.
            1. The energy in this reaction becomes available for microbiological growth and reproduction, and the Nitrite-N is then available for the second step in nitrification.
            2. Nitrite oxidizing bacteria (NOB) does the majority of converting the available Nitrite-N (NO2) molecules and available dissolved oxygen to Nitrate-N and energy.
    d. The measure of the success of the nitrification process is generally deemed complete when the remaining Nitrite-N concentrations are less than 0.5 mg/L, and when Nitrate-N and Ammonia-N concentrations are less than 3 mg/L.

3. Denitrification
    a. Denitrification is an additional step required beyond nitrification, to reduce the Nitrate-N to Nitrogen gas.
    b. Denitrification is completed by heterotrophic bacteria, which both consumes BOD and conducts the denitrification reactions.
        i. Heterotrophic bacteria consume BOD as a food source by utilizing dissolved oxygen to oxidize BOD.
            1. However, if dissolved oxygen is not present or available, the denitrifying bacteria will utilize

        other available sources of oxygen, such as Nitrate-N.
    2. Therefore, anoxic environments are required where dissolved oxygen is not present is called anoxic, which is a required condition to promote and maintain the denitrifying bacteria.

- c. Heterotrophic BOD consuming denitrifiers have relatively fast reproduction and population growth rates as compared to autotrophic nitrifiers.
- d. As part of the denitrification reaction, base (OH-) produced has the potential to buffer a portion of the acid (H+) produced during nitrification.

7.1.7 Key Shortcut Nitrogen Removal Factors

1. Nitrite Shunt
    - a. This is a partial nitrification process that prevents the formation of nitrate and converting nitrite directly to nitrogen gas.
    - b. This is completed by inhibiting nitrite oxidizing bacteria and favoring ammonia-oxidizing bacteria.
        - i. Requires variations in the control of these reactions inside the biological wastewater treatment process, focused on providing:
        - ii. Higher temperatures.
        - iii. Shorter solids retention times (SRT).
        - iv. Controlling dissolved oxygen concentrations.
    - c. The main advantage is a reduction of 25 percent of the dissolve oxygen requirement, where 75 percent of the oxygen required

in the aerobic nitrification process is required to convert Ammonium ions to nitrite ions, and the remaining 25 percent of the oxygen demand is to convert nitrite ions to nitrate ions.

    i. Energy savings is in part due to there are less nitrogen species to be oxidized, but also because the denitrification rate is approximately 1.5 to 2 times faster in this shortcut, as compared with the conventional NdN.
    ii. A faster denitrification rate also provides cost savings, as a smaller anoxic reactor is required.

d. A secondary advantage is carbon savings, as 40 percent of the carbon required to convert nitrate ions to nitrite ions during the anoxic heterotrophic process would not be required. The remaining 60 percent of the required carbon is then utilized for the conversion of nitrite ions to nitrogen gas and nitrous oxides.
e. Key factors to be considered with the Nitrite Shunt process are bacteria growth rates, temperature, SRT, free ammonia, pH, and dissolved oxygen.
f. Wastewaters with higher ammonia concentrations, and higher temperatures are well fit for Nitrite Shunt process.
g. Aerobic ammonia-oxidizing bacteria grow faster at temperatures higher than 59 degrees Fahrenheit (15o C) than nitrite-oxidizing bacteria, and the accumulation of sufficient oxidizing-bacteria, with the inhibition of nitrite-oxidizing bacteria.
h. Dissolved oxygen concentration control does not directly inhibit nitrite-oxidizing bacteria. However, the oxygen saturation

coefficients between those two types of bacteria are different, where aerobic ammonia-oxidizing bacteria have a coefficient between 0.74 and 0.99 mg/L, and nitrite-oxidizing bacteria have a coefficient between 1.4 to 1.75 mg/L.

- i. This means that in reactors where the dissolved oxygen concentrations are maintained under 1.5 mg/L, aerobic ammonia-oxidizing bacteria will grow faster, but may require greater SRT.
- j. pH control for chemical equilibrium.
  - i. Higher pH favors the ammonia species of ammonia-N, which is the preferred substrate for aerobic ammonia-oxidizing bacteria.
  - ii. Control of pH, and the potential addition of acidic or caustic chemical may be required to promote an environment for the Nitrite Shunt process.
  - iii. Ideal pH range for optimal oxygen utilization in this shortcut process is between 6.5 and 8.0 su, and generally ai to control pH at 7.0 su or higher.
- k. Other inhibitions.
  - i. Limitation of available free ammonia can inhibit nitrite-oxidizing bacteria at ranges below 1.0 and at 3.0 mg/L.
  - ii. Aerobic ammonia-oxidizing bacteria become inhibited in free ammonia concentrations between 10 to 150 mg/L.
  - iii. The presence of free nitrous acid can be more inhibitive to nitrite-oxidizing bacteria, especially during start-up.

l. Controlling the pH, temperature, dissolved oxygen, and SRT leads to nitrite accumulation and higher free nitrous acid concentrations supporting a stabilized Nitrite Shunt process.
m. This process is best fit for wastewaters with low carbon to nitrogen (C:N) concentrations, and higher temperatures, and typically implemented as a side stream process, such as for the treatment of anaerobic digestion, thickening, and dewatering supernatant.
n. Target applications for Nitrite Shunt processes are those that are high in ammonium and ammonia concentrations, such as:
    i. Scalping WRF's that discharge waste streams back to the collection system for downstream treatment and disposal.
    ii. Anaerobically treated sludge centrate.
    iii. High strength industrial discharges.
    iv. Sludge composting, co-digestion, thermal drying, hydrothermal oxidation, and thermal hydrolysis waste streams.
o. Operational considerations.
    i. In the presence of sufficient carbon, the potential carbon savings may be offset from additional demand to oxidize the carbon.
    ii. Less aeration may not be able to sustain adequate dissolved oxygen concentration, or the required mixing.

iii. Potential savings of 25 percent aeration, 40 percent carbon savings, 40 percent less sludge production and 20 percent lower carbon dioxide emissions
    1. However, as the overall treatment type and configurations increase is complexity, or magnitude of reliance on a fixed film process, the savings will decrease.

iv. Average 50 percent savings as compared with a MBR process, up to 80 percent savings as compared with stripping technologies.

v. Potential for mainstream implementation.
    1. However, there are inherent conditions in mainstream effluent, including lower nitrogen concentrations and lower temperatures, which are not ideal for the selection nitrite-oxidizing bacteria.
    2. Key factors to improve effectiveness for mainstream treatment are:
        a. Controlling dissolved oxygen concentrations, residual ammonia, transient anoxia, and SRT.

vi. The opportunity to promote the growth and dominance of nitrite-oxidizing bacteria, at lower dissolved oxygen concentrations, in the presence of sufficient organic carbon is theoretically feasible for mainstream applications.

        vii. Free ammonia concentrations that are too low for inhibition of nitrite-oxidizing bacteria can be addressed by alternating aerobic, anoxic cycles to maintain residual ammonium ion concentrations.
        viii. Transient anoxia is a measured control approach for aerobic SRT, which introduces a process time lag for nitrite-oxidizing bacteria, between the anoxic to aerobic conditions.
            1. Potentially effective for high-strength wastewaters, and low strength mainstream plug-flow type processes.
        ix. SRT control is paramount.
            1. Effectiveness managed with a relatively low SRT, such that aerobic ammonia-oxidizing bacteria do not get washed out, but nitrite-oxidizing bacteria can get washed out.
                a. Oxidation of ammonium ions is maximized based on the influent quality and C:N ratios by optimizing the SRT.
        x. Effluent will contain nitrite, which can be a higher chemical demand when chlorine is used for disinfection.
        xi. Residual ammonium ions anticipated to be present from maintaining the nitrite-oxidizing bacteria reactions, are conducive for chloramination disinfection, and would reduce chlorine demand of nitrite.

2. SHARON® Shunt Process

a. Single reactor system, aerobic environment.
b. External carbon source typically required.
c. Uses the pH of the wastewater to control the process.
d. No sludge retention.
e. High temperatures and a short retention time.
    i. Promotes nitrite oxidizers to be washed out.
    ii. Formation of nitrite is isolated which equates to a savings of up to 25% in oxygen requirement.
f. Nitrate can also be removed but in an anoxic environment and successfully denitrified.
    i. Required 40% more carbon than for conversion of nitrite.
g. Nitrification process creates an acid, and the ammonium ion oxidation process stops when the pH of the wastewater stream being treated approaches 6.5 su.
    i. At that point the ammonium ion begins to oxidize and there will be insufficient free ammonium ions in the process for the growth of the Nitrosomonas.
    ii. Based on actual implementation, the need for the addition of a base is required to provide a stable range.
h. As a single reactor process, wasting of WAS is required.
    i. The time to select and promote the growth of aerobic ammonia-oxidizing bacteria is longer.
        1. Therefore, recovering that bacteria from WAS is required, and typically configured as a RAS stream.

        i. Does not require equalization of side streams, as solids are not retained, but can be recycled, as part of the process.

3. ANITA^{TM} Shunt Process
    a. Typically implemented into conventional activated sludge and SBR's.
    b. Inhibits the transformation of NO_2 to NO_3 in the nitrification process, while reducing the quantity of sludge produced.
    c. Effective for effluents with ammonium ion concentration greater than 100 mg/L.
    d. Energy savings of 25% due to lower oxygen requirements, and a 40% reduction in chemical addition costs.
    e. Equalizes nitrogen and side stream influent, as part of the SBR process.
    f. There are a variety of projected savings regarding long-term operations and maintenance expenditures, including less energy for aeration, faster denitrification reaction rates, and smaller footprints for anoxic reactors. However, there are other factors that can prevent the full actualization of theoretical projected savings in the nitrite shunt process.
4. Deammonification
    a. A series of reactions to oxidize nitrite to nitrate, and then to nitrogen gas, after nitrification.
    b. Deammonification is a direct, or shortcut, reaction where ammonium ions are converted to nitrite and directly to nitrogen gas.

c. Process is facilitated by aerobic ammonia oxidizing bacteria, which are selectable by controlling key process parameters of:
    i. Ammonia load, dissolved oxygen, pH, SRT and temperature.
    ii. Deammonification reaction continues for anaerobic ammonia oxidation, facilitated by chemolithoautotrophic anaerobic ammonia oxidizing bacteria, by utilizing nitrite.

d. Potential savings of 60 percent of traditional NdN oxygen demand, 40 percent less sludge production and up to 100 percent less organic electron donor.

e. Target wastewater characteristics are:
    i. Lower COD to total ammonia-N ratio.
    ii. High ammonia-N concentration
    iii. Low fluctuation in concentrations.

f. Deammonification demonstrated in side stream applications, with less dominance in mainstream.

g. Successful with average efficiencies of 75 percent total inorganic nitrogen removal and 80 percent ammonia reduction.

h. Such side stream application beneficial when COD: NH_4^+ ratio is 2:1 or less.

i. Deammonification started as a single-reactor techniques, and later a two-stage process was implemented.

j. ammonia oxidizing bacteria is the rate-limiting step for both Single and two stage deammonification reactors.

k. Mainstream application is limited by:

        i. Optimal operational control regarding competitive conditions.
        ii. Strategies between anaerobic ammonium-oxidizing bacteria and nitrite oxidizing bacteria relating to promoting and maintain sufficient populations of autotrophic and organotrophic bacteria diversity as part of the same overall reaction.

5. Simultaneous Nitrification - Denitrification (SNdN)
    a. Key factors include:
        i. Bulk liquid oxygen concentration. Conditions may vary throughout the reactor and may not be easily controlled or predicted.
        ii. Availability of organic carbon, and part of the balance with the bulk liquid oxygen concentration, which also needs to be sufficient to support nitrification reactions.
        iii. Microenvironments, where gradients of dissolved oxygens concentrations may have preferential pathways for diffusion, variable fluid dynamics related to mixing and suspension.
            1. The environment will be nonhomogeneous and complete SNdN may not fully occur at all point, and the rate of those reactions will vary.
    b. Reaction rates in the SNdN process are typically less than optimal for nitrification and denitrification, as the available biomass and substrate is shared between those reactions.

    i. Denitrification rates are lower as substate consumption in aerobic portions of the floc are lower.
    ii. Nitrification rates are lower due to targeted lower dissolved oxygen concentrations, either to balance the denitrification reaction, or in swing zone or conditions between aerobic and anoxic.
    iii. This is overcome in SNdN by providing longer HRT's.
    iv. Wastewater treatment processes that are best suited to accommodate longer HRT's and provide higher volumes, are oxidation ditches and SBR's.

c. The rates that both reactions will occur are a function of the floc density, size, structure, n kinetics, COD loading, SRT, and the dissolved oxygen concentration in the bulk liquid.
d. Nitrification SRT is inversely proportional to the specific growth rate and based on ammonia-N concentration, in comparison with the dissolved oxygen concentration.
e. Nitrate reduction rate in an anoxic reactor or floc, is inversely proportional to the specific growth rate and based on COD concentrations for denitrification.
f. Lower dissolved oxygen concentration provides more efficient denitrification, with longer SRT's.
g. SNdN is considered a shortcut technique as both reactions are occurring simultaneously but not all the benefits of the other shortcut techniques may not be fully actualized due to the need for longer SRT and greater volumes to achieve desired nitrogen removal efficiencies.

h. SNdN reactions require a range of influent COD to nitrogen (COD:N) ratio of six to 10, and bulk liquid dissolved oxygen concentrations within the reactor of 0.3 to 0.7 mg/L
i. SNdN Nitrite Shunt process has an advantage, where less COD is required. Wastewater treatment facilities that are deficient in COD are a good fit.

7.2 Decision Matrices

The summary of core factors listed above were distilled into decision matrices for traditional and nitrogen shortcut technique implementation, based on influent raw wastewater characteristics, biotic and abiotic factors regarding the ecology, microbiology, and operational considerations.

The application of the matrices below are meant to be used as a summary tool by academia and practitioners in conceptual and preliminary design, or optimization of wastewater treatment processes that require nitrogen removal, to help guide decisions regarding when, and how to implement shortcut nitrogen removal techniques, which factors are critical and are to be further evaluated during and after conceptual designs.

These matrices and recommendations are meant to promote the ease of a more widely adapted approach, of including shortcut nitrogen removal techniques in alternatives evaluation for all applications of greenfield, retrofit, expansion, or optimization of wastewater treatment plants that require nitrogen removal. The following matrices and recommendations are not all encompassing, but can provide quick guidance for high level consideration, and to validate consideration of including different wastewater treatment nitrogen removal techniques, as one begins to plan the approach for evaluating the best fit alternatives, considering effectiveness, initial capital investment, and the long-term cost to operate and maintain.

7.2.1 Existing Wastewater Treatment Facility Considerations

Understanding the ecology is key to identifying and creating niches to promote and sustain the right microbiology, conditions, and remove targeted pollutants. When evaluating existing wastewater treatment facilities, for optimization or expansion, it is recommended that one first understand the current ecology as follows:

1. Define the community structure.
2. Define the potential community function.
3. Define the observed community function.
4. Define the abiotic and biotic factors, and interactions between microbiology and the environment.

The results of the questions above should be used in conjunction with the matrices presented below.

7.2.2 Influent Wastewater Characteristics

Focusing on the predicted or actual influent wastewater characteristics can help guide decisions regarding the feasibility of implementing shortcut nitrogen removal techniques, and identify key design criteria to be evaluated, as graphically depicted in Figure 42 below.

There are many influent wastewater characteristics that influent decisions about wastewater treatment processes. However, the key factors, as identified above were temperature, C:N, loading concentrations (BOD and Ammonium). Based on those key factors, a series of matrices were prepared and depicted in Figure 42 below for quick guidance on decisions.

As depicted in Figure 42, C:N ratios can be as low as 2.9 for Nitrite Shunt implementation, and where Deammonification does not require any carbon.

Additionally, the higher the temperature, the more effective Nitrite Shunt and Deammonification can be as compared with traditional NdN.

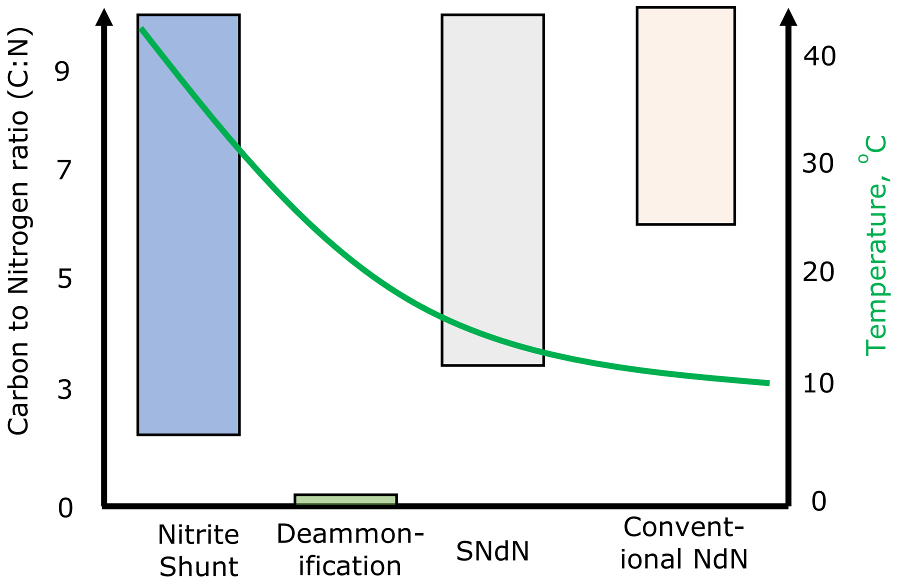

Figure 42 C:N and Temperature Matrix

The next key factors to consider with making decisions about what type of traditional, or shortcut nitrogen removal techniques focuses on the loading, of both BOD, and Ammonia-N. A quick guide to understanding the best fit for consideration of those alternative is graphically depicted in Figure 43 and Figure 44 below.

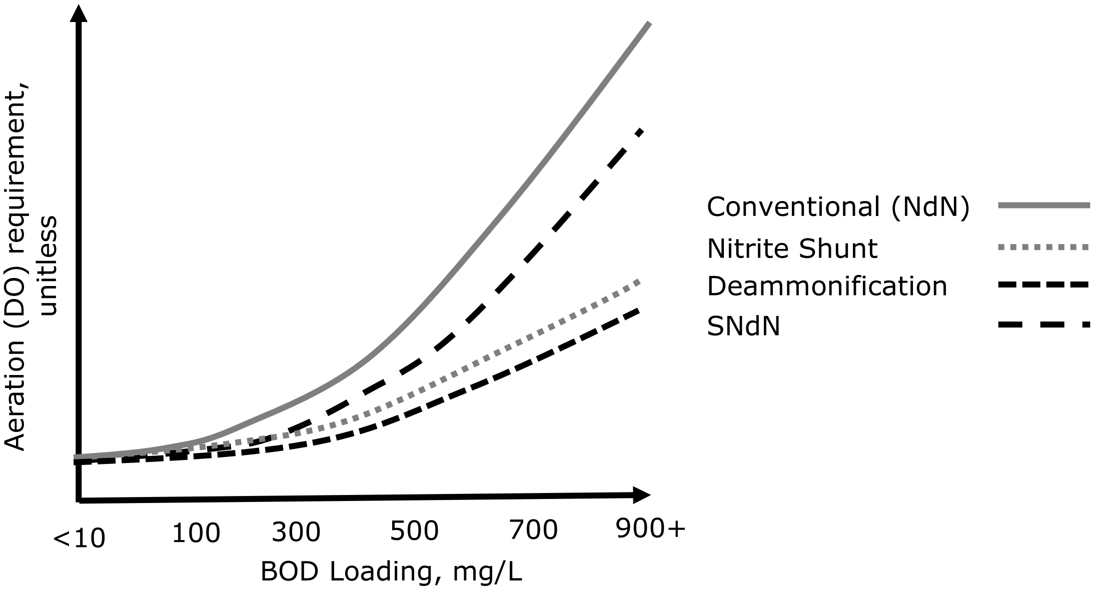

Figure 43 Ammonia-N Loading, Aeration Requirements

As depicted in Figure 43 above, there is an anticipated 20 percent to 25 percent savings in Nitrite Shunt and Deammonification processes as compared with traditional NdN, and SNdN can return a savings of 10 percent or greater.

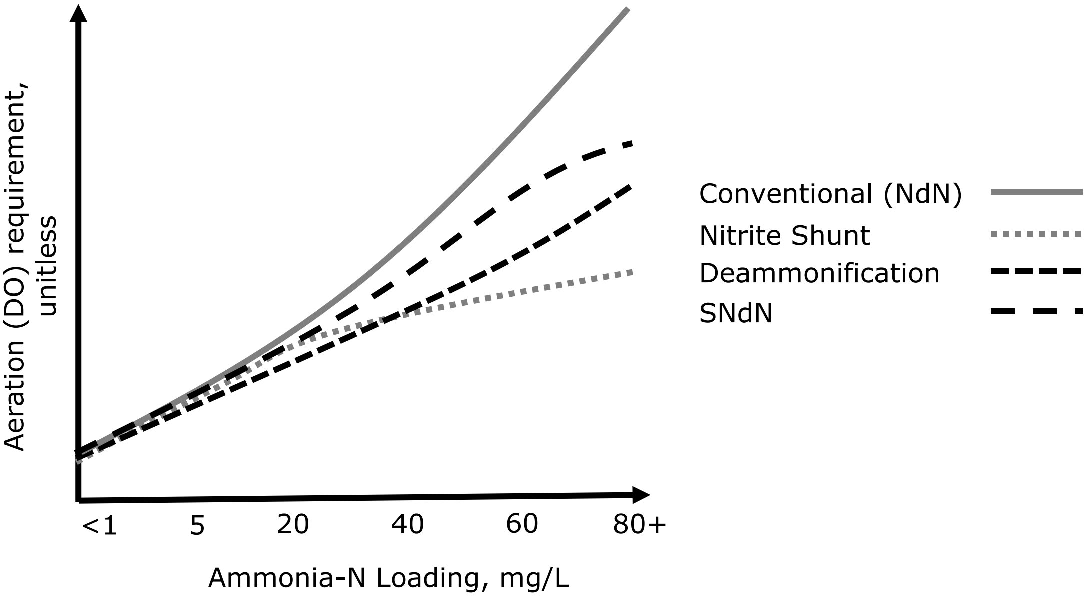

Figure 44 Ammonia-N Loading, Aeration Requirement by Nitrogen Removal Technique Matrix

As depicted in Figure 44 above, there is similar savings, in the aeration required for nitrogen removal.

<u>7.2.3 Operational control considerations</u>

Understand the boundaries of key operational parameters help define the design criteria for early evaluations, and include SRT, and dissolved oxygen. Control of dissolved oxygen concentrations within and across wastewater treatment unit processes, vary based on the desired reaction, treatment process type, configuration, and influent wastewater loading characteristics, as depicted in Figure 45 below.

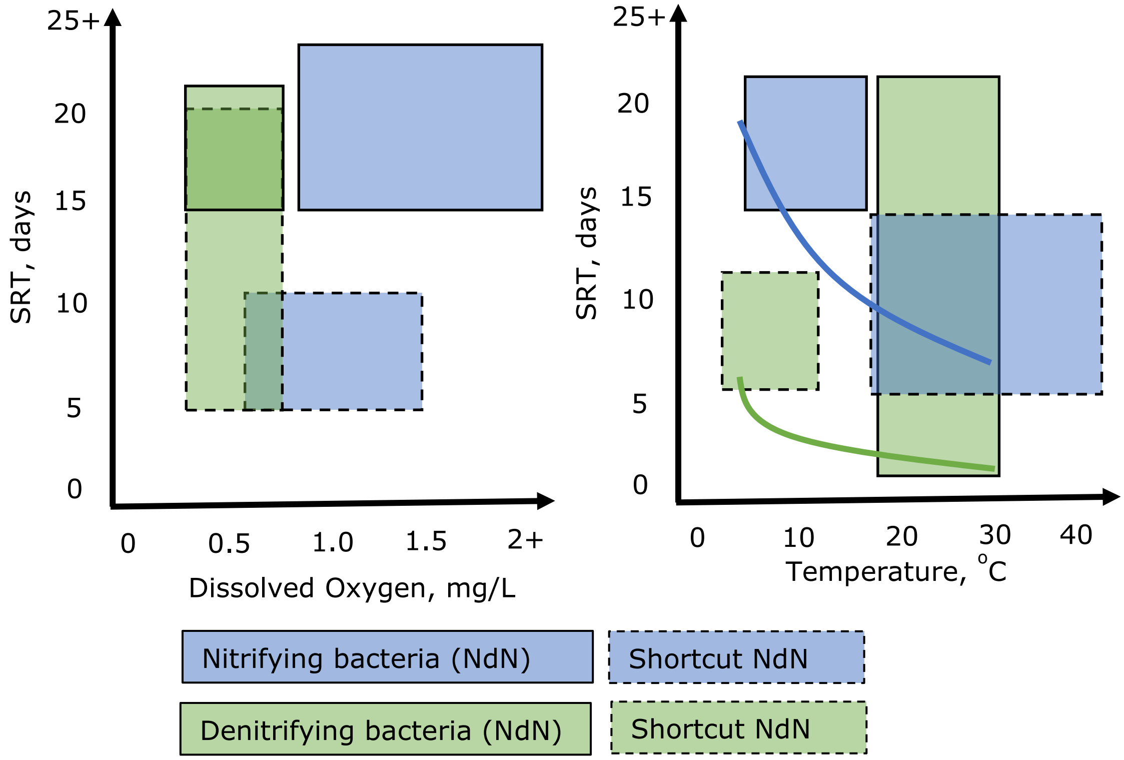

Figure 45 SRT and DO Control Matrix

As depicted in the Figure 45 above, shortcut nitrogen removal techniques can be accomplished with precise control of DO, in ranges from 0.3 to <1.5 mg/L and will need to vary based on the process selected, and during different stages in the process. A strategy to management DO concentrations, and the effectiveness of the nitrogen removal process, is to combine DO monitoring and control, and ORP monitoring, as the ORP value can provide additional insights, and more accurately define the success of the treatment strategy. Recommended ORP values, based on the nitrogen removal step, or shortcut technology implemented in depicted in Figure 46 below.

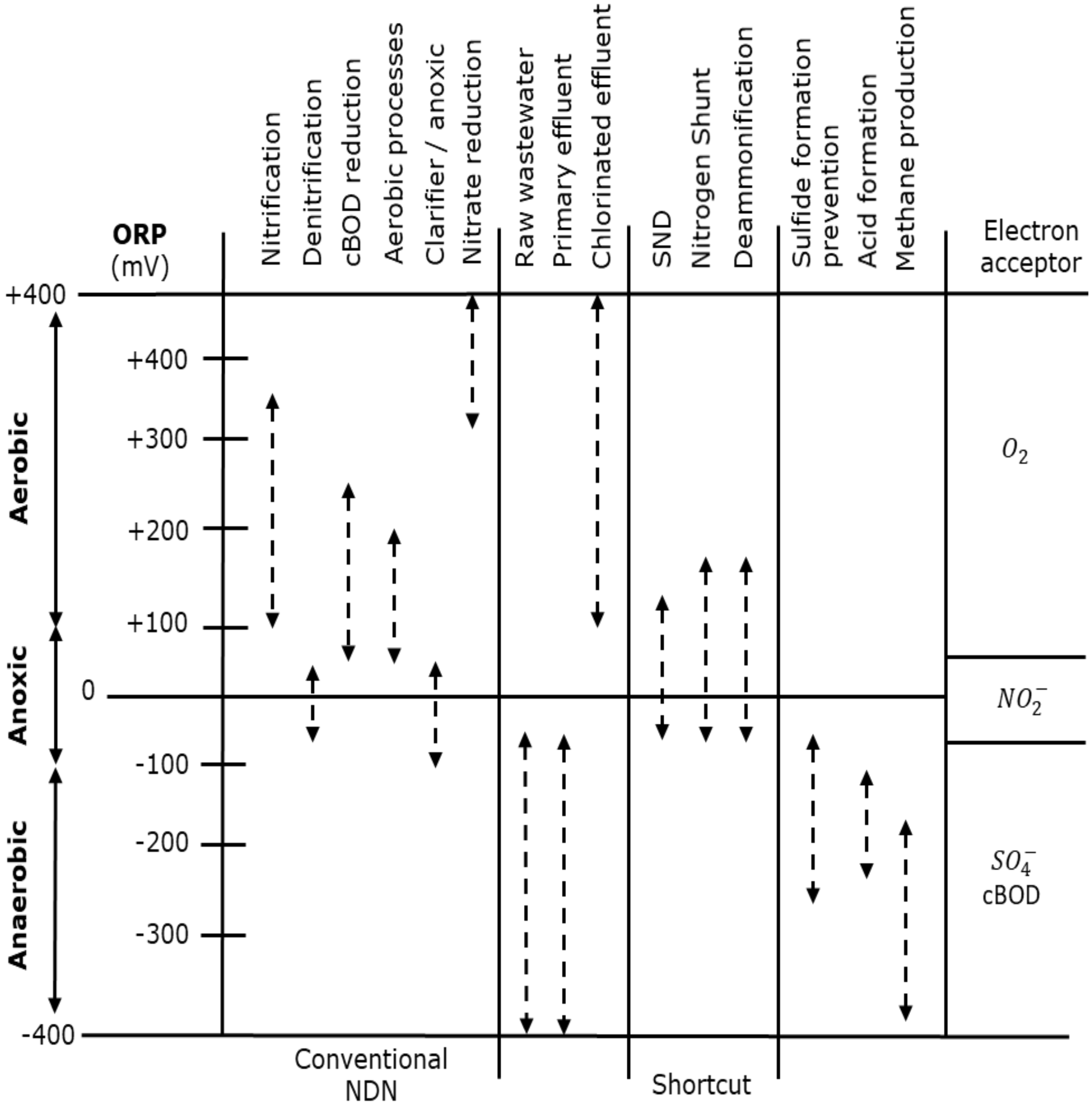

Figure 46 ORP Values in Wastewater Treatment (Trygar, 2011)

Awareness of potential or actual inhibition of other compound concentrations should be considered during design and modeling, as the accumulation of certain reaction byproducts can partially inhibit the growth and reproduction of the desired nitrifying and denitrifying bacteria, or even lead to irreversible inhibition, as graphically depicted in Figure 47 below.

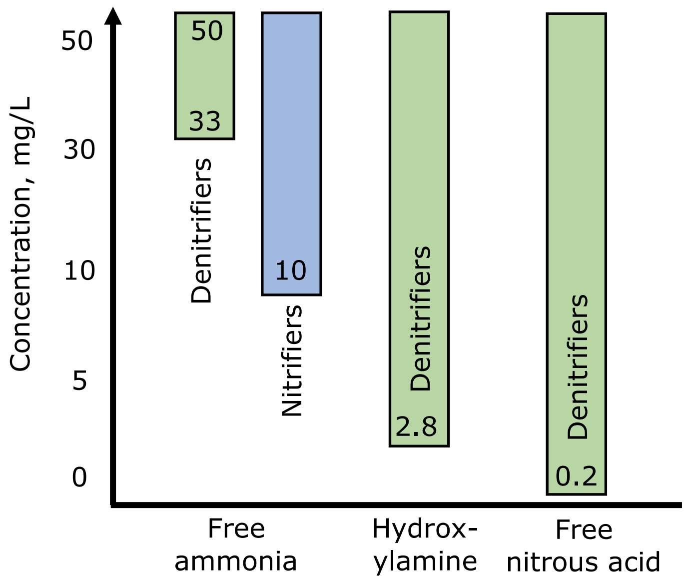

Figure 47 Other Compound Inhibitions

These decision matrices are intended for use as a general guideline to focus decision makers considerations on a short list of which techniques to consider and compare with traditional biological wastewater treatment techniques, and to identify, at a conceptual level, which parameters will be key for later conceptual analysis and preliminary design or optimization. Each application of biological wastewater treatment is unique, and therefore the direction provided in the matrices above may not fit all considerations.

7.3 Shortcut versus Traditional NdN Comparison

Existing shortcut nitrogen removal techniques were identified and discussed that documented reduced energy and carbon demands relating to potential long-term costs to operate and maintain biological wastewater treatment processes, and in performance efficiency with the benefit of nutrient removal. However, the summation and comparison of projected initial capital expenditures, along with the long-term operations and maintenance costs related with nitrogen shortcut techniques versus traditional nitrification-denitrification biological wastewater treatment processes has not been previously quantified extensively. This section is focused on the development of wastewater treatment models, footprint, and cost models for the following nitrogen removal and wastewater treatment scenarios, which are then compared with the decision matrices presented above.

7.3.1 Influent Raw Wastewater Characteristic Considerations

Recent trends of municipal and industrial wastewater influent loading have generally revealed increasing concentrations of key regulated constituents for treatment, including BOD, TSS, nitrogen compounds, and others, without parallel increased in the quantity of influent flow. This change may be in part due to downstream impacts from successes in water conservation efforts in communities, whereby, less water is discharged, but equal loading of wastes is discharged for wastewater treatment. This change could also be due in part to an increase in the magnitude of wastes that are discharge throughout communities. An example of such increase is depicted in the figures below, where the parameters used at the time of design 20 years ago or more, are different than the actual loading conditions observed at the present. The data presented below represents an existing 1.0 MGD capacity water reclamation facility, serving typical municipal wastewater treatment needs, which includes approximately 60 percent of the flow from domestic discharges, 5 percent from institutions, and the remaining 35 percent from retail, commercial and industrial dischargers. The facility was commissioned in 2001 and is currently experience a flow of approximately 40 percent of design capacity.

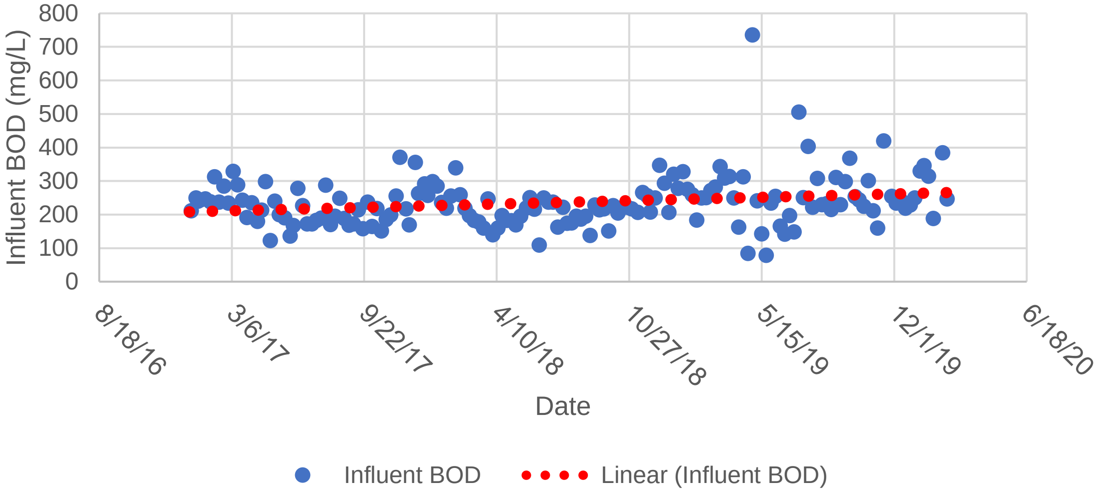

Figure 48 Influent Loading Variation, BOD

As depicted in Figure 48 above, the average influent BOD concentrations are generally within the design criteria of 250 mg/L. However, the actual range of peak and minimum concentrations have been observed the be at or below 100 mg/L, and over 300 mg/L, with a projection of increasing concentration.

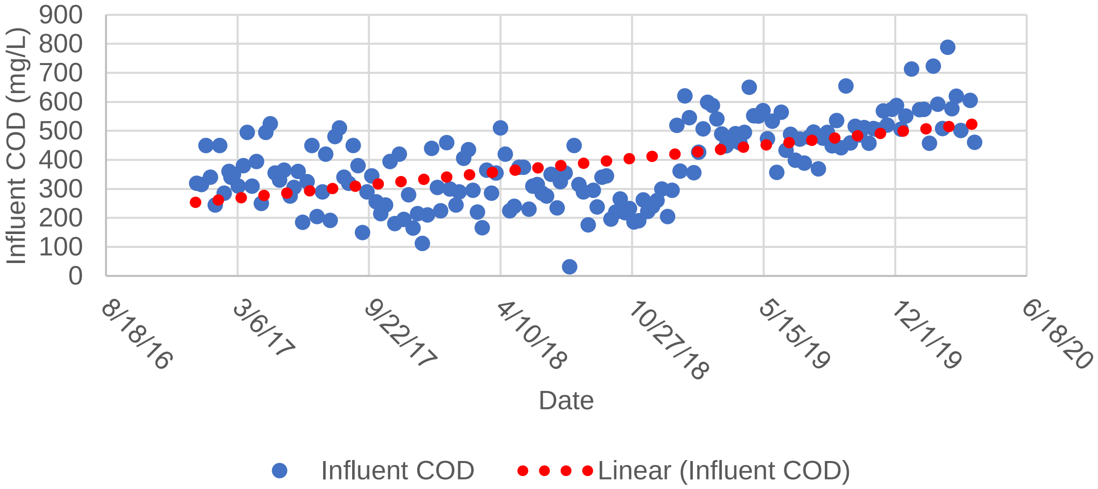

Figure 49 Influent Loading Variation, COD

As depicted in Figure 49 above, the average influent COD concentrations, both peak, average, and minimum concentrations, have surpassed the design criteria of 400 mg/L and represent a clear projection of increasing concentration.

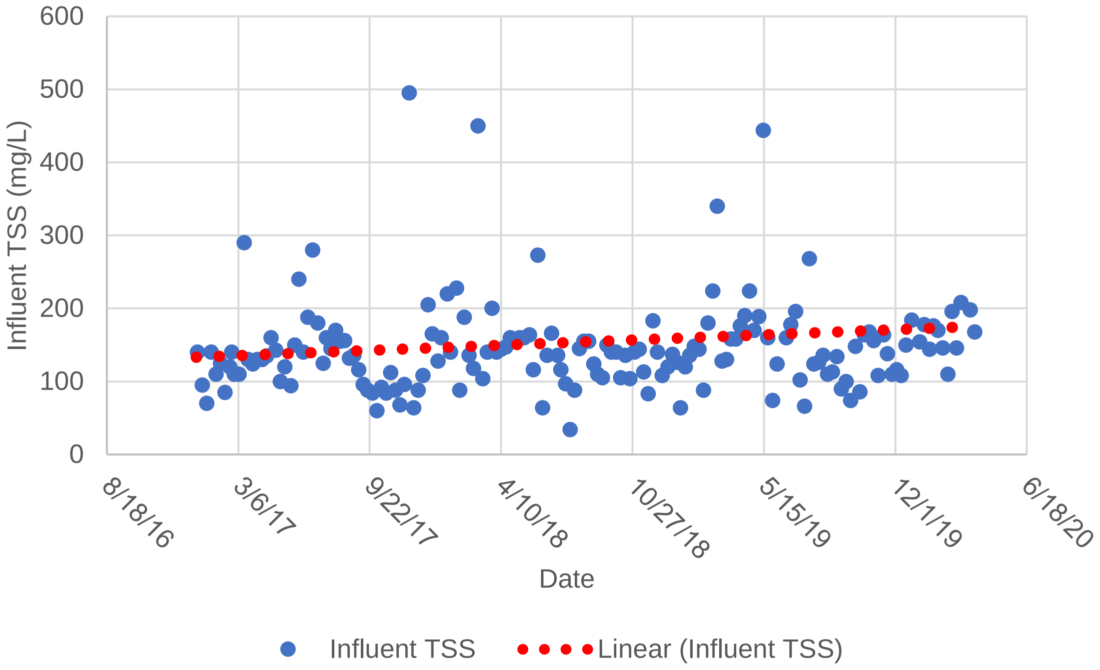

Figure 50 Influent Loading Variation, TSS

As depicted in the figure above, the average influent TSS concentrations are generally within the design criteria of 250 mg/L. However, the actual range of peak and minimum concentrations have been observed the be at or below 100 mg/L, and over 300 mg/L, with a projection of increasing concentrations towards the initial design criteria.

When just those three constituents are considered in whole, this reveals that waste characteristics of the biochemical and carbonaceous oxygen demands are increasing disproportionally to the flow increase and will have non-parallel increases in the TSS concentrations. This means the available substrate for food and energy of the microbiological communities, along with other key factors are varying beyond what was designed to meet and sustain performance and compliance.

This demonstrates that we need to rethink the theoretical values used as a baseline for the design of biological wastewater treatment processes, and account for more variability in future loading conditions in the presence of reduce flow and/or increased loading.

In the presence of increasing and variable discharges of nitrogen wastes collected for wastewater treatment, traditional NdN mainstream process can become challenging to achieve and sustain effluent discharge compliance requirements. Therefore, in the model scenarios presented below, a range of loading conditions is used to accommodate known current conditions, and a predicted range of future conditions, where the loading may increase or decrease, which will provide more resiliency in the success of those treatment systems but will introduce greater costs that will need to be offset by multiple points of optimization.

7.3.2 Wastewater treatment model Scenarios

Modeling is all about boundaries, where those that can accommodate minimum and maximum conditions, can generally accommodate average and other conditions between those boundaries. Therefore, four main model scenarios were utilized for analysis, which considered uniform flow, with variable loading defined as sub-scenarios “A” and “B”, based on different wastewater temperatures, and sub-scenarios “C” and “D”, based on high and low loading conditions.

For simplicity in evaluation, only one set of wastewater treatment effluent goals were established, aligned with actual local, regional, and national effluent quality goals, and as based up the location of the of the water reclamation facility from which the example data was obtained.

Influent Criteria	A – Low Temp	B- High Temp	C – high loading	D- low loading
Flow, MGD	1.0	1.0	1.0	1.0
BOD, mg/L	250 – 700	250 – 700	700	100
COD, mg/L	125 – 1,400	125 – 1,400	1,400	125
BOD/COD ratio	0.5 – 0.8	0.5 – 0.8	~0.5	~0.8
BOD equivalent of VSS, lb BOD/lb VSS	1.42 – 3.85	1.42 – 3.85	3.85	1.42
TSS, mg/L	100 – 400	100 – 400	400	100
TKN, mg/L	50 – 100	50 – 100	100	50
Total Phosphate, mg/L	0 – 6	0 – 6	6	0
Temperature, degrees F	59	30	30 – 59	30 – 59
Alkalinity, mg/L as $CaCO_3$	100 – 400	100 – 400	400	100
pH, su	6.8 – 7.8	6.8 – 7.8	7.8	6.8

Table 9 Model Influent Criteria Scenarios

Effluent Parameters	Criteria
BOD, mg/L	< 5
Turbidity, NTU	< 2
Total Nitrogen, mg/L	<10
Nitrate, mg/L	< 5
pH, su	6.8 – 7.8
Fecal Coliform, CFU	Non-detect 4out of 7 days, 23 max. single day

Table 10 Model Effluent Goals

In addition to the influent and effluent criteria presented above, a series of process configuration scenarios were developed to quantify and compare the difference between traditional NdN processes with nitrogen shortcut techniques. These process configuration scenarios are aligned with those presented in the decision matrices above and were develop on the following uniform assumptions applied to each scenario:

- Influent pumping is required.
- Tertiary filters are required to meet turbidity goals, when MBR is not used.
- Ultra-violet (UV) light is used for disinfection, no chemical disinfection is included.
- Effluent pump station is required to disposal and/or reuse of effluent.
- Aerobic digestion of solids is included.
- Solids handling is included for dewatering.
- Solids disposal is assumed to be hauled to a landfill, after dewatering.
- Standby power generation is required and assumed to be a diesel-powered generator.
- No other ancillary support systems are included, such as general site improvements beyond the treatment and conveyance processes, building, access, security, landscaping, etcetera.
- Hard costs for construction overhead, administration, mobilization, profit, and taxes are based on defined percentages of the hard construction subtotal.
- Soft cost relating to planning, engineering, regulatory approvals, and contractor procurement are based on defined percentages of the hard construction subtotal.

	Scenarios	A	B	C
1	Oxidation ditch (OD); Single reactor	Traditional NdN	Side stream Nitrite Shunt	Simultaneous nitrification-denitrification (SND)
2	Sequence batch reactor (SBR); Single reactor	Traditional NdN	Side stream Nitrite Shunt	Mainstream Anammox/ biocatalyst
3	Modified Ludzack-Ettinger (MLE); Separate reactor	Traditional NdN	Side stream Nitrite Shunt	Mainstream Anammox/ biocatalyst

Table 11 Wastewater Treatment Process Configuration Scenarios

Utilizing the influent and effluent criteria presented above, a series of combined hydraulic, chemical, and biological wastewater treatment models were developed utilizing the BioWin Process Simulator® software, version 6.2.2.2403, by EnviroSim Associates Ltd. 2010 to 2020. The configuration of each of the process configuration scenarios are depicted in the figures below.

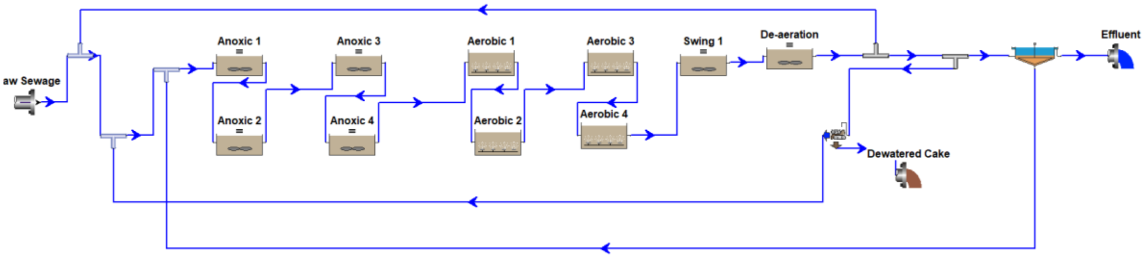

Figure 51 Scenario 1 – Oxidation Ditch (OD)

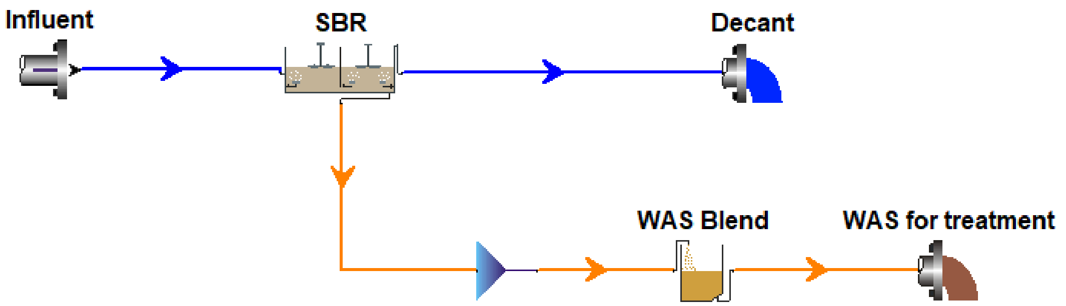

Figure 52 Scenario 2A – Sequence Batch Reactor (SBR)

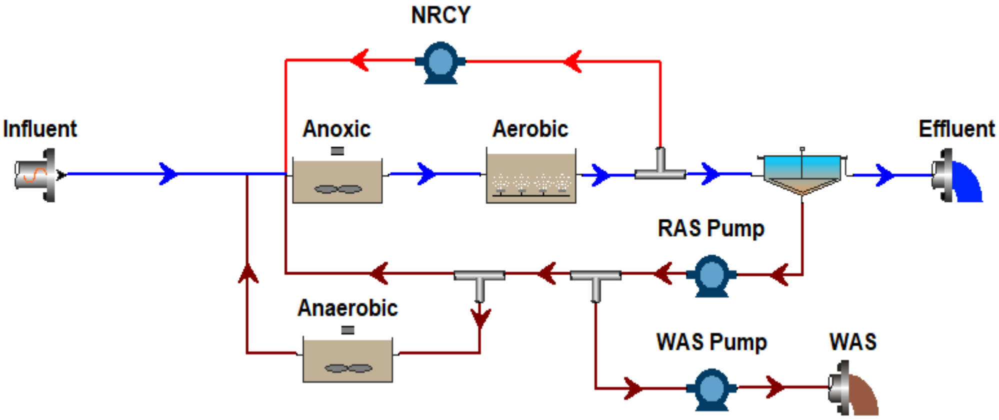

Figure 53 Scenario 3A – Modified Ludzack-Ettinger (MLE)

The results from those analysis, meeting the target effluent goals, and based on the varying influent wastewater characteristics, identified the required size of basins and consumables (energy, carbon, alkalinity) for each alternative, which was then used to complete conceptual budgetary estimates and projections. The outcome of that modeling effort, and subsequent cost estimating are summarized in the conclusions chapter.

CHAPTER 8

PRACTITIONER EXAMPLES

8.1 Utilizing Fundamentals in Understanding Process Deficiencies

Experiences where the fundamentals of microbial selection, and comprehension of the dominant factors that promote nitrogen removal techniques, applied as a practitioner, can provide context for the ability to optimize wastewater treatment processes. Two examples from the experience of the author, summarize different deficient conditions, and the approaches used to evaluate and rectify each, along with a summary of the outcome of those efforts.

8.1.1 Decentralized Remote Commercial WWTP Optimization

An SBR type process was implemented to provide wastewater treatment services for a remote community, with more than 90 percent of flow and loading from commercial, restaurant, and public restroom facilities, with the remaining 10 percent from a transient-community residence. An example of the nitrogen loading at the influent, effluent, and within the SBR is depicted in Figure 54 below.

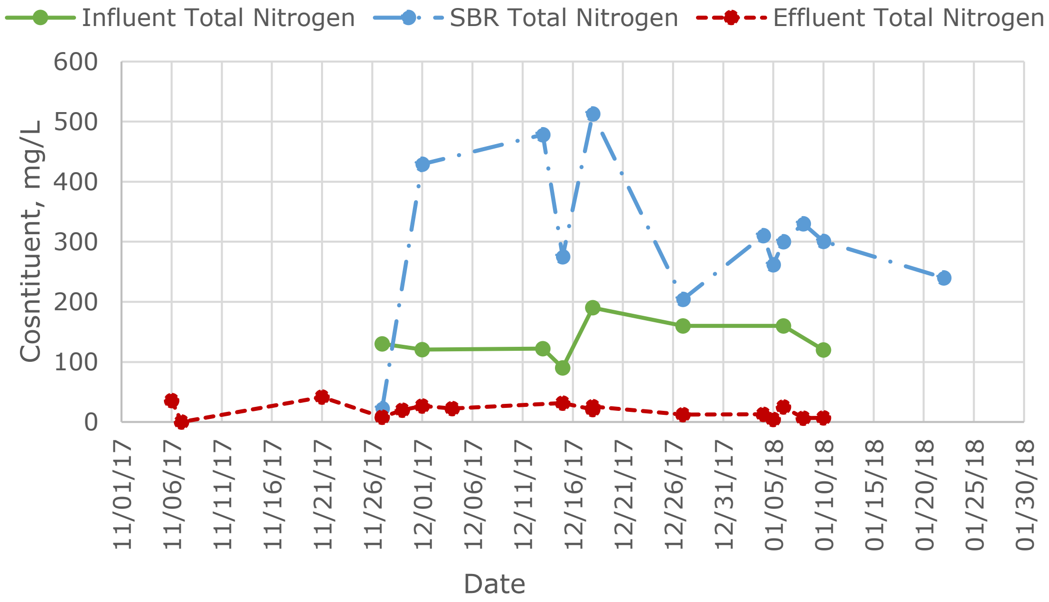

Figure 54 Example 1 – Total Nitrogen Loading

As depicted in Figure 54 above, the total nitrogen in the influent wastewater was consistently greater than 100 mg/L, and total nitrogen concentrations within the SBR biological reactor were generally between 200 mg/L and 500 mg/L. Average daily flow to this facility was less than 100,000 gallons per day (gpd). Regulatory and process goals included BOD and TSS reduction, with set limits for effluent turbidity, total nitrogen, and a minimum chlorine residual, prior to discharge for aquifer recharge. After numerous years of inconsistent compliance, various mechanical improvements and operational control changes, the decision and commitment were made to get to the root of the problem, which included several main elements that contributed to the lack of ability to complete the nitrification and denitrification processes.

First, through microscopy efforts, it was determined there was no visual confirmation of adequate living microbiology, and a lack of higher order life microbiology such as protozoa or rotifers. The results found only filamentous material. An images from that initial microscopy effort, representative of each sample taken from within the SBR aeration, anoxic and decant basis is depicted in Figure 55 below.

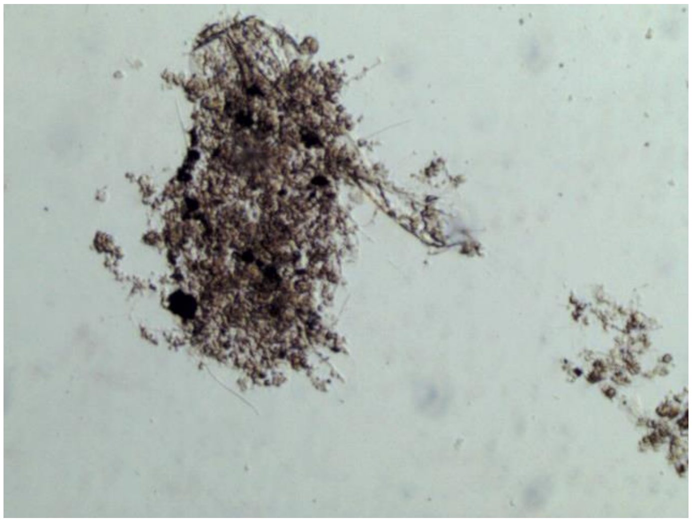

Figure 55 Example of Existing Conditions

This was an initial indicator that either there were insufficient bacteria or other carbon and energy source to feed higher order organisms, and/or there was insufficient hydraulic or solids retention time to propagate desired biology, and/or there were other ecological and abiotic factors such as temperature or pH, that were inhibiting the ability to sustain the desired and required microbiology to perform all the required wastewater treatment processes.

Second, through sampling and laboratory analysis efforts, the concentrations of key parameters in the raw influent wastewater were able to be qualified, which focused on the following:

- Ammonium ion (NH_4^+).
- Ammonia (NH_3).
- Total Kjeldahl Nitrogen (TKN).
- Nitrate (NO_3^-).
- Nitrite (NO_2^-).
- Total Volatile Suspended Solids (TVSS).
- Carbonaceous Oxygen Demand (COD).
- Biochemical Oxygen Demand (BOD).
- Total Suspended Solids (TSS).
- Total Dissolved Solids (TDS).
- Total Organic Carbon (TOC).
- pH.
- Alkalinity.

The results of those analysis verified that there was exceptionally high ammonium, BOD, and COD, and low TOC and alkalinity. Based on those results, the C:N ratio was calculated as less than 1.0. Recalling that a C:N ratio of greater than

5, is a minimum to promote efficient nitrogen removal. Also recalling that nitrification is completed by aerobic autotrophic bacteria, which utilizes nitrogenous biochemical oxygen demand and alkalinity as a food and energy source, or can create its own food source through chemosynthesis, which uses ammonium or nitrite and oxygen. Additionally, denitrification is an anoxic process, conducted by heterotrophic bacteria that use carbon as a food and energy source.

Another contributing factor identified through sampling and analysis was that the concentrations of TSS retained within the SBR system was up to eight times greater than the influent concentrations as depicted in Figure 56 below.

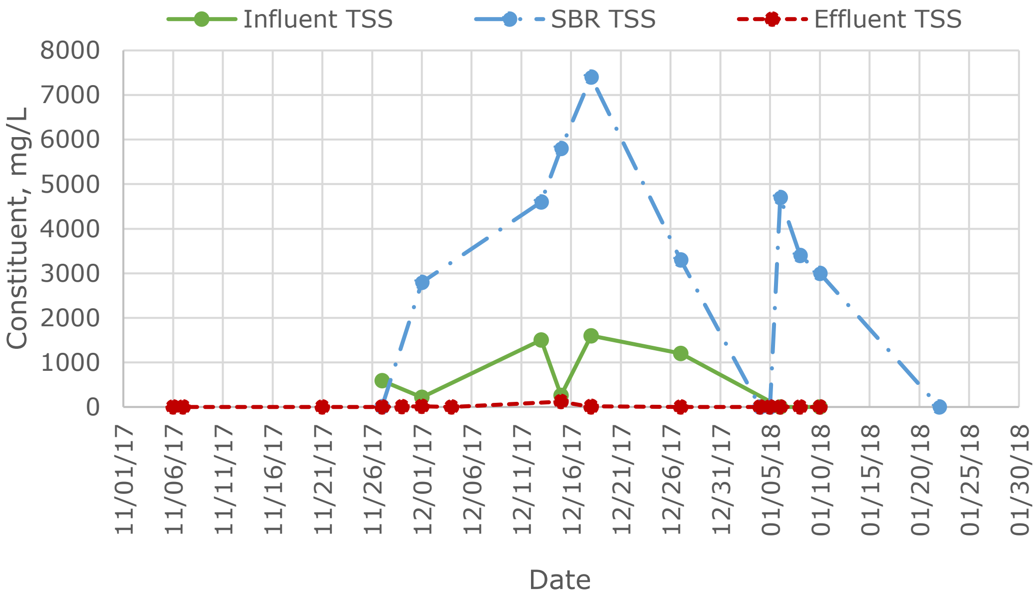

Figure 56 Example 1 – Total Suspended Solids Loading

Noting that the influent TSS concentrations were near 1,000 mg/L, which is high, but is also a quick indicator that sufficient sludge is not being wasted or removed from the system. This can indicate a failure or lack of optimized control of recycling and wasting systems, which was evaluated and addressed later.

The third step was a series of settleometer tests on each basin of the SBR reactor, to measure how well the treated wastewater settles may be for an operator of suspended activated sludge wastewater treatment facilities.

The settleometer test also provides the benefit of monitoring the health of the microbiology in the system and provide insights and confirmation for operators to make decisions of when to waste the sludge, and to determine the returned-sludge flow rate. The settleometer results can also be used to calculate sludge volume index (SVI), which helps determine the health of the floc as well as the severity of poor settling (bulking) events.

Floc forming bacteria may not be the specific focus of developing and sustaining nitrifying and denitrifying bacteria. However, key operational control insights can be gained from a settleometer test, on the ecology of the system, dominant microbiology, and health of the microbiology, when used in consideration of the following guidance.

1. If the floc settles rapidly but leaves pin floc in solution or on the top, this may indicate an "old sludge". This can result in poor performance and efficiency and lead to turbidity and TSS passing into the effluent.
    a. If present, the operator should increase wasting rates.
2. If the floc does not compact as much as normal, there may be young sludge, filamentous or non-filamentous bulking.
    a. Young sludge will also have a high oxygen uptake rate and many bacteria cells in free solution outside the floc.
        i. In this case, it will take more time for the microbiology to develop and may indicate increased loadings.
3. Filaments and non-filaments are a different situation than young sludge. Each filament and type of bulking have various factors.

The results of this sites initial settleometer test results are depicted in Figure 57 below.

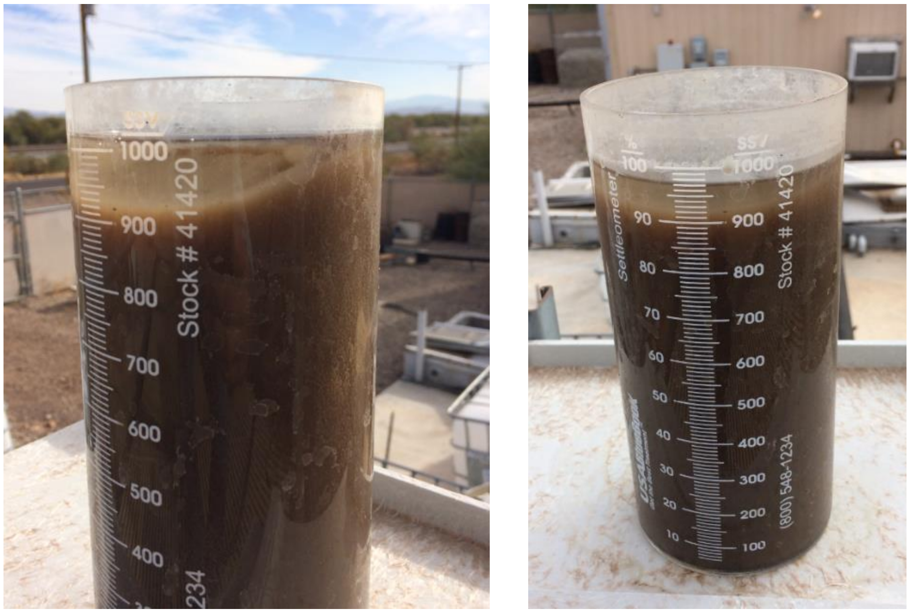

Figure 57 Initial Settleometer Test Results

As depicted in the images in Figure 57 above, the sludge does not settle quickly, or more than 10 percent. Those results, in combination with microscopy efforts, revealed most of the sludge was non-filamentous bulking, and old sludge, with almost no living microbiology.

At this point, based on the limited findings summarized above, and without further evaluation of the physical infrastructure, control strategies, or other support systems, the following was determined as a root cause of the deficiencies:

1. There is insufficient carbon to promote denitrification.
2. The system may have been experiencing period of nitrification, which were only adding to the concentrations of high nitrate in the effluent.
3. There is insufficient microbiology to promote almost any of the desired biological treatment of wastewaters.

That was determined within less than 1-week of effort.

Based on those results, the following corrective action were implemented:

1. Many of the solids that had been built up in the system were removed, until the TSS concentration within the SBR reactor was near the concentration of the influent raw wastewater.
2. The facility was "re-seeded" with recycled activated sludge (RAS), from a local, well performing wastewater treatment plant, with similar nitrogen removal requirements.
3. A supplemental, and external carbon source was added.
    a. MicroC® 1000 solution was utilized, which is a non-hazardous, carbohydrate based liquid solution.
    b. This was dosed to the SBR bioreactor at a rate to maintain a C:N of greater than 6.

As a result of those efforts and approach, new microbiology was introduced, old filamentous and non-filamentous sludge was removed, and carbon was added, within an additional week. Monitoring and sampling occurred daily, in which nitrification was measured to begin occurring within two days, and denitrification began within five days. Examples of the observed microbiology is depicted in Figure 58 below, which also reveals the reduced filamentous material in the bulk solution.

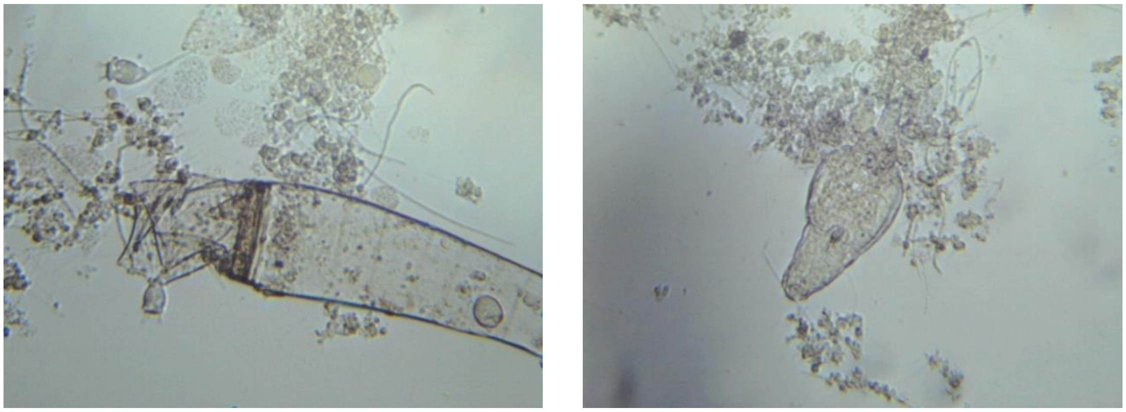

Figure 58 Microbiology Microscopy Results after External Carbon Addition

An image of the resulting settleometer test results, after the corrections as listed above, are depicted in Figure 59 below.

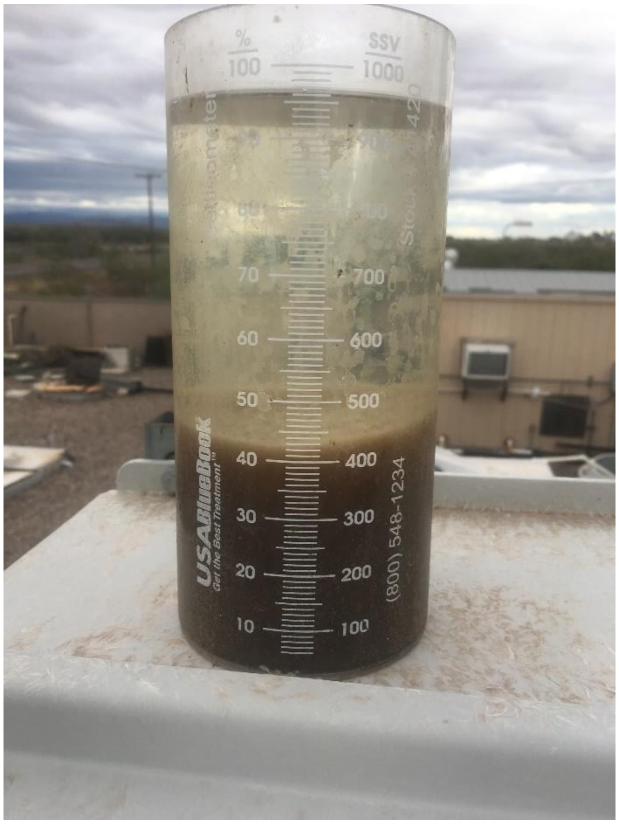

Figure 59 Final Settleometer Test Results

In this example, fully compliant effluent was able to be achieved within less than 18 days from start of the evaluation, prior to evaluating, improving, or optimizing the mechanical or instrumentation systems, and control strategies. This demonstrates that a focus on the fundamental, and dominant factors of microbial selection can be used for making early decisions for both optimization and for design.

Additionally, the specific facility, and influent conditions may be an ideal opportunity to implement a deammonification nitrogen shortcut technique, which adds value by not requiring external carbon source, or potentially, any carbon, and based on the capacity of less than 100,000 gpd, a side stream implementation may be highly cost affordable.

8.1.2 Centralized Urban WWTP with High Industrial Intensity Optimization

In this example, an Oxidation Ditch (OD) style wastewater treatment process was implemented in an urban area, with an average day capacity greater than 10 MGD and less than 20 MGD. The service area of this facility consisted of generally 70 percent residential and domestics flow and loading, and 30 percent industrial and commercial flow and loading. However, there is a single industrial wastewater generator, than represents approximately 10 percent of the flow to the OD wastewater treatment facility.

Regulatory and process goals included BOD and TSS reduction, with set limits for effluent turbidity, total nitrogen, and a minimum chlorine residual, prior to discharge for aquifer recharge. Recently that facility has experience elevated concentrations of total nitrogen in the effluent and were concerned about the ability to sustain compliant performance. Again, focusing on the biotic, abiotic, and ecological factors of the facility, a similar approach was used, as described above in the first example, to evaluate and determine the root causes.

In this example, the first step completed was an extensive sampling and laboratory analysis of the quality and concentrations of constituents in the influent raw wastewater to the facility, and of the effluent from the target industry. These results supported the ability to conduct a mass balance of flow and loading to quickly identify any major concerns.

Next, those results were compared with the intended design capacities and performance of each unit processes to identify any early concerns or limitation, for later focus on microbiological factors. Key findings of that early analysis included the following:

1. The OD WWTP was experiencing approximately 80 percent of BOD influent loading capacity, while experiencing approximately 60 percent of flow capacity.
2. The private industry was contributing approximately 50 percent of the BOD capacity of the OD WWTP.
3. The OD WWTP was experiencing an influent TKN loading of 50 to 70 percent of capacity.
4. The private industry was contributing approximately 10 percent of the TKN capacity of the OD WWTP.

The existing oxidation ditches processes utilized mechanical surface aerators were rated to provide a maximum dissolved oxygen to the biological reactor of approximately 70,000 lbO_2/day. Based on the elevated loadings, during reduced flow to the OD WWTP, the required dissolved oxygen to support the reduction of the BOD and TKN, is approximately 500 lbO_2/day, higher than what is possible for the existing system to provided.

Based on the magnitude of the existing OD bioreactors, where both the nitrifying process is conducted in the aerated zone of the reactors, and denitrifying is designed to occur in the anoxic zones of the OD, the only quick fix for this situation was to reduce the concentration of BOD and TKN discharged to the existing OD WWTP.

The long-term solutions, focusing on optimizing the biological treatment process, identified that a simultaneous nitrification-denitrification (SNdN) shortcut nitrogen technique could provide both initial capital and long-term cost savings. The OD configuration lends itself to promoting the SNdN technique, but it is important to recall that nitrification and denitrification reaction rates may be less efficient, since the available biomass and substrate will be shared between those reactions. However, nitrification rates are typically lower due to targeted lower dissolved oxygen concentrations to support denitrification, and to balance swing zone or conditions between aerobic and anoxic. This can be overcome in processes with longer HRT's, where there is sufficient volume to overcome lower nitrogen removal reaction rates.

This example also demonstrates the importance of providing flexibility in setting design criteria for greenfield, and expansion type wastewater products, where the right flexibility is available for when wastewater treatment flows, and loading change in the future, in additional to being a candidate for the SNdN short nitrogen removal technique.

CHAPTER 9

CONCLUSION

9.1 Thesis Outcome

Based on the results of the models and analysis as conducted and described above, conceptual plan view footprints for each process configuration scenario were developed, to scale, which quantified the physical infrastructure required. For each, it was assumed the upstream processes such as influent lift station, screening and grit removal, intermediate hydraulic processes, and downstream processes, such as digestion, solids handling, dewatering and disposal, disinfection or deactivation, and effluent disposal were the same across all alternatives, and therefore the footprint or cost required for those processes were not evaluated. The results are summarized below.

1. When mainstream nitrogen shortcut techniques are implemented, the overall footprint required is reduced by less than five percent as compared with the traditional SND process configurations.
    a. The projected capital savings was less than 5 percent of the total capital cost as compared with the traditional NdN.
    b. However, the savings in energy and consumables was projected as much as 26 percent and could equate to a return on investment (ROI) in less than 5-years.
2. When a side stream nitrogen shortcut technique is added to the process, this increased the footprint and infrastructure by as much as 15 percent, with an additional 12 percent added to the total capital cost as compared with the traditional NdN.

        a. However, the savings in energy and consumables was projected as much as 18 percent and could equate to a return on investment (ROI) in less than 10-years.
        b. Therefore, the real cost savings will rely upon the reduction of energy and consumables over the lifetime of the facility.
    3. Adding or retrofitting an existing OD or SBR to implement nitrogen shortcut technique had no increase or decrease to the footprint.
        a. However, the savings in energy and consumables was projected as much as 14 percent and could equate to a return on investment (ROI) in less than 10-years.

9.2 Thesis Conclusion

Nitrogen removal in wastewater treatment is a required public service that benefits the health and well-being of communities and the environment. Optimization of those processes to reduce both the initial capital cost, and long-term cost to operate is required to provide a sustainable solution, and to meet current and future needs.

Various nitrogen shortcut techniques, including nitrite shunt, biocatalyst, nitritation, deamminification, and simultaneous nitrification-denitrification each have potential and actual benefits to address the challenges at hand. Whether implemented in a mainstream or side stream configuration, or to an existing or new wastewater treatment plant, these process can provide significant reduction in the long-term cost to operate, due to lower energy and consumable requirements, fast reaction times resulting in shorter solids retention times, and improvement efficiency in nitrogen removal from wastewater.

The key outcome from the efforts discussed above, is that proper comprehension of the microbiological ecological, kinetics, and thermodynamics, both for design or optimization, and during operations is paramount to achieving and sustaining the savings and improved performance projected with the implementation of nitrogen shortcut techniques.

Current and future projected concerns regarding increased BOD and nitrogen loading, without parallel flow increases, to wastewater treatment plants will decrease the resiliency of existing facilities and should be accounted for in the design of new facilities, or optimization and expansion of existing facilities.

Nitrogen shortcut techniques currently can provide demonstrated benefits and success when implemented in a side stream application, and when used to modify existing OD and SBR in a mainstream application. Further research, improvement and testing is required for other mainstream applications, but holds promise as a viable improvement for nitrogen removal.

9.3 Recommended future efforts

Recommendation for a continuation of this research, and to improve the body of knowledge regarding the implementation and operations of nitrogen shortcut techniques in wastewater treatment, focused on the following items, highlighted in the Chapters above, and as an identified need, based on the efforts and outcome of this research.

1. Existing wastewater treatment plants consider a more robust laboratory sampling and analysis of both influent wastewater characteristics, and in-plant variables, and cataloging of microscopy efforts, to best characterize the ecology and microbiological conditions and environments.
    a. This would support the ability to evaluate the feasibility to implement savings and optimization relating to nitrogen

    removal and provide improved comprehension for improving efficiency.

2. Improvements to models that combine biological, chemical, and physical treatment processes of wastewater, with sufficient hydraulic analysis, need to expand the ability to evaluate speciations of various compounds and chemicals, and include and three dimensional (3D) computational dynamics evaluations to support thermodynamic evaluations and calculations.
    a. Such modeling efforts will also need to improve on defining and measuring thermodynamic yield estimation and biomass growth yield with respect to the electron donor, by various pathways.
3. Further detailed conceptual foot printing and cost estimating should be conducted to refine the precision of cost implications, and to identify other trends between short-, and long-term costs to implement and operate.

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