Chapter 6
Nitrification-Denitrification

6.1 INTRODUCTION

Nitrification-denitrification is a two-step biological process that transforms potentially hazardous ammonia and nitrate into harmless dinitrogen (N_2) gas. **Table 6.1** displays some potential benefits of nitrification-denitrification. Nitrogen in this gaseous form makes up 78 percent of the earth's atmosphere (Los Alamos National Laboratories, 2003). The first step, nitrification, is the conversion of ammonia to nitrate. Denitrification is the second step, where nitrate is converted to dinitrogen gas.

Nitrification-denitrification is powered by microorganisms. The nitrifying and denitrifying bacteria exist naturally in the environment so normally no special seeding or inoculation is required for the process to occur. In fact, when untreated manure is applied to land, these microorganisms work as part of the natural cycling of nitrogen in the ecosystem. In the soil, the nitrifying bacteria convert the nitrogen

Nitrification: The conversion of ammonia to nitrite and then to nitrate by the autotrophic aerobic bacteria *Nitrosomonas* and *Nitrobacter*, respectively.

Denitrification: The conversion of nitrate to dinitrogen gas by heterotrophic facultative bacteria.

Table 6.1: Potential nitrification-denitrification benefits.

Chapter Number	Chapter Name	Treatment Process	Reduce Nitrogen	Reduce Phosphorus	Reduce Biochemical Oxygen Demand	Stabilize Manure	Reduce Manure Volume	Reduce Pathogens	Reduce Manure Gases	Reduce Odor	Reduce Ammonia Volatilization	Operate at Low Temperatures	Minimal Footprint	Low Energy Requirement	Create Biogas	Create Value-Added Products
6	Nitrification-Denitrification	Nitrification-denitrification	✓		✓						✓					

Lagoon: A shallow pond where sunlight, oxygen, and bacteria degrade and transform compounds in manure.

Acid rain: Any form of precipitation with a pH less than 5.6, the normal pH of rain. Nitrogen oxides (NO_x) and sulfur dioxide (SO_2) released into the atmosphere, generally by anthropogenic sources, turn into acids, lowering the pH of precipitation. Acid rain is harmful to vegetation, soils, and waterbodies.

Eutrophication: A process where a water body becomes nutrient-enriched and eventually unable to sustain plant and animal life.

in manure to plant-available forms of nitrogen, such as nitrate. The denitrifying bacteria convert nitrate to dinitrogen gas. The key is simply to provide an environment where the naturally existing bacteria can flourish.

Nitrification-denitrification may take place in separate reactors linked in series or in a single reactor. Reactors may be open facilities, such as **lagoons**, or closed tank style reactors. This chapter addresses the process as two separate stages—nitrification and then denitrification. It is important to remember that these are microbial processes occurring on a microscopic level; microbiology and chemical reactions drive this treatment technology. These reactions are introduced as well as system design and design considerations.

Manure with a low organic matter content is best treated by nitrification-denitrification. Organic matter may inhibit the first step in the treatment process, so farms with existing solids separation processes, which reduces the amount of organic matter in the liquid fraction, are best suited for this treatment technology. Farms producing large volumes of manure but without sufficient land for nitrogen assimilation can greatly benefit from nitrification-denitrification.

6.2 ADVANTAGES AND DISADVANTAGES OF NITRIFICATION-DENITRIFICATION

Nitrification-denitrification is just one of many manure treatment technologies. There is no single manure treatment technology that will work for every farm—the treatment goals must be weighed against the economic, management, and maintenance requirements. As always, a system of treatment processes, rather than a single unit process, will provide a more robust manure management plan and meet more treatment objectives.

6.2.1 ADVANTAGES

Nitrification-denitrification is a proven treatment method for removing nitrogen from agricultural and municipal wastewater. Gaseous ammonia, an air pollutant, and liquid ammonia and nitrate, both water pollutants, are converted to non-polluting dinitrogen during nitrification-denitrification. Gaseous ammonia has a basic pH but reacts with acidic sulfur dioxide in the atmosphere, increasing local **acid rain** deposition. Liquid ammonia is toxic to fish and is a significant contributor to fish kills. Nitrate can cause **eutrophication.**

This unit process is easily added to existing treatment processes to create a comprehensive manure management treatment system.

6.2.2 DISADVANTAGES

Nitrification-denitrification only removes nitrogen from the manure. Although other biological processes may simultaneously occur, the primary treatment results when using this technology is reduced nitrogen levels. Removing nitrogen from the waste stream diminishes the value of treated manure for use as nitrogen-rich fertilizer.

Nitrification is an oxygen-intensive process, requiring electrical and mechanical inputs for aeration. These inputs increase energy costs and maintenance requirements. The optimal temperature ranges for nitrification and denitrification processes are greater than the ambient temperatures in cold climates; heating may be required during winter months to achieve consistent levels of treatment.

Depending on the design, nitrification-denitrification can be costly, and the two-step process must be completed. Otherwise, ammonia and nitrate will endure, posing air and water quality hazards.

6.3 NITRIFICATION PROCESS

There are two stages in the nitrification process, both of which are performed by **aerobic autotrophic** bacteria. First, ammonia is converted to nitrite by the bacteria *Nitrosomonas*. **Equation 6.1** shows the chemical equation for the conversion of ammonia to nitrite.The nitrite is quickly converted to nitrate by the bacteria *Nitrobacter*. This conversion is so fast that persistent levels of nitrite are rarely found in nature. Nitrate is the form of nitrogen that plants uptake. **Equation 6.2** illustrates the conversion of nitrite to nitrate, while **Equation 6.3** shows the overall nitrification process.

Nitrosomonas and *Nitrobacter* are the bacteria most commonly responsible for nitrification. Other nitrifying autotrophs exist as well. **Table 6.2** displays other autotrophs capable of nitrification.

Table 6.2 Other autotrophic bacteria capable of the nitrification processes (Metcalf and Eddy, 2003).

Ammonia to nitrite	Nitrite to nitrate
Nitrosococcus	*Nitrococcus*
Nitrosospira	*Nitrospira*
Nitrosolobus	*Nitrospina*
Nitrosorobia	*Nitroeystis*

6.3.1 TYPES OF NITRIFICATION DESIGNS

Nitrification can either occur in suspended growth or attached growth systems. Nitrification can take place in one reactor or in multiple reactors situated in series. The reactors may be either ponds or tanks.

a. Suspended Growth Processes

In suspended growth processes, the nitrifying bacteria are kept in suspension with the liquid wastewater. Aerators are used to keep the **biomass** from settling to the bottom of the reactor and to maintain a homogenous mixture. The aeration not only ensures contact between the bacteria and the waste, but also serves as an oxygen source.

b. Attached Growth Processes

In attached growth systems, the nitrifying bacteria are attached to a medium, such as polymer gel pellets. The nitrifying bacteria are encapsulated in pellets permeable to ammonia and oxygen. The pellets are typically 3.2 to 6.4 millimeters ($^1/_8$ to $^1/_4$ of an inch) wide and are typically constructed from polyethylene glycol and polyvinyl alcohol (Becker, 2003). The pellets generally take up seven to 15 percent of the nitrifying tank's volume and are kept inside the tank by a screened structure. **Figure 6.1** is a photograph of pellets used in attached growth nitrification.

Plastic mesh or rocks are alternatives to pellets. The plastic or rocks provide

Figure 6.1: Pellets used in an attached growth nitrification (H. Becker, 2001).

Equation 6.1: Conversion of ammonia to nitrite by Nitrosomonas (Crites and Tchobanoglous, 1998).

$$2NH_4 + 3O_2 \rightarrow 2NO_2 + 2H_2 + 2H_2O$$

Equation 6.2: Conversion of nitrite to nitrate by Nitrobacter (Crites and Tchobanoglous, 1998).

$$2NO_2 + O_2 \rightarrow 2NO_3$$

Equation 6.3: Overall conversion from ammonia to nitrate by Nitrosomonas and Nitrobacter (Crites and Tchobanoglous, 1998).

$$2NH_4^+ + 4O_2 \rightarrow 2NO_3^- + 2H_2^- + 2H_2O$$

Aerobic: An oxygenated environment or requiring an oxygenated environment to survive.

Autotrophic: Organisms that make their own food by using either solar or chemical energy. The bacteria responsible for nitrification use the chemical energy released during ammonia oxidation to make their food.

Biomass: The total dry mass of an individual or population.

Biochemical oxygen demand (BOD): A measure of the amount of oxygen needed by aerobic microorganisms to break down solids and organic matter present in wastewater. Although BOD is not a specific compound, it is defined as a conventional pollutant under the federal Clean Water Act. The BOD_5 test is a five-day laboratory test to determine the amount of oxygen available for biochemical oxidation in a sample.

Heterotrophic: Organisms that cannot make their own food and must obtain energy by consuming other organisms or their organic products; a consumer or a decomposer in the food chain.

a surface for the nitrifying bacteria to cling to, forming a biofilter. Biofilters require periodic backwashes to dislodge any excess biosolids that may accumulate. **Figure 6.2** is a diagram of biofilter nitrification.

The wastewater may be introduced to the nitrifying tanks either from the top or from the bottom. When the liquid is introduced from the top, the nitrifying tank may be called a trickling filter—the wastewater trickles down through the growth medium. If the liquid is introduced from the bottom it may be known as an upflow biofilter. The wastewater may be recycled through the nitrification system to achieve greater conversion of ammonia to nitrate.

In attached growth systems, the majority of the **biochemical oxygen demand** (BOD) must be eliminated before the nitrifying organisms are established. If there is a high organic matter content **heterotrophic** bacteria will flourish, out-competing the nitrifying autotrophic bacteria.

6.3.2 NITRIFICATION DESIGN CONSIDERATIONS

There are several things to consider when designing a nitrification system. Nitrification can be a long and difficult process for the microorganisms hard at work, and their environment must be controlled to provide the best living conditions for them as possible. Temperature, pH, aeration, and loading rates all affect the ability of nitrifying bacteria to thrive and convert ammonia to nitrate.

a. Temperature and pH

Temperature and pH are also important factors. The temperature range should be between 10 and 30° C (50 and 86° F), depending on the treatment length (Grady et al., 1998). The pH should be kept within the range of 7.5 to 8.6 (Crites and Tchobanoglous, 1998).

b. Aeration

Nitrification is an oxygen-intensive process, so the wastewater must be well aerated. Nitrifying tanks are mechanically aerated to provide the required oxygen for the nitrifying bacteria. Tanks may be aerated in different ways, including from the bottom. Aeration may also be used to keep bacteria afloat in suspended growth processes.

c. Loading Rate

The wastewater loading rate for the nitrification process is based on several factors, including the treatment goals, ambient temperature, rate of aeration, and the **chemical oxygen demand (COD)** of the manure. High ammonia levels may overwhelm the bacteria responsible for conversion.

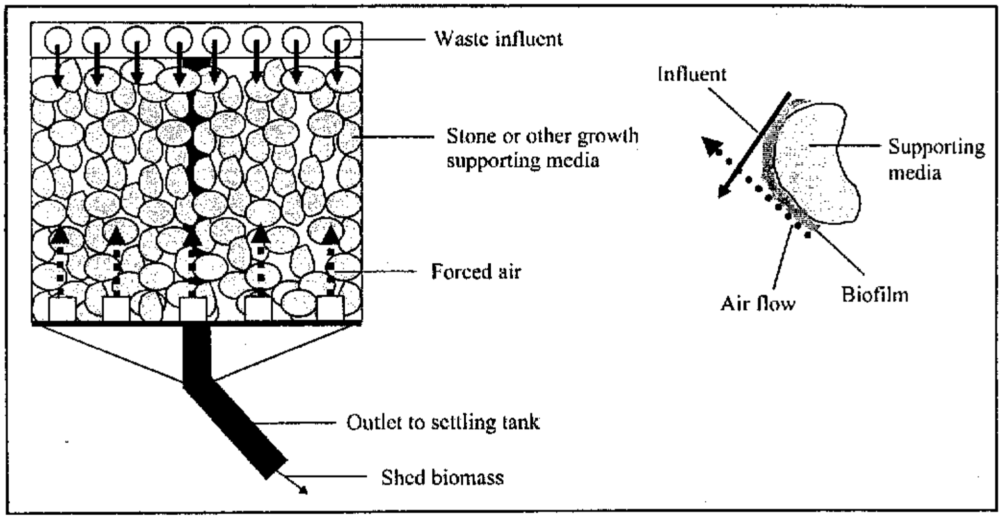

Figure 6.2: Biofilter nitrification (adapted from Miner et al., 2000).

Care must be taken to allow the bacteria to slowly acclimate to the ammonia-rich environment. For high-strength swine wastewater, it can take up to four weeks for the bacterial population to acclimate. During acclimation, the bacteria are establishing themselves in the reactor environment and growing. After acclimation, bacteria can treat nitrogen-laden waste for up to ten years (Becker, 2003).

Nitrification can also be difficult if the manure contains high concentrations of organic matter. Concentrations of organic compounds, such as oximes, as low as two mg/L can inhibit nitrifying bacteria (Baily and Love, 1999).

d. Retention Time

The nitrifying autotrophic bacteria grow more slowly than **heterotrophic** bacteria. The extra time for growth requires much longer **hydraulic retention time (HRT)** for treatment than are found in other treatment technologies.

6.4 DENITRIFICATION PROCESS

Denitrification is the second stage of the biological removal of nitrogen from manure. In denitrification, the bacteria convert nitrate to dinitrogen gas. Dinitrogen gas is **inert** and can be released harmlessly to the atmosphere; the atmosphere is composed primarily of dinitrogen gas.

There are both autotrophic and heterotrophic denitrifying bacteria, but most denitrification in nature is conducted by heterotrophic bacteria. *Achromobacter, Bacillus,* and *Pseudomonas* are the most common denitrifying bacteria.

Special enzymes in the denitrifying bacteria convert the nitrate to nitrogen gas. These enzymes are usually present all the time, but they are inhibited by oxygen. **Facultative** organisms, such as the heterotrophic bacteria responsible for denitrification, can thrive in environments that are not fully aerated, as well as in completely **anaerobic** environments. In these **anoxic** environments, facultative bacteria can use nitrate, sulfate, or carbonate as an **electron acceptor** instead of oxygen. In denitrification, nitrate is the electron acceptor used by the facultative microorganisms. **Equation 6.4** is a simplified chemical equation for denitrification.

Other bacteria that are capable of denitrification are *Acinetobacter, Agrobacterium, Alcaligenes, Arthrobacter, Chromobacterium, Corynebacterium, Flavobacterium, Hypomicrobium, Moraxella, Neisseria, Paracoccus, Propionibacterium, Rhizobium, Halobacterium,* and *Methanomonas.*

Equation 6.4: Denitrification (Crites and Tchobanoglous, 1998).

$$2NO_3^{+} + 5H_2 + 2H^{+} \rightarrow N_2 + 6H_2O$$

6.4.1 TYPES OF DENITRIFICATION DESIGNS

Like nitrification, denitrification systems may either be suspended growth or attached growth systems. **Figure 6.3** is a photograph of an anoxic zone of a treatment lagoon. When nitrification and denitrification occur in different reactors the nitrified liquid is sent to a denitrifying reactor, often via a **clarifier.** It is possible to convert up to 80 percent of nitrate into dinitrogen gas during denitrification (Vanotti et al., 2003).

Chemical oxygen demand (COD): A measure of the oxygen-consuming capacity of inorganic and organic matter present in wastewater. COD may be higher than BOD because the chemical oxidant may react with substances that bacteria do not consume.

Heterotrophic: Organisms that cannot make their own food and must obtain energy by consuming other organisms or their organic products; a consumer or a decomposer in the food chain.

Hydraulic retention time (HRT): The amount of time that a substance is retained in a reactor, representing the time required to treat the substance.

Inert: A material or compound that does not chemically react with other elements.

Figure 6.3: Anoxic zone for denitrificatioon (J. Robbins, 2004).

Facultative: An environment that contains both oxygenated and oxygen-free regions or microorganisms that can live in both oxygenated and oxygen-free environments. When oxygen is not available, these organisms can switch from respiration to fermentation.

Anaerobic: An oxygen-free environment or requiring an oxygen-free environment to survive.

Anoxic: Lacking oxygen.

Electron acceptor: A substance that accepts electrons and is reduced during an oxidation-reduction reaction. Examples of electron acceptors used by bacteria are oxygen (aerobes), nitrate (nitrate reducing bacteria), and sulfate (sulfate reducers).

Clarifier: A physical unit designed to settle and remove biosolids from wastewater.

6.4.2 Denitrification Design Considerations

As with nitrification, a denitrification system must take into consideration several factors in order to promote a healthy population of denitrifying bacteria. Oxygen level, temperature, pH, availability of carbon, and nitrate loading rate all impact the success of denitrification.

a. Oxygen Level

The presence of oxygen will inhibit denitrification; denitrification occurs in anoxic or anaerobic environments. High levels of oxygen must be excluded from denitrification systems.

b. Temperature and pH

The optimal temperature for denitrification is 12°C (53.6°F) (Crites and Tchobanoglous, 1998). This temperature is on the low end of the acceptable temperature range for nitrification to occur, requiring the nitrified waste to cool somewhat before denitrification. The pH for denitrification can range from 6.5 to 7.5, slightly lower than that for nitrification (Crites and Tchobanoglous, 1998).

c. Carbon Content

Denitrifying bacteria use nitrate as an electron acceptor. They require carbon for energy production and cell synthesis. However, nitrified waste is typically low in carbon since organic matter inhibits nitrification. An external source of carbon may be required for denitrification. Methanol is a common supplemental source of carbon and may even be generated on the farm.

d. Loading Rate

Since denitrifiers utilize nitrate as an electron acceptor, the availability of nitrate affects the health and growth of the denitrifying population. Nitrate must be provided at a rate to satisfy the denitrifying bacteria.

6.5 Combined Nitrification-Denitrification Processes

Combining nitrification and denitrification into one reactor can save on capital and energy costs and eliminate intermediate steps such as clarifiers. However, combined nitrification-denitrification systems require special attention to loading and to start-up procedures in order to properly acclimate both the nitrifying and denitrifying bacteria. **Figure 6.4** is a diagram of the overall

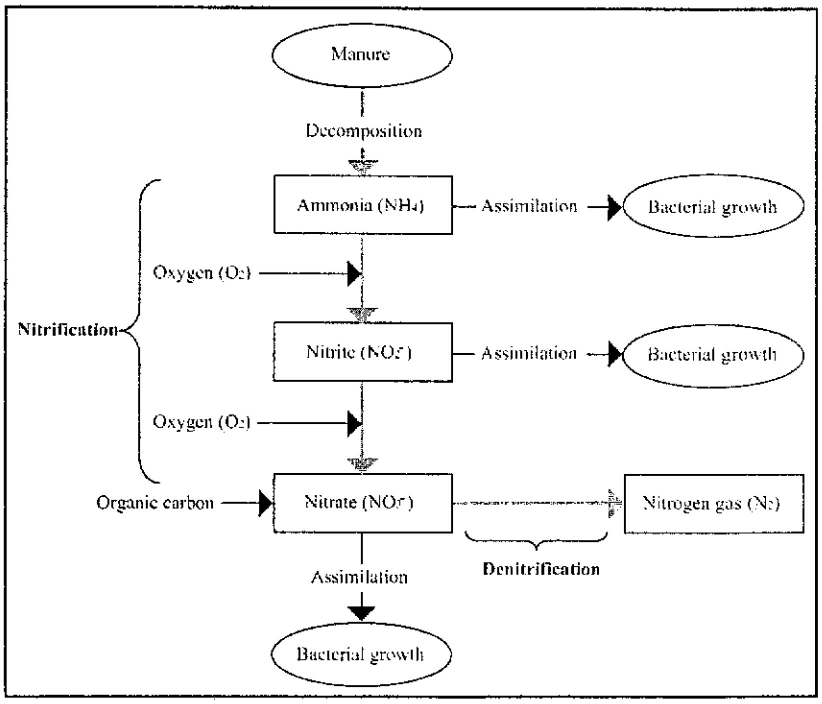

Figure 6.4: Nitrification-denitrification flow chart (adapted from Crites and Tchobanoglous, 1998).