Onsite Nitrogen Removal

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Citation

Chemistry of Nitrogen

Nitrogen can exist in nine various forms in the environment due to seven possible oxidation states:

<table>
<thead>
<tr>
<th>Nitrogen Compound</th>
<th>Formula</th>
<th>Oxidation State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic nitrogen</td>
<td>Organic-N</td>
<td>-3</td>
</tr>
<tr>
<td>Ammonia</td>
<td>NH$_3$</td>
<td>-3</td>
</tr>
<tr>
<td>Ammonium ion</td>
<td>NH$_4^+$</td>
<td>-3</td>
</tr>
<tr>
<td>Nitrogen gas</td>
<td>N$_2$</td>
<td>0</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>N$_2$O</td>
<td>+1</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>NO</td>
<td>+2</td>
</tr>
<tr>
<td>Nitrite ion</td>
<td>NO$_2^-$</td>
<td>+3</td>
</tr>
<tr>
<td>Nitrogen dioxide</td>
<td>NO$_2$</td>
<td>+4</td>
</tr>
<tr>
<td>Nitrate ion</td>
<td>NO$_3^-$</td>
<td>+5</td>
</tr>
</tbody>
</table>
Because of the various oxidation states that can change in the environment, it is customary to express the forms of nitrogen in terms of nitrogen rather than the specific chemical compound: (e.g., Organic-N, NH$_3$-N, NH$_4^+$-N, N$_2$-N, NO$_2^-$-N, and NO$_3^-$-N.)

Thus, for example, 10 mg/L of NO$_3^-$-N is equivalent to 45 mg/L of NO$_3^-$ ion.
The Nitrogen Cycle in Soil-Groundwater Systems

As shown in Figure 1, transformation of the principal nitrogen compounds in soil-groundwater systems (Organic-N, NH$_3$-N, NH$_4^+$-N, N$_2$-N, NO$_2^-$-N, and NO$_3^-$-N) can occur through five key mechanisms in the environment:

- Fixation
- Ammonification
- Synthesis
- Nitrification
- Denitrification
Figure 1: The nitrogen cycle in soil and groundwater
Adapted from U.S. EPA (1993)
Nitrogen Fixation

- Nitrogen fixation is the conversion of nitrogen gas into nitrogen compounds that can be assimilated by plants. Biological fixation is the most common, but fixation can also occur by lightning, and through industrial processes:

- Biological: \( \text{N}_2 \rightarrow \text{Organic-N} \)

- Lightning: \( \text{N}_2 \rightarrow \text{NO}_3^- \)

- Industrial: \( \text{N}_2 \rightarrow \text{NO}_3^- \) or \( \text{NH}_3/\text{NH}_4^+ \)
Ammonification

- Ammonification is the biochemical degradation of Organic-N into NH$_3$ or NH$_4^+$ by heterotrophic bacteria under aerobic or anaerobic conditions.

- Organic-N + Microorganisms $\rightarrow$ NH$_3$/NH$_4^+$

- Some Organic-N cannot be degraded and becomes part of the humus in soils.
Synthesis

- Synthesis is the biochemical mechanism in which $\text{NH}_4^+$-N or $\text{NO}_3^-$-N is converted into plant Organic-N:
  
  - $\text{NH}_4^+ + \text{CO}_2 + \text{green plants} + \text{sunlight} \rightarrow \text{Organic-N}$
  
  - $\text{NO}_3^- + \text{CO}_2 + \text{green plants} + \text{sunlight} \rightarrow \text{Organic-N}$
Nitrogen fixation is also a unique form of synthesis that can only be performed by nitrogen-fixing bacteria and algae:

\[ N_2 \rightarrow \text{Organic-N} \]
Nitrification

- Nitrification is the biological oxidation of $\text{NH}_4^+$ to $\text{NO}_3^-$ through a two-step autotrophic process by the bacteria *Nitrosomonas* and *Nitrobacter*:

**Nitrosomonas**

- **Step 1:** $\text{NH}_4^+ + \frac{3}{2}\text{O}_2 \rightarrow \text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O}$

**Nitrobacter**

- **Step 2:** $\text{NO}_2^- + \frac{1}{2}\text{O}_2 \rightarrow \text{NO}_3^-$
Nitrification

- The two-step reactions are usually very rapid and hence it is rare to find nitrite levels higher than 1.0 mg/L in water.

- The nitrate formed by nitrification is, in the nitrogen cycle, used by plants as a nitrogen source (synthesis) or reduced to N₂ gas through the process of denitrification.

- Nitrate can, however, contaminate groundwater if it is not used for synthesis or reduced through denitrification as shown in Figure 1.
Denitrification

- NO$_3^-$ can be reduced, under anoxic conditions, to N$_2$ gas through heterotrophic biological denitrification as shown in the following unbalanced equation:

**Heterotrophic Bacteria**

- NO$_3^-$ + Organic Matter $\rightarrow$ N$_2$ + CO$_2$ + OH$^-$ + H$_2$O
The denitrification equation is identical to the equation for the biological oxidation of organic matter with the exception that \( \text{NO}_3^- \) is used as an electron acceptor instead of \( \text{O}_2 \):

\[
\text{Heterotrrophic Bacteria} \\
\text{O}_2 + \text{Organic Matter} \rightarrow \text{CO}_2 + \text{OH}^- + \text{H}_2\text{O}
\]
Denitrification

- A large variety of heterotrophic bacteria can use nitrate in lieu of oxygen for the degradation of organic matter under anoxic conditions.

- If $O_2$ is present, however, the bacteria will preferentially select it instead of $NO_3^-$. Thus it is very important that anoxic conditions exist in order that $NO_3^-$ will be used as the electron acceptor.

- A carbon source is required as the electron donor for denitrification to occur.
Denitrification

- Autotrophic denitrification is also possible with either elemental sulfur or hydrogen gas used as the electron donor by autotrophic bacteria as shown in the following unbalanced equation:

\[
\text{Autotrophic Bacteria} \\
\text{NO}_3^- + \text{CO}_2 + \text{Inorganic Electron Donor} \rightarrow \text{N}_2 + \text{Oxidized Electron Donor (Sulfur or H}_2\text{ gas)}
\]
Environmental Effects of Nitrogen Discharges

- Health Effects from Groundwater Contamination with Nitrates
  - Methemoglobinemia
  - Carcinogenesis
  - Birth Defects

- Surface Water Pollution with Nitrogen
  - Eutrophication
  - Oxygen Demand through Nitrification
  - Ammonia Toxicity to Aquatic Organisms
Sources of Nitrogen Discharges to Groundwater

Agricultural Activities:

- A significant source of nitrate in groundwater.

- Nitrate can enter groundwater at elevated levels by:
  - Excessive or inappropriate use of nitrogen-based nutrient sources:
    - Commercial fertilizers
    - Animal manures
    - Types of crops utilized
  - Crop irrigation that leads to nitrate leaching
  - Inappropriate livestock manure storage
Sources of Nitrogen Discharges to Groundwater

Septic Tank-Soil Absorption Systems:

- Contamination of groundwater with nitrates from septic tank-soil absorption systems is a problem in many parts of the US.

- The build-up of nitrate in groundwater is one of the most significant long-term consequences of onsite wastewater disposal.

- As an example, the annual nitrogen contribution for a family of four from a septic-tank soil absorption system on a quarter acre lot could be as high as 50 lbs. per year.
Sources of Nitrogen Discharges to Groundwater

Septic Tank-Soil Absorption Systems:

- The annual nitrogen requirement for a quarter acre of Bermuda grass is also about 50 lbs. per year, which could also be close to the annual nitrogen production of a family of four.

- The nitrogen from the septic tank-soil absorption system, however, is not uniformly distributed throughout a lawn and is typically discharged at a depth below which plants can utilize it.

- Nitrogen exists as Organic-N and NH$_3$-N/NH$_4^+$-N in septic tank effluent, and is usually transformed into nitrate as the wastewater percolates through the soil column. Also, the nitrogen loading from high housing densities can greatly exceed any potential plant uptake of nitrogen even if the effluent was uniformly distributed for plant uptake.
Control of Nitrogen Discharges from Onsite Systems

Public health and water pollution control agencies have tried to limit the number of onsite systems in a given area by:

- Quantifying nitrogen loadings
- Examining alternative onsite technologies that provide nitrogen removal
Hantzsche-Finnemore Mass Balance Equation:

- The Hantzsche-Finnemore Equation estimates nitrate loadings to groundwater based upon the measured factors of rainfall, aquifer recharge, septic system nitrogen loadings, and denitrification.
Hantzsche-Finnemore Mass Balance Equation:

\[ n_r = \frac{l \cdot n_w \cdot (1-d) + R \cdot n_b}{(l + R)} \]

- \( n_r \) = NO\textsubscript{3} \cdot N concentration in groundwater, mg/L
- \( l \) = volume of wastewater entering the soil averaged over the gross developed area, in/yr
- \( n_w \) = Total-N concentration of wastewater, mg/L
- \( d \) = fraction of NO\textsubscript{3} \cdot N lost to denitrification
- \( R \) = average recharge rate of rainfall, in/yr
- \( n_b \) = background NO\textsubscript{3} \cdot N concentration, mg/L
Hantzsche-Finnemore Mass Balance Equation:

The number of gross acres per dwelling unit to ensure that groundwater NO$_3$-N will not exceed 10 mg/L can be calculated from the following equation:

\[ A = \frac{0.01344W[n_w - d\cdot n_w - 10]}{R(10 - n_b)} \]

- \( A \) = gross acres/dwelling unit
- \( W \) = average daily wastewater flow per dwelling unit, gallons
Nitrogen Dynamics in Septic Tank-Soil Absorption Systems

Wastewater Characteristics:

- The mass loading of nitrogen in domestic wastewater averages from 4 to 18 lbs. of Total-N per capita per year.

- Untreated domestic wastewater typically contains 20 to 85 mg/L Total-N, with the majority occurring as a mixture of NH$_3$-N/NH$_4^+$-N (12-50 mg/L) and Organic-N (8-35 mg/L)
Nitrogen Dynamics in Septic Tank-Soil Absorption Systems

Because the carbon to nitrogen ratio of wastewater is typically on the order of 4:1 to 6:1, there will be excess nitrogen after secondary biological treatment (BOD removal) that cannot be assimilated by microorganisms as shown in the following unbalanced equation:

\[
\text{bacteria} \\
\text{COHNS} + \text{O}_2 + \text{Nutrients} \rightarrow \text{CO}_2 + \text{NH}_4^+ + \text{C}_5\text{H}_7\text{NO}_2 + \text{end products} \\
\text{Organic} \quad \text{new bacterial} \\
\text{Matter} \quad \text{cells}
\]
Nitrogen Dynamics in Septic Tank
Soil Absorption Systems

Septic Tanks:

- The removal of Total-N within septic tanks is on the order of 10 to 30%, with the majority being removed as particulate matter through sedimentation or flotation processes.

- Because of the septic tank’s anaerobic environment, nitrogen exists principally as Organic-N and NH$_3$-N/NH$_4^+$-N (TKN).
Nitrogen Dynamics in Septic Tank-Soil Absorption Systems

Subsurface Absorption Trenches:

- Nitrogen can undergo several transformations within and below subsurface absorption trenches:
  - Adsorption of NH$_4^+$-N in the soil
  - Volatilization of NH$_3$-N in alkaline soils at a pH above 8.0
  - Nitrification and subsequent movement of NO$_3^-$-N towards the groundwater
  - Biological uptake of both NH$_3$-N/NH$_4^+$-N and NO$_3^-$-N
  - Denitrification if the environmental conditions are appropriate
Sequential Nitrification/Denitrification Processes (Figure 2):

- Sequential nitrification/denitrification processes form the basis of all biological nitrogen removal technologies that have been used or proposed for onsite wastewater treatment.

- Aerobic processes are first used to remove BOD and nitrify organic and $\text{NH}_4^+$-N.

- Anoxic processes are then used to reduce $\text{NO}_3^-$-N to $\text{N}_2$ gas, either using the wastewater as a carbon source or an external carbon source.
Figure 2: Biological Nitrification / Denitrification in Onsite Wastewater Treatment
Adapted from University of Rhode Island (1995)
Classification of Biological Nitrogen Removal Systems:

- Table 1 gives a summary of onsite nitrogen removal systems that have been reported in the literature.

- Using the terminology of wastewater engineering, the systems outlined in Table 1 are categorized according to whether they are suspended growth or attached-growth processes.

  - Suspended-growth processes are biological treatment processes in which the microorganisms responsible for treatment are maintained in suspension within the liquid, usually through mechanical or diffused-air aeration.

  - Attached-growth processes are those in which the microorganisms responsible for treatment are attached to an inert medium such as sand or plastic trickling filter media.
## Treatment Processes for Onsite Nitrogen Removal

### Table 1
Examples of Onsite Biological Nitrogen Removal from the Literature

<table>
<thead>
<tr>
<th>Technology Examples</th>
<th>Total-N Removal Efficiency, %</th>
<th>Effluent Total-N mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Suspended Growth:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic units w/pulse aeration</td>
<td>25-61</td>
<td>37-60</td>
</tr>
<tr>
<td>Sequencing batch reactor</td>
<td>60</td>
<td>15.5</td>
</tr>
<tr>
<td><strong>Attached Growth:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single Pass Sand Filters (SPSF)</td>
<td>8-50</td>
<td>30-65</td>
</tr>
<tr>
<td>Recirculating Sand/Gravel Filters (RSF)</td>
<td>15-84</td>
<td>10-47</td>
</tr>
<tr>
<td>Multi-Pass Textile Filters</td>
<td>14-31</td>
<td>14-17</td>
</tr>
<tr>
<td>RSF w/Anoxic Filter</td>
<td>40-90</td>
<td>7-23</td>
</tr>
<tr>
<td>RSF w/Anoxic Filter w/External Carbon Source</td>
<td>74-80</td>
<td>10-13</td>
</tr>
<tr>
<td>RUCK System</td>
<td>29-54</td>
<td>18-53</td>
</tr>
</tbody>
</table>
Biological Nitrification

Process Chemistry:

- Nitrification is a two-step autotrophic process (nitrifiers use CO$_2$ instead of organic carbon as their carbon source for cell synthesis) for the conversion of NH$_4^+$ to NO$_3^-$-N. During this energy yielding reaction some of the NH$_4^+$ is synthesized into cell tissue giving the following overall oxidation and synthesis reaction:

\[
1.00\text{NH}_4^+ + 1.89\text{O}_2 + 0.08\text{CO}_2 \rightarrow 0.98\text{NO}_3^- + 0.016\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.95\text{H}_2\text{O} + 1.98\text{H}^+
\]

**Autotrophic**

**Bacteria**  
new bacterial cells
Biological Nitrification

Process Chemistry:

The previous balanced equation shows that:

- For each mole of NH$_4^+$ oxidized, 1.89 moles of oxygen are required and 1.98 moles of hydrogen ions will be produced.

- In mass terms, 4.32 mg of O$_2$ are required for each mg of NH$_4^+$-N oxidized, with the subsequent loss of 7.1 mg of alkalinity as CaCO$_3$ in the wastewater, and the synthesis of 0.1 mg of new bacterial cells.

Biological Nitrification

Process Microbiology:

- Nitrifying organisms exhibit growth rates that are much lower than those for heterotrophic bacteria.
- As a result, the rate of nitrification is controlled first by concurrent heterotrophic oxidation of CBOD; as long as there is a high organic (CBOD) loading to the system, the heterotrophic bacteria will dominate. (See Figure 3.)
- Nitrification systems must thus be designed to allow sufficient detention time within the system for nitrifying bacteria to grow.
- After competition with heterotrophs, the rate of nitrification will be limited by the concentration of available NH$_4^+$-N in the system.
Where a sufficient number of nitrifying organisms are present, nitrification can occur as shown by the dotted curve.

Nitrification is usually observed to occur from 5 to 8 days after the start of the BOD incubation period.

Figure 3: Carbonaceous and Nitrogenous Biochemical Oxygen Demand
Adapted from Metcalf and Eddy (1991)
Biological Nitrification

Process Microbiology:

- Figure 4 shows the relationship between fraction of nitrifying organisms in suspended-growth wastewater treatment (activated sludge) and the BOD$_5$/TKN ratio.

- At low BOD$_5$/TKN ratios (0.5 to 3) the population of nitrifying bacteria is high and nitrification should not be influenced by heterotrophic oxidation of CBOD; this type of nitrification process is termed separate-stage nitrification. At higher BOD$_5$/TKN ratios, the fraction of nitrifying organisms in the system is much lower due to heterotrophic competition from oxidation of CBOD; this process is termed single-stage nitrification. Examples of single-stage and separate-stage nitrification are shown in Figure 5.
Figure 4: Percent Nitrifiers vs. BOD/TKN Ratio
Figure 5: Single-Stage and Separate-Stage Nitrification
Biological Nitrification

Dissolved Oxygen Requirements and Organic Loading Rates:

- **Suspended Growth Systems**
  
  - The concentration of DO has a significant effect on nitrification in wastewater treatment.

  - Although much research has been performed, practical experience has shown that DO levels must be maintained at approximately 2.0 mg/L in suspended-growth (aerobic) systems, especially when NH$_4^+$-N loadings are expected to fluctuate widely; this is likely to be the case in domestic onsite wastewater systems.
Biological Nitrification

Dissolved Oxygen Requirements and Organic Loading Rates:

- **Attached-Growth Systems.**
  - DO levels must be maintained at levels that are at least 2.7 times greater than the NH$_4^+$-N concentrations in order to prevent oxygen transfer through the biofilm from limiting nitrification rates.
  - This is usually overcome in practice by using lower organic surface loadings than what would be normally applied for CBOD removal to allow for growth of nitrifying organisms; otherwise the heterotrophic organisms will dominate the bacterial film within the attached-growth media.
  - For trickling filters, for example, the organic loading rate for nitrification is only about 1/5 to 1/8 of the CBOD loading for CBOD removal.
  - Recirculation of effluent through the attached growth media, and use of special media, such as trickling filter plastic media with high specific surface areas, are also used to lower organic surface loadings and to promote high oxygen transfer rates.
Biological Nitrification

- Table 2 shows design organic loading rates for various attached-growth systems to achieve nitrification.
- Unfortunately, organic loading rates for onsite attached-growth systems are not well defined even for CBOD removal, let alone nitrification.
- The more commonly used hydraulic loading rates show mixed results for nitrification as cited in the literature.
- This is no doubt due, at least in part, to varying organic loading rates that were not taken into consideration since the CBOD$_5$ of septic tank effluent can vary greatly, ranging from less than 100 to 480 mg/L.
## Biological Nitrification

### Table 2

Design Loading Rates for Attached Growth Systems to Achieve >85% Nitrification

<table>
<thead>
<tr>
<th>Process</th>
<th>Hydraulic Loading Rate, gpd/ft²</th>
<th>Organic Loading Rate, lbs. BOD/ft²-day</th>
<th>State of Knowledge for Design</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trickling Filters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rock Media</td>
<td>30-900</td>
<td>0.04-0.12</td>
<td>Well Known</td>
</tr>
<tr>
<td>Plastic Media</td>
<td>288-1700</td>
<td>0.10-0.25</td>
<td>Well Known</td>
</tr>
<tr>
<td><strong>Sand Filters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single Pass</td>
<td>0.4-1.2</td>
<td>0.000135-0.002</td>
<td>Lesser Known</td>
</tr>
<tr>
<td>Recirculating</td>
<td>3-5</td>
<td>0.002-0.008</td>
<td>Lesser Known</td>
</tr>
</tbody>
</table>
Biological Nitrification

pH and Alkalinity Effects:

- The optimum pH range for nitrification is 6.5 to 8.0.
- Nitrification consumes about 7.1 mg of alkalinity (as CaCO$_3$) for every mg of NH$_4^+$-N oxidized.
- In low alkalinity wastewaters there is a risk that nitrification will lower the pH to inhibitory levels.
Biological Nitrification

pH and Alkalinity Effects:

- Figures 6 and 7 graphically show the loss of alkalinity with nitrification for septic tank effluent that percolated through the soil column and was measured at a two-foot depth with suction lysimeters.

- In this particular example, the alkalinity decreased from an average of approximately 400 mg/L to 100 mg/L as CaCO$_3$ in order to nitrify an average of about 40 mg/L organic-N and NH$_4^+$-N.

- Figure 8 shows the theoretical relationship of the fraction of TKN that can be nitrified as a function of initial TKN and alkalinity in the wastewater.
Figure 6: Alkalinity Concentrations in Septic Tank Effluent and Vadose Zone Receiving Nitrified Effluents

Alkalinity, mg/L as Calcium Carbonate

Date

Figure 7: Nitrate Nitrogen Concentrations in Septic Tank Effluent and Vadose Zone Receiving Nitrified Effluents
Figure 8: Nitrification as a Function of Initial TKN and Alkalinity

TKN, mg/L as N

Fraction of TKN Nitrified

Alkalinity = 400 mg/L as CaCO₃

Alkalinity = 300 mg/L as CaCO₃

Alkalinity = 200 mg/L as CaCO₃

Alkalinity = 100 mg/L as CaCO₃
Biological Nitrification

Temperature Effects:

- Temperature has a significant effect on nitrification that must be taken into consideration for design.

- In general, colder temperatures require longer cell residence times in suspended-growth systems and lower hydraulic loading rates in attached-growth systems due to slower growth rates of nitrifying bacteria.
Biological Nitrification

Inhibitors:

- Nitrifying bacteria are much more sensitive than heterotrophic bacteria and are susceptible to a wide range of organic and inorganic inhibitors as shown in Table 3.

- There is a need to establish a methodology for onsite wastewater systems for assessing the potential for, and occurrence of, nitrification inhibition.

- Figure 9 illustrates the effect of an inhibitor on nitrification in a septic tank/recirculating trickling filter system; in this particular case a carpet cleaning solvent that was flushed down the toilet contaminated the septic tank and destroyed the nitrifying bacterial population in the attached-growth media. If this system had not been continuously monitored, the effects of the inhibitor on nitrification would have passed unnoticed.
## Biological Nitrification

**Table 3: Examples of Nitrification Inhibitors**

<table>
<thead>
<tr>
<th>Inorganic Compounds</th>
<th>Organic Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>Acetone</td>
</tr>
<tr>
<td>Free Cyanide</td>
<td>Carbon Disulfide</td>
</tr>
<tr>
<td>Perchlorate</td>
<td>Chloroform</td>
</tr>
<tr>
<td>Copper</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Mercury</td>
<td>Phenol</td>
</tr>
<tr>
<td>Chromium</td>
<td>Ethylenediamine</td>
</tr>
<tr>
<td>Nickel</td>
<td>Hexamethylene diamine</td>
</tr>
<tr>
<td>Silver</td>
<td>Aniline</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Monoethanolamine</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td></td>
</tr>
<tr>
<td>Sodium cyanide</td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td></td>
</tr>
<tr>
<td>Hydrazine</td>
<td></td>
</tr>
<tr>
<td>Sodium cyanate</td>
<td></td>
</tr>
<tr>
<td>Potassium chromate</td>
<td></td>
</tr>
</tbody>
</table>
A carpet cleaning solvent was introduced into the system after 120 days in operation.
Biological Nitrification

Inhibitory Effects:

- Since heterotrophic bacteria are much more resilient than nitrifying bacteria, and because many of the inhibitory compounds are biodegradable organics, inhibitory effects can oftentimes be controlled by designing separate-stage nitrification systems.

- In separate-stage systems the CBOD is first removed along with any biodegradable inhibitory compounds; the nitrifying organisms, which are in effect protected in the second stage, are then used to nitrify the low-CBOD, high-NH$_4^+$-N effluent.
Biological Denitrification

Process Description:

- Denitrification is a biological process that uses NO$_3^-$ as the electron acceptor instead of O$_2$ to oxidize organic matter (heterotrophic denitrification) or inorganic matter such as sulfur or hydrogen (autotrophic denitrification) under anoxic conditions.
- In the process NO$_3^-$ is reduced to N$_2$ gas.
- Because the principal biochemical pathway is a modification of aerobic pathways (i.e., NO$_3^-$ is used as the electron acceptor instead of O$_2$), the denitrification process is said to occur under anoxic conditions as opposed to anaerobic conditions (where obligate anaerobic organisms would be present). Denitrifying bacteria, whether heterotrophic or autotrophic, are facultative aerobes and can shift between oxygen respiration and nitrate respiration.
Biological Denitrification

Process Description:

- For heterotrophic denitrification, the carbon source can come from the original wastewater, bacterial cell material, or an external source such as methanol or acetate.

- For autotrophic denitrification, which is common in water treatment but not wastewater treatment, the electron donor can come from elemental sulfur or hydrogen gas.

- The possible process configurations for heterotrophic denitrification are shown in Figure 10.
a) Wastewater as a Carbon Source

b) Bacterial Cells as Carbon Source

c) External Carbon Source

Figure 10: Heterotrophic Denitrification Configurations
Biological Denitrification

Heterotrophic Denitrification: Wastewater as the Carbon Source

- The following unbalanced equation illustrates the process when wastewater or bacterial cell material is used as the carbon source:

\[
\text{Heterotrophic} \\
\begin{align*}
a \text{COHNS} + b \text{NO}_3^- & \rightarrow c \text{N}_2 + d \text{CO}_2 + e \text{C}_5\text{H}_7\text{O}_2\text{N} + f \text{OH}^- + g \text{H}_2\text{O} + \text{end products} \\
\text{organic} & \quad \text{Bacteria} & \quad \text{bacterial} \\
\text{matter} & \quad \text{cells} & \quad \text{cells}
\end{align*}
\]
Biological Denitrification

Heterotrophic Denitrification: Wastewater as the Carbon Source

- The reduction of 1 mg of NO$_3^-$ is equivalent to 2.86 mg of O$_2$.

- Thus, for example, a wastewater with an ultimate BOD (BOD$_L$) of 200 mg/L could potentially reduce almost 70 mg/L of NO$_3^-$-N if the wastewater were used as the carbon source.

- This does not happen in practice, however, because a portion of the organic carbon in the wastewater must be used for cell synthesis and not nitrate reduction.
Heterotrophic Denitrification: Wastewater as the Carbon Source

- For complex organic matter such as wastewater, the stoichiometric equivalency can range from 3.46-5.07 mg BOD$_L$/mg NO$_3^-\cdot$N, with 4.0 mg BOD$_L$/mg NO$_3^-\cdot$N used as a rule of thumb.

- In terms of BOD$_5$, this amounts to 2.72 mg BOD$_5$/mg NO$_3^-\cdot$N for $k$ (base e) = 0.23 d$^{-1}$.
Heterotrophic Denitrification: Wastewater as the Carbon Source

- Figure 11, which assumes the "rule of thumb" stoichiometric equivalency of 4.0 mg BOD$_L$/mg NO$_3^-$ N (2.72 mg BOD$_5$/mg NO$_3^-$ N), shows total nitrogen removal as a function of initial TKN and wastewater BOD$_5$.

- In this figure it is assumed there is sufficient alkalinity for nitrification, and that $k = 0.23$ d$^{-1}$. It is obvious from Figure 11 that nitrogen removal by denitrification using wastewater as the carbon source is highly feasible for an initial TKN of 40 mg/L or less, but becomes more problematic as the initial TKN increases in relation to BOD$_5$. 
Figure 11: Nitrogen Removal as a Function of Initial TKN and Wastewater BOD$_5$ When Wastewater is Used as Carbon Source.

(It is assumed there is sufficient alkalinity, the initial TKN is nitrified, and that the stoichiometric equivalency is $4.0 \text{ mg BOD}_5/\text{mg NO}_3^-\text{N} = 2.72 \text{ mg BOD}_5/\text{mg NO}_3^-\text{N}$ for $k = 0.23 \text{ d}^{-1}$.)
Biological Denitrification

Heterotrophic Denitrification: External Carbon Source

- Where there is insufficient CBOD left in the wastewater to serve as an electron donor for denitrification, an external carbon source must be supplied.

- Although there are many possibilities, methanol and acetate have been studied the most and their stoichiometry is well known.
Biological Denitrification

Heterotrophic Denitrification: External Carbon Source

**Methanol:**

\[
\text{Heterotrophic} \\
\text{NO}_3^- + 1.08\text{CH}_3\text{OH} + 0.24\text{H}_2\text{CO}_3 \rightarrow 0.47\text{N}_2 + 0.056\text{C}_5\text{H}_7\text{O}_2\text{N} + \text{HCO}_3^- + 1.68\text{H}_2\text{O}
\]

**Acetate:**

\[
\text{Heterotrophic} \\
\text{NO}_3^- + 0.87\text{CH}_3\text{COO}^- + \text{H}^+ \rightarrow 0.46\text{N}_2 + 0.08\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.87\text{HCO}_3^- + \text{H}_2\text{O} + 0.44\text{CO}_2
\]
Heterotrophic Denitrification: Process Microbiology

- The heterotrophic denitrifying bacteria are facultative aerobes that can use either oxygen or nitrate (under anoxic conditions) as an electron acceptor for the oxidation of organic matter.

- Denitrifiers are commonly found in nature and are ubiquitous in wastewater.

- Generally, denitrification processes perform similarly to aerobic processes designed for CBOD removal.
Biological Denitrification

Heterotrophic Denitrification: Process Microbiology

- When an adequate carbon source is available, the principal problem associated with denitrification is the achievement of anoxic conditions.
- The dissolved oxygen concentration controls whether or not the denitrifying bacteria use $\text{NO}_3^-$ or $\text{O}_2$ as the electron acceptor.
- Dissolved oxygen must not be present above certain maximum levels or the denitrifying bacteria will preferentially use it for oxidation of organic matter rather than $\text{NO}_3^-$. 
- As a result, the design of anoxic zones is one of the most important factors in denitrification processes.
Biological Denitrification

Heterotrophic Denitrification: pH and Alkalinity Effects

- Theoretically, 3.57 mg of alkalinity as CaCO$_3$ is produced for each mg of NO$_3$-N reduced to N$_2$ gas when the wastewater is used as the carbon source.

- Thus denitrification can recover approximately half of the alkalinity lost in nitrification and can help overcome pH drops in low alkalinity waters. Because denitrifying organisms are heterotrophic, they normally will be affected by pH changes in the same way heterotrophic bacteria are affected.
Heterotrophic Denitrification: Temperature Effects

- The data from the literature suggest that denitrification rates can be significantly affected by temperature drops below 20 °C, with the denitrification rate at 10 °C ranging from 20% to 40% of the rate at 20°C.

- It can be expected that this decrease is similar to that encountered for heterotrophic organisms removing CBOD and should be taken into consideration for designs in cold climates.
Biological Denitrification

Heterotrophic Denitrification: Inhibitory Effects

- In general, denitrifiers are much more resilient than nitrifying organisms.

- Denitrifiers most likely exhibit the same characteristics as heterotrophic bacteria for CBOD removal to inhibitory compounds.
Biological Denitrification

Autotrophic Denitrification:

- It is also theoretically possible to denitrify wastewaters using autotrophic bacteria and elemental sulfur or H₂ gas as the electron donor instead of carbon.

**Sulfur as Electron Donor:**

\[
\text{Autotrophic} \\
\text{NO}_3^- + 0.11\text{CO}_2 + 0.94 \text{S} + 0.5\text{H}_2\text{O} \rightarrow 0.5\text{N}_2 + 0.02\text{C}_5\text{H}_7\text{NO}_2 + 0.94 \text{SO}_4^{2-} + 0.83\text{H}^+ \\
\text{Sulfur} \quad \text{Bacteria} \quad \text{Bacterial Cells}
\]

**H₂ as Electron Donor:**

\[
\text{Autotrophic} \\
\text{NO}_3^- + 2.82\text{H}_2 + 0.14\text{CO}_2 + \text{H}^+ \rightarrow 0.49\text{N}_2 + 0.03\text{C}_5\text{H}_7\text{NO}_2 + 3.22\text{H}_2\text{O} \\
\text{Bacteria} \quad \text{Bacterial Cells}
\]
Biological Denitrification

Autotrophic Denitrification:

- Autotrophic denitrification, while somewhat common in drinking water treatment, is not commonly used in conventional wastewater treatment, let alone onsite wastewater treatment.

- There is one example of elemental sulfur being tried in autotrophc denitrification for onsite systems in Suffolk County, New York, but this attempt ended in failure.
Biological Denitrification

Summary of Heterotrophic Denitrification Processes:

- Table 5 summarizes the three processes for heterotrophic denitrification (which are shown in Figure 10) with their advantages and disadvantages for onsite nitrogen removal.

- In summary, organic carbon can be provided in the following ways:
  - As an external carbon source to an anoxic reactor after nitrification;
  - As an internal source in the form of bacterial cells through a sequential process of aerobic and anoxic zones;
  - The influent wastewater can be used as the carbon source by recycling nitrified effluent to an anoxic reactor that precedes the aerobic nitrification reactor, operating alternating aerobic/anoxic zones on one reactor (sequencing batch reactor), or conveying the flow sequentially through alternating aerobic/anoxic zones. Denitrification reactors can be designed as suspended-growth or attached-growth processes.
# Biological Denitrification

## Table 5: Onsite Processes for Heterotrophic Denitrification

<table>
<thead>
<tr>
<th>Process</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>External Carbon Source</td>
<td>High removal rates. Denitrification easily controlled.</td>
<td>Insufficient performance data for onsite systems. Operation and maintenance data lacking. Alkalinity lost through nitrification may or may not be recovered, depending on the carbon source used.</td>
</tr>
<tr>
<td>Wastewater as Carbon Source</td>
<td>Lower energy and chemical requirements.</td>
<td>Insufficient performance data. Process difficult to control. Routine monitoring required. Operation and maintenance data lacking.</td>
</tr>
<tr>
<td>Bacterial Cells as Carbon Source</td>
<td>Lower energy and chemical requirements.</td>
<td>Insufficient performance data. Process difficult to control. Routine monitoring required. Operation and maintenance data lacking.</td>
</tr>
</tbody>
</table>
Biological Denitrification

- Denitrification reactors can be designed as suspended-growth or attached-growth processes.

- The lack of reliable performance data precludes a sound design strategy for onsite denitrification, although much valuable information exists for centralized treatment systems.

- In general, using wastewater as the carbon source has many potential advantages, such as recovery of alkalinity (≈ 50%) and diminished oxygen requirements for CBOD removal since NO\textsubscript{3}^- is used as the electron acceptor.
Process Design for Onsite Nitrogen Removal

Centralized Wastewater Treatment:

- Nitrogen removal through biological nitrification/denitrification, as practiced in centralized wastewater treatment, is generally classified as an advanced treatment process.
- Detailed information on wastewater flows and characteristics is required for successful design, operation, and trouble-shooting if nitrogen removal is to be successful.
- As a result, design and operational parameters, such as alkalinity requirements, organic loading rates necessary to achieve nitrification, and stoichiometric equivalencies for various reactions have been widely published in order to advance knowledge and improve design and operation.
Much of the published literature does not report data in terms of parameters that can be used to rigorously assess systems, compare them to other sites, and improve design and operation. As an example, the loading rates on single pass sand filter (ISF) systems have been almost exclusively expressed in terms of hydraulic loading rates; the most useful information in terms of nitrification, however, would be organic loading rates. Alkalinity concentrations are also very rarely monitored in onsite wastewater treatment studies, but are fundamental in assessing the limits on nitrification.
Process Design for Onsite Nitrogen Removal

Onsite Wastewater Treatment Systems: Key Design Factors

Wastewater Flows
  Range of Flowrates
  Diurnal Variability of Flowrates

Wastewater Characteristics
  Organic Loadings (BOD₅)
  Alkalinity and pH
  BOD₅/TKN
  Presence of Inhibitors
Process Design for Onsite Nitrogen Removal

Technological Assessment and Design Considerations.

- Figures 12 and 13, which show nitrogen removal as a function of initial TKN, alkalinity, and BOD$_5$, have been developed for the range of BOD$_5$ values (100-140 mg/L) reported for septic tank effluents with an effluent filter.

- These figures can be used for an initial technical assessment of possible removal efficiencies and design considerations for a given wastewater.
(It is assumed the BOD$_5$ = 120 mg/L, that 50% of the alkalinity lost by nitrification is recovered in denitrification, and that the stoichiometric equivalency is 4.0 mg BOD$_5$/mg NO$_3$-N = 2.72 mg BOD$_5$/mg NO$_3$-N for $k = 0.23$ d$^{-1}$.)
Figure 13 Nitrogen Removal Using Wastewater as Carbon Source as a Function of TKN and Alkalinity

(It is assumed the BOD₅ = 180 mg/L, that 50% of the alkalinity lost by nitrification is recovered in denitrification, the stoichiometric equivalency is 4.0 mg BOD₅/mg NO₃⁻-N = 2.72 mg BOD₅/mg NO₃⁻-N for k = 0.23 d⁻¹.)
Examples of Onsite Nitrogen Removal Technologies

**Suspended Growth:**
- Aerobic units w/pulse aeration
- Sequencing batch reactor

**Attached Growth:**
- Single Pass Sand Filters (SPSF)
- Recirculating Sand/Gravel Filters (RSF)
- Recirculating Textile Filters
- RSF w/Anoxic Filter
- RSF w/Anoxic Filter w/external carbon source
- RUCK system
Examples of Onsite Nitrogen Removal Technologies

- **Suspended-Growth Systems** (Figure 14)
  - **Aerobic Units with Pulse Aeration.** These units are, in principal, extended aeration activated sludge systems in which aeration is periodically stopped or pulsed to promote denitrification. Operational data on these systems is lacking although nitrogen removal efficiencies have been reported to be in the range of 25-61 percent.

- **Sequencing Batch Reactor (SBR).** The SBR differs generally from aerobic units in that fill-and-draw, and alternating aerobic and anoxic cycles, are created within a single reactor; during the anoxic phase sedimentation takes place and the supernatant is pumped from the reactor. Both endogenous phase bacteria and influent wastewater serve as the carbon source. SBR technology has been demonstrated to be an excellent nitrogen control technology for large-scale systems, but there is a paucity of information for onsite systems.
Figure 14: Process Diagram Suspended-Growth Systems: Pulse Aeration and Sequencing Batch Reactors with Aerobic/Anoxic Cycles

a) Aerobic Cycle (Nitrification)

b) Anoxic Cycle (Denitrification)
Examples of Onsite Nitrogen Removal Technologies

- Attached-Growth Systems (Figure 15)

- Single Pass Sand Filters (SPSF). SPSF technology is the most studied of all proposed nitrogen removal technologies. The mechanism of nitrogen removal includes a combination of CBOD removal and nitrification within the sand medium at low organic loadings (low BOD$_5$/TKN ratio), and subsequent denitrification within anoxic microenvironments in the sand.

Total-N removal rates with SPSFs have been quoted in the literature as ranging from 8% to 50%. The greatest advantage of ISF technology is in the achievement of nitrification. The percentage of TKN nitrification in SPSF systems has been reported to range between 75% to 96%.
a) Intermittent Sand Filter (ISF)

b) Recirculating Sand Filter (RSF)

c) Recirculating Sand Filter with Anoxic Rock Filter

d) RSF with Anoxic Rock Filter and External Carbon Source

Figure 15: Process Diagram for Attached-growth Systems
Examples of Onsite Nitrogen Removal Technologies

- Attached-Growth Systems (Figure 15)

  - Single Pass Sand Filters, Continued. Unfortunately, there is a paucity of sound design data for nitrification based on organic loading rates. Most of the loading rates have been reported in terms of hydraulic loading rather than organic loading. Also, accurate data on measured loadings per unit area based on the type of distribution system used as opposed to calculated loadings are difficult to come by.

  Assuming there is sufficient alkalinity for nitrification, it can be expected that SPSF systems will always be denitrification-limited due to the lack of availability of both a carbon source and anoxic conditions.
Examples of Onsite Nitrogen Removal Technologies

- Attached-Growth Systems (Figure 15)

  - Recirculating Sand/Gravel Filters (RSF). RSF technology is also very well studied in the literature. Total-N reduction has been reported to range from 15% to 84%. RSFs can achieve high nitrification rates and consistently higher denitrification rates than ISFs because the nitrified effluent can be recycled back to a recirculation tank where it mixes with wastewater from the septic tank, thus using the incoming wastewater as a carbon source.

  As with SPSF systems, the organic loading rates for RSF systems are poorly defined in the literature. The available data suggest that organic loading rates that promote nitrification typically are in the range of 0.002-0.008 lbs. BOD₅/ft²-day. The extent of denitrification can be expected to vary widely since RSF systems have not typically been designed and operated specifically for nitrogen removal.
Examples of Onsite Nitrogen Removal Technologies

- Attached-Growth Systems (Figure 15)

  - Recirculating Sand/Gravel Filters, Continued.

  - There is no doubt RSF performance could be significantly improved for nitrogen removal with design and operational changes.

  - The recirculation tank is not generally configured to maximize the mixing of septic tank effluent with RSF effluent or to optimize the formation of anoxic conditions for denitrification.
Examples of Onsite Nitrogen Removal Technologies

- Attached-Growth Systems (Figure 15)

  - Recirculating Sand/Gravel Filters, Continued. A better design to enhance denitrification recycles the filter effluent to the inlet side of an anoxic recirculation tank, or an anoxic rock filter, where it mixes with septic tank effluent; the final effluent for discharge is then taken from the filter. This type of system has been termed "classical predenitrification". The rock filter fosters anoxic conditions by preventing hydraulic short-circuiting and allows denitrifying organisms to grow on the rock surfaces, although there could be serious problems with maintenance due to sludge accumulation.

  - Systems using this type of design have been reported in the literature. While one system exhibited a mean Total-N removal of 40% with a mean effluent concentration of 23 mg/L, another study reported Total-N removals of 80 to 90% and effluent Total-N concentrations ranging from 7-10 mg/L.
Examples of Onsite Nitrogen Removal Technologies

- Attached-Growth Systems (Figure 15)

  - Recirculating Sand/Gravel Filters, Continued. Operational changes that could improve nitrogen removal include optimizing the recirculation ratio in order to i) minimize dissolved oxygen in the recirculation tank and ii) maximize denitrification.

  The recirculation ratio for denitrification must be at least 4:1 or greater in order to remove at minimum 80% of the NO$_3^-$-N. Many RSFs in operation may be below this minimum since the range of recommended recirculation ratios is often 3:1 to 5:1. Also, very high recirculation ratios used to prevent filter drying during low-flow periods can inhibit denitrification because they cause high dissolved oxygen concentrations in the recirculation tank. The optimization of the recirculation ratio for Total-N removal has to be done on a site-specific basis.
Examples of Onsite Nitrogen Removal Technologies

- Attached-Growth Systems (Figure 15)

- Single Pass (ITF) and Multi-Pass Textile Filters (RTF). Textile filters are a relatively new technology. The design configurations and operational characteristics of single pass and multiple-pass textile filters are essentially the same as for sand/gravel filters with one important exception that has been reported in a refereed journal article: hydraulic and organic surface loading rates are much higher due to the specific surface area of the textile medium. (Leverenz, H., et al., Evaluation of High-Porosity Medium in Intermittently Dosed, Multi-Pass Packed Bed Filters for the Treatment of Wastewater, Small Flows Quarterly, Vol. 2, No. 2, pp. 28-35, 2001)
Examples of Onsite Nitrogen Removal Technologies

- Attached-Growth Systems (Figure 15)

  **Single Pass and Multi-Pass Textile Filters, Continued.**

  Unfortunately, there are a paucity of data for textile filters in relation to both nitrification and Total-N removal. One detailed study of MPTFs showed that 83-95% nitrification occurred with as much as 14-29% Total-N removal at an organic loading rate of 0.01 lbs. BOD$_5$/ft$^2$-day, and that only 59-76% nitrification occurred with from 17-31% Total-N removal at an organic loading of 0.03 lbs. BOD$_5$/ft$^2$-day.

  As with sand and gravel filters, more data are needed to adequately characterize textile media in terms of design and operational parameters for both nitrification and Total-N removal.
Examples of Onsite Nitrogen Removal Technologies

- Attached-Growth Systems (Figure 15)

  - Peat Filters. Peat filters have been used in a manner similar to intermittent sand filters, with similar hydraulic and organic loading rates. The results of a very few studies show a potential for high Total-N removal, but there is very little known about the mechanisms for adequate design.

    It can be assumed that the peat would serve as a carbon source for reduction of NO$_3^-$-N after nitrification has occurred in the filter. Unfortunately, few detailed design and operational data are available to adequately characterize the various peat media in terms of design and operational parameters for both nitrification and Total-N removal.
Examples of Onsite Nitrogen Removal Technologies

- Attached-Growth Systems (Figure 15)

  - Recirculating Sand/Gravel Filters with Anoxic Filter and External Carbon Source. This system is similar to the RSF above with an anoxic rock filter except the anoxic rock filter now follows the RSF and an external carbon source is added as shown in Figure 15. Part of the RSF effluent is recycled to the recirculation tank, and another part is discharged to the anoxic rock filter where the external carbon source is added. Total-N removal in this type of system has been reported to be very high. One detailed study on pilot scale systems showed Total-N removals of from 74 to 80% with Total-N effluent concentrations ranging from 10-13mg/L.
Examples of Onsite Nitrogen Removal Technologies

- Attached-Growth Systems (Figure 15 Continued)

- RUCK System. The RUCK system, which is shown in Figure 15, is a proprietary system that uses source separation for nitrification and denitrification. Separate collection systems are designed for greywater and blackwater, with each having its own septic tank. The blackwater, which is comprised of wastewater from toilets, showers and baths, is discharged to an SPSF for nitrification and then passes to an anoxic rock filter; the greywater, comprised of kitchen and laundry wastewater, passes from its septic tank directly to the anoxic rock filter, where it serves as the carbon source.
Figure 15 (continued): Process Diagram for Attached-Growth Systems (continued)
Examples of Onsite Nitrogen Removal Technologies

- Attached-Growth Systems (Figure 15 Continued)

- **RUCK System, Continued.** While the RUCK system has often been cited in the literature as a potential technology for nitrogen removal, there is a paucity of performance data that have been published. While the process is intended to provide at least 80% Total-N removal, results from a few studies have shown much poorer removal rates of from 29-54% Total-N removal.

  The variability in nitrogen removal efficiency is no doubt due to the complexity of the system, the variability of the quality of greywater, and the need to adjust the operation to site-specific conditions. The RUCK system likely requires significant adjustment to blackwater and greywater characteristics and site conditions to operate effectively.
Examples of Onsite Nitrogen Removal Technologies

- Shallow Trench and Subsurface Drip Irrigation Systems.

  - The use of either shallow trench or subsurface drip irrigation (SDI) systems has been proposed as an alternative means to remove Total-N in the soil column. Both systems have the potential to promote nitrogen uptake by plant roots if effluent was discharged directly within the root zone.

  - There is also a potential that both systems within the 'A' horizon of the soil could promote denitrification of nitrified effluents if there was sufficient organic matter present, either naturally or added, and if the conditions were conducive for denitrification (i.e., anoxic). This type of denitrification has been demonstrated with the use of a reactive porous media barrier using sawdust as a carbon source, which was used to denitrify nitrified septic tank effluents percolating through the soil column.
Examples of Onsite Nitrogen Removal Technologies

- **Shallow Trench and Subsurface Drip Irrigation Systems.**
  
  - To date, the results on the use of shallow trenches or SDI systems for onsite nitrogen removal is mixed at best, with removal efficiencies of Total-N ranging from 0 to 40%.
  
  - Coupling nitrogen loadings with plant uptake requires significant operational monitoring and adjustment.
  
  - Denitrification, if it is desired, cannot be easily controlled within a trench system or the soil column as it can within a treatment reactor above ground.
  
  - Monitoring of nitrogen removal in the soil column is also a significant problem since lysimeter systems have to be used, and they require some degree of sophistication in installation and sample collection.