• Denitrification

- dissimilatory reduction of $\text{NO}_3^-$ or $\text{NO}_2^-$ to $\text{N}_2$ gas
  ($\text{NO}_3^-$ or $\text{NO}_2^-$ is the electron acceptor used in energy generation)

- Denitrification is widespread among heterotrophic and autotrophic bacteria.
  (many of which can shift between oxygen respiration and nitrogen respiration)

- applied when the complete removal of N is required to prevent eutrophication.

- In order to have denitrification, the nitrogen must be of its oxidized forms,
  $\text{NO}_3^-$ or $\text{NO}_2^-$, so denitrification is frequently is coupled to nitrification,
  which is needed to create the oxidized nitrogen.

- Tertiary denitrification: the water does not contain the necessary electron donor and thus an exogenous electron donor must be provided.

- One-sludge denitrification: the water contains an electron donor that can drive denitrification.
10.1 Physiology of Denitrifying Bacteria

• Denitrification process

- It proceeds in a stepwise manner:

\[ \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2 \]

\[ \text{NO}_3^- + 2e^- + 2H^+ = \text{NO}_2^- + H_2O \quad \text{Nitrate Reductase} \]

\[ \text{NO}_2^- + e^- + 2H^+ = \text{NO} + H_2O \quad \text{Nitrite Reductase} \]

\[ 2\text{NO} + 2e^- + 2H^+ = \text{N}_2\text{O} + H_2O \quad \text{Nitric Oxide Reductase} \]

\[ \text{N}_2\text{O} + 2e^- + 2H^+ = \text{N}_2 + H_2O \quad \text{Nitrous Oxide Reductase} \]

- Very low conc. of the electron donor or too high DO can lead to accumulation of denitrification intermediates. (NO\textsubscript{2}\textsuperscript{-}, NO and N\textsubscript{2}O)

  i) a low conc. of the electron donor limits the supply of electrons to drive the reductive half-reactions.
  ii) a high DO tends to repress the nitrite and nitrous oxide reductases before the nitrate reductase is suppressed.
10.1 Physiology of Denitrifying Bacteria

- pH values outside the optimal range of 7 to 8 can lead to accumulation of intermediates.

- In low acidity waters, pH control can become an issue, because denitrification produces strong base.

\[
\text{CH}_3\text{COOH} + \frac{8}{5} \text{NO}_3^- + \frac{4}{5} \text{H}_2\text{O} \rightarrow \frac{4}{5} \text{N}_2 + 2\text{H}_2\text{CO}_3^- + \frac{8}{5} \text{OH}^- \quad [10.1]
\]

\[
4\text{H}_2 + \frac{8}{5} \text{NO}_3^- \rightarrow \frac{4}{5} \text{N}_2 + \frac{8}{5} \text{OH}^- + \frac{16}{5} \text{H}_2\text{O} \quad [10.2]
\]

- 8/5 equivalents of strong base produced when 8/5 mol of \(\text{NO}_3^- - N\) is reduced.
  \(\rightarrow\) an alkalinity increase of \((50)/(14) = 3.57\text{g as CaCO}_3/\text{g} \) \(\text{NO}_3^- - N\) consumed.

- For the acetate case, the effect is altered slightly, because 2 mol of a weaker acid \((\text{H}_2\text{CO}_3, \text{pKa} \approx 6.3)\) replace 1 mol of a weak acid \((\text{CH}_3\text{COOH, pKa} = 4.3)\)
10.1 Stoichiometric and kinetic parameters for denitrifiers

- Stoichiometric and kinetic parameters for denitrifiers

- In the early application of denitrification, intensive study was conducted for systems with relatively high NO3-N (electron acceptor) or little BOD (electron donor) levels such as *agricultural runoff (high NO3-N) and advanced treatment of secondary effluent (little BOD).*

- Thus, research addressed exogenous electron donors and carbon sources.

- Because methanol was relatively inexpensive, a very large database on methanol has been developed.

- But the large database on methanol cannot be applied directly for situations in which another organic molecule is the donor.

- In table 10.1,

  OD ; the mass of O₂ required for complete oxidation of the donor.
  (8g OD = one e- eq.   OD=BODₗ for organic donor )
### Table 10.1
Representative stoichiometric and kinetic parameters for denitrifiers
($T = 20 \, ^\circ C$)

<table>
<thead>
<tr>
<th>Electron Donor</th>
<th>Methanol</th>
<th>BOD</th>
<th>$\mathrm{H}_2$</th>
<th>$\mathrm{S}^0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-source</td>
<td>methanol</td>
<td>BOD</td>
<td>$\mathrm{CO}_2$</td>
<td>$\mathrm{CO}_2$</td>
</tr>
<tr>
<td>$f^0_s$</td>
<td>0.36</td>
<td>0.52</td>
<td>0.21</td>
<td>0.13</td>
</tr>
<tr>
<td>$g , \text{g VSS}_d/\text{g donor}$</td>
<td>0.27</td>
<td>0.26</td>
<td>0.85</td>
<td>0.10</td>
</tr>
<tr>
<td>$g , \text{g VSS}_d/\text{g OD}$</td>
<td>0.18</td>
<td>0.26</td>
<td>0.11</td>
<td>0.07</td>
</tr>
<tr>
<td>$\hat{q} , \text{g donor/g VSS}_d \cdot \text{d}$</td>
<td>6.9</td>
<td>12</td>
<td>1.6</td>
<td>8.1</td>
</tr>
<tr>
<td>$\hat{q} , \text{g OD/g VSS}_d \cdot \text{d}$</td>
<td>10.4</td>
<td>12</td>
<td>11.8</td>
<td>11.2</td>
</tr>
<tr>
<td>$K$, mg donor/l</td>
<td>9.1</td>
<td>1</td>
<td>1</td>
<td>?</td>
</tr>
<tr>
<td>mg OD/l</td>
<td>13.7</td>
<td>1</td>
<td>0.13</td>
<td>?</td>
</tr>
<tr>
<td>$b$, d$^{-1}$</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>$[\rho^\text{min}]_\text{lim} \cdot \text{d}$</td>
<td>0.55</td>
<td>0.33</td>
<td>0.76</td>
<td>1.3</td>
</tr>
<tr>
<td>$S_{\text{min}}$, mg donor/l</td>
<td>0.25</td>
<td>0.017</td>
<td>0.04</td>
<td>?</td>
</tr>
<tr>
<td>mg OD/l</td>
<td>0.38</td>
<td>0.017</td>
<td>0.005</td>
<td>?</td>
</tr>
<tr>
<td>$D$, cm$^2$/d</td>
<td>1.3</td>
<td>1.0</td>
<td>0.9</td>
<td>—</td>
</tr>
<tr>
<td>$J_R$, kg OD/1,000 m$^2$-d</td>
<td>1.5</td>
<td>0.5</td>
<td>1.2</td>
<td>?</td>
</tr>
<tr>
<td>$S_{\text{min}}^*$ (no detachment)</td>
<td>0.027</td>
<td>0.017</td>
<td>0.040</td>
<td>0.066</td>
</tr>
<tr>
<td>($b_{\text{det}} = 0.2/d$)</td>
<td>0.15</td>
<td>0.087</td>
<td>0.23</td>
<td>0.45</td>
</tr>
<tr>
<td>$K^*$</td>
<td>1.8</td>
<td>0.4</td>
<td>2.2</td>
<td>?</td>
</tr>
</tbody>
</table>

Notes: For $K^*$, $L = 40 \, \mu m$, $D_f/D = 0.8$, and $X_f = 40 \, \text{mg VSS}_d/\text{cm}^3$. ? = not yet determined — not applicable.
<table>
<thead>
<tr>
<th>Organism Type</th>
<th>Electron Donor</th>
<th>Electron Acceptors</th>
<th>C-Source</th>
<th>$f_s^0$</th>
<th>$Y$</th>
<th>$\hat{q}$</th>
<th>$\hat{\mu}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aerobic, Heterotrophs</strong></td>
<td>Carbohydrate BOD</td>
<td>O_2</td>
<td>BOD</td>
<td>0.7</td>
<td>0.49 gVSS/gBOD_L</td>
<td>27 gBOD_L/gVSS-d</td>
<td>13.2</td>
</tr>
<tr>
<td>Other BOD</td>
<td>O_2</td>
<td>BOD</td>
<td>0.6</td>
<td>0.42 gVSS/gBOD_L</td>
<td>20 gBOD_L/gVSS-d</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td><strong>Denitrifiers</strong></td>
<td>BOD</td>
<td>NO_3^-</td>
<td>BOD</td>
<td>0.5</td>
<td>0.25 gVSS/gBOD_L</td>
<td>16 gBOD_L/gVSS-d</td>
<td>4</td>
</tr>
<tr>
<td>H_2</td>
<td>NO_3^-</td>
<td>CO_2</td>
<td>0.2</td>
<td>0.81 gVSS/gH_2</td>
<td>1.25 gH_2/gVSS-d</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S(s)</td>
<td>NO_3^-</td>
<td>CO_2</td>
<td>0.2</td>
<td>0.15 gVSS/gS</td>
<td>6.7 gS/gVSS-d</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Nitrifying Autotrophs</strong></td>
<td>NH_4^+</td>
<td>O_2</td>
<td>CO_2</td>
<td>0.14</td>
<td>0.34 gVSS/gNH_4^+-N</td>
<td>2.7 gNH_4^+-N/gVSS-d</td>
<td>0.92</td>
</tr>
<tr>
<td>NO_2^-</td>
<td>O_2</td>
<td>CO_2</td>
<td>0.10</td>
<td>0.08 gVSS/gNO_2^- -N</td>
<td>7.8 gNO_2^- -N/gVSS-d</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td><strong>Methanogens</strong></td>
<td>acetate BOD</td>
<td>acetate</td>
<td>acetate</td>
<td>0.05</td>
<td>0.035 gVSS/gBOD_L</td>
<td>8.4 gBOD_L/gVSS-d</td>
<td>0.3</td>
</tr>
<tr>
<td>H_2</td>
<td>CO_2</td>
<td>CO_2</td>
<td>0.08</td>
<td>0.45 gVSS/gH_2</td>
<td>1.1 gH_2/g VSS-d</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><strong>Sulfide Oxidizing Autotrophs</strong></td>
<td>H_2S</td>
<td>O_2</td>
<td>CO_2</td>
<td>0.2</td>
<td>0.28 gVSS/gH_2S-S</td>
<td>5 gS/gVSS-d</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Sulfate Reducers</strong></td>
<td>H_2</td>
<td>SO_4^{2-}</td>
<td>CO_2</td>
<td>0.05</td>
<td>0.28 gVSS/gH_2</td>
<td>1.05 gH_2/gVSS-d</td>
<td>0.29</td>
</tr>
<tr>
<td>acetate BOD</td>
<td>SO_4^{2-}</td>
<td>acetate</td>
<td>0.08</td>
<td>0.057 gVSS/gBOD_L</td>
<td>8.7 gBOD_L/gVSS-d</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><strong>Fermenters</strong></td>
<td>sugar BOD</td>
<td>sugars</td>
<td>0.18</td>
<td>0.13 gVSS/gBOD_L</td>
<td>9.8 gBOD_L/gVSS-d</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

$Y$ is computed assuming a cellular VSS_a composition of $C_5H_7O_2N$, and $NH_4^+$ is the N source, except when $NO_3^-$ is the electron acceptor; then $NO_3^-$ is the N source. The typical units on $Y$ are presented.

$\hat{q}$ is computed using $\hat{q} = 1 e^- / eq/gVSS_d$.

$\hat{\mu}$ has units of $d^{-1}$. 
1. While the $f_s^0$ value for heterotrophs using general BOD$_L$ is only slightly smaller than $f_s^0$ for aerobic heterotrophs (around 0.6 e$^-$ eq synthesis/e$^-$ eq donor), the $f_s^0$ values for the two autotrophs are much smaller, similar to nitrifiers. The $f_s^0$ value for the one-carbon oxidizers that consume methanol is lower than for other heterotrophs. True yield values parallel the $f_s^0$ values.

2. Since $\hat{q}$ and $b$ values are roughly similar (cf. 12 g OD/g VSS$_a$-d and 0.05/d), $[\theta_x^{\min}]_{lim}$ is controlled mainly by $Y$.

3. $S_{min}$ values are less than 1 mg OD/l, which means that high residuals of BOD in the effluent are not a special problem.

4. For biofilm processes, all microbial types show high growth potential (low $S_{min}^*$) when detachment is negligible. On the other hand, the autotrophic processes are subject to growth limitations as $b_{det}$ increases.

5. The $K^*$ value for biofilm processes is not large. This means that external mass transport controls the substrate flux when the biofilm loading is in the medium- or high-load region.
In summary, the heterotrophic denitrifiers have kinetic characteristics similar to aerobic heterotrophs. Because they are facultative aerobes, the shifts from O$_2$ respiration to NO$_3^-$ or NO$_2^-$ respiration causes only a small decrease in $f_s^0$ and $Y$, which gives only modest increases in $[\theta_x^{\text{min}}]_{\text{lim}}$

$$[\theta_x^{\text{min}}]_{\text{lim}} = \frac{1}{Y\hat{q} - b}$$  \[3.27\]

Thus, denitrification processes should perform similarly to aerobic processes used for BOD$_L$ removal.

On the other hand, the kinetic characteristics of heterotrophic denitrifiers and autotrophic denitrifiers are very different. Autotrophic denitrifiers have lower $f_s^0$, are much slower growers, and thus require substantially longer SRT. \( \rightarrow \text{see table 10.1} \)

While high DO is required for maximum nitrification, high DO slows or stops denitrification. Therefore, process design and operation must reconcile these conflicting physiological characteristics.
10.1 Stoichiometric and kinetic parameters for denitrifiers

- The denitrifying bacteria often use NO$_3^-$ or NO$_2^-$ as the N source for cell synthesis.

The added electron cost of reducing the N source to the -3 oxidation state in cell reduces $f^0_s$ and the true yield.

- Using NO$_3^-$ as N source requires 8 extra electron equivalents per mole of biomass (C$_5$H$_7$O$_2$N) compared with NH$_3$ as N source.

\[ NO_3^- \rightarrow N_2 \rightarrow N^{-3} \]
\[ 5e^- \quad 3e^- \]

-C$_5$H$_7$O$_2$N requires 20 electron equivalents (N-source; NH$_4^+$) to reduce the C the oxidation state zero but it requires 28 electron equivalents to reduce the C,N when the NO$_3^-$ is N-source (see table 2.4), so OD (oxygen demand) of biomass:

\[ \frac{28e^- \text{ eq}}{\text{mol cells}} \cdot \frac{1 \text{ mol cells}}{113 \text{ g cells}} \cdot \frac{8 \text{ g } O_2}{e^- \text{ eq}} = 1.98 \text{ g OD / g cells (cell } \rightarrow \text{ NO}_3^-) \]

(compare with \[ \frac{20e^- \text{ eq}}{\text{mol cells}} \cdot \frac{1 \text{ mol cells}}{113 \text{ g cells}} \cdot \frac{8 \text{ g } O_2}{e^- \text{ eq}} = 1.42 \text{ g OD / g cells (cell } \rightarrow \text{ NH}_3^+) \])
### 2.5 Overall Reactions for Biological Growth

**Table 2.4**  
Cell formation \((R_c)\) and common electron acceptor half-reactions \((R_a)\)

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Half-reaction</th>
<th>(\Delta G^0^\circ) kJ/e(^-) eq</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell Synthesis Equations ((R_c))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium as Nitrogen Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1 (\frac{1}{5}) CO(_2) + (\frac{1}{20}) HCO(_3^-) + (\frac{1}{20}) NH(_4^+) + H(^+) + e(^-) = (\frac{1}{20}) C(_5)H(_7)O(_2)N + (\frac{9}{20}) H(_2)O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate as Nitrogen Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-2 (\frac{1}{28}) NO(_3^-) + (\frac{5}{28}) CO(_2) + (\frac{29}{28}) H(^+) + e(^-) = (\frac{1}{28}) C(_5)H(_7)O(_2)N + (\frac{11}{28}) H(_2)O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrite as Nitrogen Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-3 (\frac{5}{26}) CO(_2) + (\frac{1}{26}) NO(_2^-) + (\frac{27}{26}) H(^+) + e(^-) = (\frac{1}{26}) C(_5)H(_7)O(_2)N + (\frac{10}{26}) H(_2)O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinitrogen as Nitrogen Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-4 (\frac{5}{23}) CO(_2) + (\frac{1}{46}) N(_2) + H(^+) + e(^-) = (\frac{1}{23}) C(_5)H(_7)O(_2)N + (\frac{8}{23}) H(_2)O</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Common Electron-Acceptor Equations ((R_a))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-14 Oxygen (\frac{1}{4}) O(_2) + H(^+) + e(^-) = (\frac{1}{2}) H(_2)O</td>
<td></td>
<td>-78.72</td>
</tr>
<tr>
<td>I-7 Nitrate (\frac{1}{5}) NO(_3^-) + (\frac{6}{5}) H(^+) + e(^-) = (\frac{1}{10}) N(_2) + (\frac{3}{5}) H(_2)O</td>
<td></td>
<td>-72.20</td>
</tr>
<tr>
<td>I-9 Sulfate (\frac{1}{8}) SO(_4^{2-}) + (\frac{19}{16}) H(^+) + e(^-) = (\frac{1}{16}) H(_2)S + (\frac{1}{16}) HS(^-) + (\frac{1}{2}) H(_2)O</td>
<td>20.85</td>
<td></td>
</tr>
<tr>
<td>O-12 CO(_2) (\frac{1}{8}) CO(_2) + H(^+) + e(^-) = (\frac{1}{8}) CH(_4) + (\frac{1}{4}) H(_2)O</td>
<td>23.53</td>
<td></td>
</tr>
<tr>
<td>I-4 Iron (III) Fe(^{3+}) + e(^-) = Fe(^{2+})</td>
<td></td>
<td>-74.27</td>
</tr>
</tbody>
</table>
10.2 Tertiary Denitrification

- **Denitrification processes:**
  - Tertiary denitrification: *exogenous electron donor* is needed and added.
  - One sludge denitrification: *donor already present* in the wastewater

**Tertiary denitrification**

- Water or wastewater containing $\text{NO}_3^-$ or $\text{NO}_2^-$, but little or no electron donor.
  i) Agricultural runoff contaminated with nitrogen fertilizers
  ii) Drinking water supplies in agricultural regions (*high N-contaminated raw water*)
  iii) Effluent from aerobic biological process (i.e., secondary treatment)
10.2 Tertiary Denitrification

- Electron donor: the rate limiting step in almost all circumstances

i) Organic electron donor

- Most commonly supplied
- They promote the accumulation of heterotrophic denitrifiers.
- In terms of physiology and kinetics, very similar to the aerobic heterotrophs used for BOD oxidation → Similar design criteria
- Historically methanol was chosen for its economic benefits, not because it is a better "exogenous" electron donor than any other choice.

\[
0.1667\text{CH}_3\text{OH} + 0.1561\text{NO}_3^- + 0.1561\text{H}^+ \\
\rightarrow 0.00954\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.0733\text{N}_2 + 0.3781\text{H}_2\text{O} + 0.119\text{CO}_2
\]

- Waste streams from the food processing and beverage industry are a good choice because of high BOD conc. & very high C/N ratio.
→ low N is desirable because the goal of denitrification is total removal of N.
ii) Inorganic electron donor

- $\text{H}_2$ is an excellent electron donor for autotrophic denitrification
  - low cost per electron equivalent compared to organic compounds
  - less biomass production than with heterotrophs
  - no reduced nitrogen ($\text{NH}_4^+$) added
  - lack of safe and efficient $\text{H}_2$ transfer system \textit{(disadvantage)}

\textit{It recently stimulated to develop a membrane-dissolution device.}

- Reduced sulfur

  - most common of reduced S is elemental sulfur, S(s).
  - Normally embedded in a solid matrix that includes a solids base, such as CaCO$_3$, because the oxidation of S(s) generates strong acid.

\[
\text{S(s)} + \frac{6}{5} \text{NO}_3^- + \frac{2}{5} \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + \frac{3}{5} \text{N}_2 + \frac{4}{5} \text{H}^+ \quad [10.3]
\]
Example 10.1  Stoichiometry of denitrification reactions

Denitrification scheme

• Heterotrophic with methanol
• Heterotrophic with acetate
• Autotrophic with H₂

Parameters

• $f_s^0 : 0.36, 0.52, 0.21$
• SRT : 15 days

Compute the overall Stoichiometric reactions for each system
Example 10.1 Stoichiometry of denitrification reactions

1. Calculate the $f_s$, $f_e$

2. Cell synthesis (Rc) with NO$_3$ (Equation C-2, Table 2.4, same for all) 

3. Acceptor equation (Ra) (Equation I-7, Table 2.2, same for all) 

4. Donor equation (Rd) (methanol, acetate, H$_2$, Table 2.3) 

5. Compute the stoichiometric equation

\[
\begin{align*}
    f_s &= f_s^0 \frac{1 + (1 - f_d) b \theta_x}{1 + b \theta_x} \quad [3.33] \\
    R &= f_e R_a + f_s R_c - R_d \quad [2.37]
\end{align*}
\]
Example 10.1 Stoichiometry of denitrification reactions

i) Heterotrophic with Methanol

Assumption: $b = 0.05$/day(Table 10.1), $fd=0.8$ for all system

1. Calculate $fs$, $fe$

<table>
<thead>
<tr>
<th></th>
<th>$fs$</th>
<th>$fe$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.267</td>
<td>0.733</td>
</tr>
</tbody>
</table>

2. Cell synthesis equation

$$\frac{1}{28} \text{NO}_3^- + \frac{5}{28} \text{CO}_2 + \frac{29}{28} \text{H}^+ + e^- \rightarrow \frac{1}{28} \text{C}_5\text{H}_7\text{O}_2\text{N} + \frac{11}{28} \text{H}_2\text{O}$$

3. Acceptor equation

$$\frac{1}{5} \text{NO}_3^- + \frac{6}{5} \text{H}^+ + e^- \rightarrow \frac{1}{10} \text{N}_2 + \frac{3}{5} \text{H}_2\text{O}$$

4. Donor equation

$$\frac{1}{6} \text{CO}_2 + \text{H}^+ + e^- \rightarrow \frac{1}{6} \text{CH}_3\text{OH} + \frac{1}{26} \text{H}_2\text{O}$$
Example 10.1 Stoichiometry of denitrification reactions

\[ f_s R_c \quad 0.267 \times \left( \frac{1}{28} \text{NO}_3^- + \frac{5}{28} \text{CO}_2 + \frac{29}{28} \text{H}^+ + e^- \rightarrow \frac{1}{28} \text{C}_5\text{H}_7\text{O}_2\text{N} + \frac{11}{28} \text{H}_2\text{O} \right) \]

\[ f_e R_a \quad 0.733 \times \left( \frac{1}{5} \text{NO}_3^- + \frac{6}{5} \text{H}^+ + e^- \rightarrow \frac{1}{10} \text{N}_2 + \frac{3}{5} \text{H}_2\text{O} \right) \]

\[ -R_d \quad \frac{1}{6} \text{CH}_3\text{OH} + \frac{1}{26} \text{H}_2\text{O} \rightarrow \frac{1}{6} \text{CO}_2 + \text{H}^+ + e^- \]

\[ 0.1667 \text{CH}_3\text{OH} + 0.1561 \text{NO}_3^- + 0.1561 \text{H}^+ \rightarrow 0.00954 \text{C}_5\text{H}_7\text{O}_2\text{N} + 0.0733 \text{N}_2 + 0.3781 \text{H}_2\text{O} + 0.119 \text{CO}_2 \]
Example 10.1  Stoichiometry of denitrification reactions

i) Heterotrophic with methanol

\[ 0.1667 \text{CH}_3\text{OH} + 0.1561\text{NO}_3^- + 0.1561\text{H}^+ \rightarrow 0.00954\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.0733\text{N}_2 + 0.3781\text{H}_2\text{O} + 0.119\text{CO}_2 \]

ii) Heterotrophic with acetate

\[ 0.125\text{CH}_3\text{COO}^- + 0.1438\text{NO}_3^- + 0.1438\text{H}^+ \rightarrow 0.0122\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.0658\text{N}_2 + 0.125\text{HCO}_3^- + 0.0639\text{CO}_2 + 0.1542\text{H}_2\text{O} \]

iii) Autotrophic with H\(_2\)

\[ 0.5\text{H}_2 + 0.1773\text{NO}_3^- + 0.0246\text{CO}_2 + 0.1773\text{H}^+ \rightarrow 0.00493\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.00862\text{N}_2 + 0.5714\text{H}_2\text{O} \]

Table 10.2.
### Table 10.2
Summary of stoichiometry for various denitrification reactions at $T = 20 \, ^\circ\text{C}$ (Example 10.1)

<table>
<thead>
<tr>
<th>Reaction Type</th>
<th>Heterotrophic with Methanol</th>
<th>Heterotrophic with Acetate</th>
<th>Autotrophic with $\text{H}_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_s$</td>
<td>0.267</td>
<td>0.342</td>
<td>0.138</td>
</tr>
<tr>
<td>Electron equivalents in donor</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Electron equivalents in biomass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ($= f_s$)</td>
<td>0.267</td>
<td>0.342</td>
<td>0.138</td>
</tr>
<tr>
<td>in C ($= \frac{20}{28} \cdot f_s$)</td>
<td>0.191</td>
<td>0.244</td>
<td>0.099</td>
</tr>
<tr>
<td>in N ($= \frac{8}{28} \cdot f_s$)</td>
<td>0.076</td>
<td>0.098</td>
<td>0.039</td>
</tr>
<tr>
<td>$\text{NO}_3^-$ consumed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mol</td>
<td>0.1561</td>
<td>0.1438</td>
<td>0.1773</td>
</tr>
<tr>
<td>$e^-$ eq as acceptor ($= f_e$)</td>
<td>0.733</td>
<td>0.658</td>
<td>0.862</td>
</tr>
<tr>
<td>$e^-$ eq as N source</td>
<td>0.076</td>
<td>0.098</td>
<td>0.039</td>
</tr>
<tr>
<td>$e^-$ eq total</td>
<td>0.809</td>
<td>0.756</td>
<td>0.901</td>
</tr>
<tr>
<td>Net $\text{H}^+$ consumed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{H}^+$ equivalents</td>
<td>0.1561</td>
<td>0.1438</td>
<td>0.1773</td>
</tr>
<tr>
<td>Key ratios</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g OD/g $\text{NO}_3^-$-N</td>
<td>3.66</td>
<td>3.97</td>
<td>3.22</td>
</tr>
<tr>
<td>g alk as CaCO$_3$/g $\text{NO}_3^-$-N</td>
<td>3.57</td>
<td>3.57</td>
<td>3.57</td>
</tr>
<tr>
<td>g VSS/g $\text{NO}_3^-$-N</td>
<td>0.490</td>
<td>0.685</td>
<td>0.224</td>
</tr>
<tr>
<td>g VSS/g OD ($= Y_n$)</td>
<td>0.135</td>
<td>0.172</td>
<td>0.0696</td>
</tr>
</tbody>
</table>
1. The observed yield of denitrifiers \( f_s \) declines significantly from acetate to methanol to \( \text{H}_2 \) as the donor.

2. 4.3 ~ 13 % of e- eq of \( \text{NO}_3^- \)-N consumed is used as the N source in synthesis.

   The great % (13 %) is associated with the greatest \( f_s \) (0.342).

   For acetate, e- eq as N-source =0.342 x (8/28) = 0.098 (eqns. at p.502)

   \[
   \% \text{ of e- eq} = \left( \frac{0.098}{0.756} \right) \times 100 = 13 \%
   \]

3. 28.6 % of the oxygen demand of the biomass is invested in reducing the N-source (\( \text{NO}_3^- \)) to the -3 oxidation state.

4. For heterotrophic nitrification with acetate requires an input of

   \[\sim 4\text{g BOD}_L / \text{g NO}_3^- -\text{N} \text{ and produces } \sim 0.69 \text{ g VSS of biomass (sludge wasting rate)} \text{ and } 3.6 \text{ g as CaCO}_3 \text{ of alkalinity (buffer requirement)}.\]
Tertiary denitrification: the water does not contain the necessary electron donor and thus an exogenous electron donor must be provided.

One-sludge denitrification: the water contains an electron donor that can drive denitrification.

- One-sludge denitrification (= single-sludge = combined denitrification) uses the BOD in the influent of a wastewater to drive denitrification.

- Two goals in one-sludge denitrification:
  1) Providing aerobic conditions that allow full nitrification
  2) Providing anoxic conditions and Reserving BOD (organic electron donor) for denitrification.

*1) and 2) seems to conflict with each other, but they should be done simultaneously. Consequently, the task is to how to reconcile them.
Benefits:

- No exogenous electron donor needs to be added.
  → chemical costs are reduced over tertiary denitrification.

- Some of the influent BOD is oxidized with nitrate as the electron acceptor (not O$_2$).
  → aeration costs are reduced compared to alternative systems that oxidize all BOD and nitrify the reduced-nitrogen forms in the influent with O$_2$.

- Full or nearly full N removal is achieved → protecting receiving waters at risk from cultural eutrophication.
10.3.1 Basic One-Sludge Strategies

- Influent wastewater contains TKN.
- In one sludge denitrification (not one reactor), the TKN must be oxidized to \( \text{NO}_3^- \)-N without oxidizing all the BOD before denitrification takes place.

Total Kjeldahl nitrogen (TKN) = organic BOD and reduced nitrogen (NH4+)

- Despite a wide range of engineering configurations, all one-sludge processes rely on one or more of three basic strategies.

- Three basic strategies for reserving organic electron donor while nitrification takes place:

  1) **Biomass storage and decay**
  2) **Classical predenitrification**
  3) **Simultaneous nitrification with denitrification**
The synthesis of biomass stores electron equivalents that originally came from the BOD and can be released through endogenous respiration to drive denitrification.

\[ \text{TKN} \rightarrow \text{NO}_3^- - \text{N} \]

BOD: partly oxidized and partly stored (synthesis)

\[ \text{NO}_3^- - \text{N} \rightarrow \text{N}_2 \]

Biomass as electron donor (endogenous respiration)
10.3.1 1) **Biomass storage and decay**

- It is called *Wuhrmann biomass decayer* *(Swiss engineer K. Wuhrmann, 1964)*

- Biomass storage and decay has limited applicability by itself and is not often employed as a stand-alone process due to two shortcomings:

  1) endogenous respirations has slow kinetics \( b = 0.05/d \)

    → a high conc. of MLVSS *(operating problems with settler and recycle)*
    and a long HRT in a anoxic tank
    → high capital costs are necessary

  2) the decay of biomass always releases \( \text{NH}_4^+ \)-N from the anoxic step although at concentrations lower than in the influent.
10.3.1 \textit{2) Classical predenitrification}

- The first tank (anoxic); the influent BOD (electron donor) is directly utilized for denitrification.

- The second tank (aerobic); the influent TKN is nitrified to $\text{NO}_3^-$ and any remained BOD is oxidized.

- The nitrate formed in the aerobic tank is recycled to an anoxic tank ($Q_{r2}$; large)

Directly utilizes the influent BOD (electron donor) for denitrification

\begin{align*}
\text{TKN} & \rightarrow \text{NO}_3^- - \text{N} \\
\text{Remained BOD: aerobically consumed}
\end{align*}

\textbf{Fig. 10.1 (b) classical predenitrification}
- Fractional removal of $N = \sim \frac{Qr^2}{Q + Qr^2}$

$Q$: plant flow rate
$Qr^2$: mixed-liquor recycle flow rate

- The large recycle flow of $\text{NO}_3^-$ from the second to the first tank is necessary, because $\text{NO}_3^-$ not recycled leaves in the effluent.

- Recycle ratios of 400 percent or more are employed to bring enough $\text{NO}_3^-$ back to the anoxic tank so that total $N$ removals are substantial.
Widespread use worldwide;

**- Advantages:**

i) direct use of influent BOD for denitrification  
   → reduces aeration costs for the removal of BOD  
ii) faster kinetics than with *biomass storage and decay*  
iii) no release of NH$_4^+$-N in the effluent

**- Disadvantage:**

i) large mixed-liquor recycle rate  
   → increases costs of piping and pumping
Three factors allow all reactions to occur simultaneously.

1) Various nitrogen reductases using N as e⁻ acceptor are repressed only when the D.O. conc. is well above 1 mg/L.

2) However, inhibition of the nitrogen reductase is not severe when the D.O. conc. is less than 1 mg/L.

\[
NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2
\]

\[
NO_3^- + 2e^- + 2H^+ = NO_2^- + H_2O \quad \text{Nitrate Reductase}
\]

\[
NO_2^- + e^- + 2H^+ = NO + H_2O \quad \text{Nitrite Reductase}
\]

\[
2NO + 2e^- + 2H^+ = N_2O + H_2O \quad \text{Nitric Oxide Reductase}
\]

\[
N_2O + 2e^- + 2H^+ = N_2 + H_2O \quad \text{Nitrous Oxide Reductase}
\]
Three factors allow all reactions to occur simultaneously.

3) D.O. conc. is depressed inside the aggregates that normally form in treatment systems; thus, denitrification can occur inside the floc (or biofilm), as long as the electron donor (BOD) penetrates inside.

- When D.O. concentration is poised at a suitably low level (< ~ 1 mg/L), anoxic denitrification can occur in parallel to the aerobic reactions of nitrification and aerobic BOD oxidation.

Fig. 10.1 (c) simultaneous nitrification with denitrification
- 100 % N removal by simultaneous nitrification with denitrification has been documented (Rittmann and Langeland, 1985), and small amounts of denitrification probably occur in most activated sludge systems that nitrify and have D.O. conc. below saturation (deSilva, 1997)

- Simultaneous nitrification with denitrification offers all the advantages of predenitrification, but overcomes the main disadvantage, the high recycle rate.

- Maintaining a low D.O. conc. throughout one reactor creates an infinitely high recycle ratio and allows essentially 100 percent N removal.

- **Drawback**

  : We do not yet know the combinations of SRT, HRT, and D.O. conc. that guarantee reliability.
Common features of all one-sludge processes:

- one community (or one sludge) of microorganisms carries out all the reactions.

- The heterotrophs switch back and forth between aerobic and anoxic (endogenous) respiration, or they do both simultaneously.

- Because the nitrifiers are slow growing autotrophs, their growth rate controls the SRT needed. SRTs greater than 15 days are required in most cases, sometimes much longer SRTs are used.

- The longer SRTs provide an added safety factor for the nitrifiers, who experience periods of low or zero DO.

- The long SRTs mean that accumulation of inert suspended solids is important.
Common features of all one-sludge processes:

- A practical outcome of a long SRT is that the HRT (in settler) needs to increase in order to keep the MLSS conc. within reasonable limits dictated by settler performance. That’s why settler parameters for one-sludge denitrification is similar to those used for extended aeration activated sludge.

- HRTs for predinitrification and simultaneous nitrification with denitrification are at least 10 h for typical sewage, and 24 h or greater are used in some instances.

- To overcome the limitations of one-sludge denitrification, the Barnard process, sequencing batch reactor (SBR), and biofilm systems are developed.
10.3.2 Variations on the Basic One-Sludge processes

**Barnard process (1975)** by Dr. J. Barnard of South Africa

- Influent TKN : 50 mg/L
- recycle ratio ($Qr^2$) from reactor 2 to 1: 400%
- **Reactor 1**: denitrification rate is 100% of recycled input and 80% of the influent TKN ($Qr^2=400\%$)
- **Reactor 2**: $\text{NO}_3^-$-N/L leaving : 10 mg/L (20%)
- **Reactor 3**: endogenous decay of cells
  - 0.3 mg $\text{NH}_4^+$-N is released per mg $\text{NO}_3$-N due to endogenous cell decay
  - Because 10 mg/L of $\text{NO}_3$-N is converted to $\text{N}_2$ gas, Reactor 3 releases 3 mg NH$_3$-N/L
- **Reactor 4** releases 3 mg $\text{NO}_3$-N/L

**Final effluent TKN** : 3 mg $\text{NO}_3$-N/L

**Total removal rate** : 94%
10.3.2 Variations on the Basic One-Sludge processes

**Barnard process**

- Well established worldwide one-sludge denitrification process (> 90% N removal)

- **Drawbacks**
  - need many tanks
  - long HRT
  - significant mixed-liquor recycle (400%) between reactors 2 and 1

![Fig. 10.2 Schematic of the Barnard process](image-url)

50 mg/L TKN

Influent

1. Anoxic pre-denitrification
   \[ \theta \approx 3 \text{ h} \]
   (based on Q)

2. Aerobic nitrification
   \[ \theta \approx 11 \text{ h} \]

Mixed liquor recycle

3. Anoxic denitrification
   \[ \theta \approx 3 \text{ h} \]

4. Aerobic nitrification
   \[ \theta \approx 1 \text{ h} \]

3 mg NH$_4^+$-N/L

10 mg/L

3 mg NO$_3^-$-N/L

Effluent

Settler

Sludge recycle

Sludge wasting

Fig. 10.2 Schematic of the Barnard process
외부환경요인 (원수수질 등)에 따른 처리효율의 변동
외부탄소원 및 대사산물에 의한 처리수의 재오염 가능
높은 회수율 (Water Recovery) 성취 과제
Disadvantage of “Membrane–dissolution process”

i) 독립영양 미생물 (autotrophs)의 느린 성장 속도 및 낮은 활성에 의한 제거 효율 저하

ii) 미생물 및 대사산물에 의한 처리수의 재오염 (후처리 필요)

iii) 막내부의 수소 부분압 상승에 의한 폭발위험성

Autotrophic Membrane Bioreactor for nitrate removal

“Membrane-dissolution process” (Bruce Rittmann, Arizona State Univ.)
Ion Exchange Membrane Bioreactor, IEMB

(J.G. Crespo, Nova de Lisboa Univ., Portugal)
 Ion Exchange Membrane Bioreactor, IEMB
(J.G. Crespo, Nova de Lisboa Univ., Portugal)

- Non-porous and dense ion-exchange membrane
- Driving force: Concentration difference
  - Electrical Potential Difference
  - Counter current-ion transport
- Used to remove the toxic heavy metal and to soften the drinking water

- Principle of Donnan Dialysis

K^+ K^+ Cl^- Cl^- NO_3^- NO_3^-
10.6 Ion Exchange Membrane Bioreactor, IEMB
(J.G. Crespo, Nova de Lisbona Univ., Portugal)

**- Principle of IEMB**

![Diagram showing the principle of IEMB](image)

- **K^+**
- **HCO_3^-**
- **Cl^-**
- **NO_3^-**
- **NO_2^-**

**Equation:**

\[ \text{N}_2 + \text{CO}_2 \leftrightarrow \text{NO}_2^- \leftrightarrow \text{NO}_3^- \]

**Annotations:**

- **Tan so won**
- **+Milaengyang bun**
- **+Counter-ion (Cl^-)**