Nitrogen Metabolism and The Nitrogen Cycle

READING: BOM-12

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   Nitrate Reduction and Denitrification
   Biochemistry of Dissimilative Nitrate Reduction
   Other Properties of Denitrifying Prokaryotes

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   Nitrogen Fixation
   Denitrification
   Ammonia Fluxes, Nitrification, and Ammox

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   Biochemistry of Dissimilative Nitrate Reduction
   Other Properties of Denitrifying Prokaryotes

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   Bioenergetics and Enzymology of Nitrification
   Other Nitrifying Prokaryotes
   Carbon Metabolism and Ecology of Nitrifying Bacteria

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   Ammonia- and Nitrite-Oxidizers
   Ecology, Isolation, and Culture
24.3 The Nitrogen Cycle

Modeling: Compartments
- Reservoirs
- Fluxes
- Residence Times
- Balance

Most cycling is carried out by living organisms, particularly bacteria.

Mean residence time of N\(_2\) in the atmosphere is >40 million years; compared to <1 year for soil inorganic N.

Most soil N is in form of organic compounds (R-NH\(_2\)), not inorganic ions.

Nitrogen cycling involves changes in oxidation state.
Nitrogen Fixation \[ \text{N}_2 \rightarrow \text{NH}_3/\text{NH}_4^+ \]

Biological
Non-biological
Industrial (Haber-Bosch)

Denitrification (=Dissimilatory Reduction)

Assimilatory Reduction \[ \text{NO}_2^- \rightarrow \text{NH}_3/\text{NH}_4^+ \]
Disimilatory Reduction \[ \text{NO}_3^- \rightarrow \text{NH}_3/\text{NH}_4^+ \text{ N}_2 \text{ N}_2\text{O NO} \]

Ammonia Fluxes, Nitrification, and Ammox

Ammonification \[ \text{R-NH}_2 \rightarrow \text{NH}_3/\text{NH}_4^+ \]
Nitrification \[ \text{NH}_3/\text{NH}_4^+ \rightarrow \text{NO}_3^- \]
21.6 Anaerobic Respiration: General Principles

21.7 Nitrate Reduction and Denitrification

Biochemistry of Dissimilative Nitrate Reduction

In Fig. 21.14(a) and (b) notice that the components of the E.coli electron transport chain differ somewhat in detail from those you learned for the mitochondrial electron transport chain. These differences reflect the evolutionary divergence between E. coli and the bacterial ancestor of the mitochondrion.

In Fig. 21.14(c) note that the Nitrate Reductase (NR) of Pseudomonas stutzeri is different that the E. coli NR because nitrate is reduced in the periplasm rather than the cytoplasm. Also, the NR of P. stutzeri appears to partition electron flow to the 4 separate reductase steps.

Assimilatory vs Dissimilatory Nitrate Reduction

<table>
<thead>
<tr>
<th></th>
<th>Assimilatory Nitrate Reduction</th>
<th>Dissimilatory Nitrate reduction (Denitrification)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>cytoplasm</td>
<td>cell membrane</td>
</tr>
<tr>
<td>Goal</td>
<td>nitrogen for assimilation to</td>
<td>terminal electron acceptor for electron transport</td>
</tr>
<tr>
<td></td>
<td>biomass (R-NH₂)</td>
<td></td>
</tr>
<tr>
<td>Regulation</td>
<td>repressed by NH₃</td>
<td>repressed by O₂</td>
</tr>
<tr>
<td>End Products</td>
<td>NH₃</td>
<td>NO, N₂O, N₂O₅, N₂, NO₂⁻ (NH₃⁻)</td>
</tr>
</tbody>
</table>

Assimilatory Nitrate Reduction

Dissimilatory Nitrate reduction (Denitrification)

\[
\begin{align*}
\text{Assimilatory Nitrate Reduction} & \\
\text{Nitrate Reductase} & \\
\text{Nitrite Reductase} & \\
\end{align*}
\]

\[
\begin{align*}
& \text{O} \quad \text{O} \\
& \text{R-C-COH} \quad \text{amination/} \\
& \text{transamination} \quad \text{H-O} \\
& \text{NH}_2 \quad \text{H}_2 \quad \text{O} \\
& \alpha\text{-keto acid} \\
\end{align*}
\]
Assimilatory vs. Dissimilatory Nitrate Reduction

**Assimilatory Reduction**
- Nitrate reductase
- \( NO_3^- \) to \( NO_2^- \)
- Nitrite reductase
- \( NO_2^- \) to \( NO \)
- \( NO \) to \( N_2 \)
- \( NH_4^+ \) to \( R-NH_2 \)

**Dissimilatory Reduction**
- Nitrate reductase
- \( NO_3^- \) to \( NO_2^- \)
- Nitrite reductase
- \( NO_2^- \) to \( NO \)
- \( NO \) to \( N_2 \)
- \( NH_4^+ \) to \( NH_4^+ \)

**Reactions**
- Assimilatory: nitrogen linked to electron transport, not coupled to electron transport
- Dissimilatory: nitrogen linked to electron transport, coupled to electron transport

**Notes**
- Assimilatory can do one or both depending on conditions.

**Remarks**
- Results from overfertilization or poor irrigation in agricultural soils.
20.12 Nitrification
Bioenergetics and Enzymology of Nitrification
Carbon Metabolism and Ecology of Nitrifying Bacteria

15.3 The Nitrifying Bacteria
Ammonia- and Nitrite-Oxidizers
Ecology, Isolation, and Culture

Nitrification is the biogeochemical process by which ammonia \([\text{NH}_3]\) or its equivalent, ammonium ion \([\text{NH}_4^+]\), is oxidized to nitrate \([\text{NO}_3^-]\).

Nitrification is considered a problem in agricultural ecosystems because it indirectly leads to removal of nitrogen from the rhizosphere. \text{NH}_4^+ is retained by soils because it readily adsorbs to anionically charged clays. \text{NO}_3^- , on the other hand, is not adsorbed to soil particles and therefore is readily leached from soils – particularly from tropical soils due to the abundant rainfall. An associated problem is nitrate contamination of groundwater by runoff from heavily fertilized agricultural fields subjected to intense cultivation by modern methods. Treatment with the synthetic chemical nitrapyrin ("N-Serve") can reduce nitrification because it inhibits a specific bacterial enzyme required for ammonia oxidation. Currently, plant biochemical ecologists are exploring the possibility that plants excrete natural chemicals, such as terpenes, that inhibit nitrification.

Nitrification is almost entirely the result of bacterial metabolism. The bacteria responsible, the "nitrobacteria", are aerobic chemolithotrophs, as the net reaction below suggests. They are also autotrophs and all use the Calvin-Benson cycle to fix \(\text{CO}_2\). Nitrobacteria are virtually ubiquitous in soil, freshwater and marine environments. They are difficult to grow in pure laboratory cultures, however, because they grow very slowly and the growth yield is low. Cultures are often overgrown by heterotrophic bacterial contaminants that metabolize organic compounds released by the nitrobacteria.

Nitrification is actually a two-step process, with each step mediated by a specific subgroup of the nitrobacteria.

\textbf{Nitrobacteria = Nitrosofying Bacteria + Nitrifying Bacteria}

\begin{align*}
\text{STEP 1} & \quad \text{Nitrosofying Bacteria} \quad \text{NH}_3 + \frac{3}{2} \text{O}_2 \quad \longrightarrow \quad \text{NO}_2^- + \text{H}^+ + \text{H}_2\text{O} \quad \{\Delta 6\text{e}^-\} \\
\text{STEP 2} & \quad \text{Nitrifying Bacteria} \quad \text{NO}_2^- + \text{H}_2\text{O} + \frac{1}{2} \text{O}_2 \quad \longrightarrow \quad \text{NO}_3^- + \text{H}_2\text{O} \quad \{\Delta 2\text{e}^-\} \\
\text{NET NITRIFICATION} & \quad \text{NH}_3 + 2 \text{O}_2 \quad \longrightarrow \quad \text{NO}_3^- + \text{H}_2\text{O} + \text{H}^+ \quad \{\Delta 8\text{e}^-\}
\end{align*}

It is interesting to note that the nitrite \([\text{NO}_2^-]\) produced by the nitrosofying bacteria is toxic to most cells, including nitrobacteria. However, nitrite rarely accumulates to significant levels in natural environments because nitrobacteria usually occur in intimate association with each other so that nitrite is quickly consumed by nitrifying bacteria as soon as it is produced by nitrosofying bacteria. Nitrite toxicity is a problem when Nitrosofying bacteria are grown in pure cultures and this adds to the difficulties of laboratory cultivation mentioned above.
NITROSOFYING BACTERIA; Ammonia Oxidizers

**AMMONIA MONOOXYGENASE**

\[ \text{NH}_3 + \text{O}_2 + 2 \text{H}^+ + 2 \text{e}^- \rightarrow \text{NH}_2\text{OH} + \text{H}_2\text{O} \]

**HYDROXYLAMINE OXIDOREDUCTASE**

\[ \text{NH}_2\text{OH} + \text{H}_2\text{O} \rightarrow \text{NO}_2^- + 5 \text{H}^+ + 4 \text{e}^- \]

**ELECTRON TRANSPORT**

\[ 2 \text{e}^- + 2 \text{H}^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{H}_2\text{O} (+\Delta p) \]

**NET**

\[ \text{NH}_3 + \frac{3}{2} \text{O}_2 \rightarrow \text{NO}_2^- + \text{H}^+ + \text{H}_2\text{O} \]

\[ \Delta G'' = -287 \text{ kJ/mole} \]

\[ \Delta E'' = +0.44 \text{ V} \]

Fate of \( \Delta 6\text{e}^- \):
- \( \Delta 2\text{e}^- \) to reduce \( \frac{1}{2} \text{O}_2 \) *directly* in monooxygenase reaction
- \( \Delta 2\text{e}^- \) electron transport to \( \frac{1}{2} \text{O}_2 \); \( \Delta 2 \text{H}^+ \)
- \( \Delta 2\text{e}^- \) to reduce \( \frac{1}{2} \text{O}_2 \) *indirectly* in monooxygenase reaction

The monooxygenase reaction is noteworthy because molecular oxygen is a substrate for the enzyme and is reduced by 4 electrons. Only 2 of the electrons come from ammonia directly, the other two come from the oxidation of a special cytochrome. In the third reaction of the metabolic scheme a final 2 electrons from nitroxyl intermediate are used to reduce the cytochrome again. Of the 6 electrons available, only two participate in electron transport, limiting the ATP yield. (Note the absence of substrate level phosphorylations.)

Representative Genera:

*Nitrosononas* sp.
*Nitrosococcus* sp.

Compare to Fig. 20.32
NITRIFYING BACTERIA; Nitrite Oxidizers

\[
\begin{align*}
\text{NO}_2^- + \text{H}_2\text{O} & \rightarrow \text{NO}_3^- + 2 \text{H}^+ + 2 \text{e}^- \\
2 \text{H}^+ + 2 \text{e}^- + \frac{1}{2} \text{O}_2 & \rightarrow \text{H}_2\text{O}
\end{align*}
\]

\[
\text{NO}_2^- + \text{H}_2\text{O} + \frac{1}{2} \text{O}_2 \rightarrow \text{NO}_3^- + \text{H}_2\text{O}
\]

\[\Delta G^\circ = -76 \text{ kJ/mole}\]
\[\Delta E^\circ = +0.42 \text{ V}\]

\[\Delta 2\text{e}^- \text{ electron transport to O}_2 \text{ through cytochromes; } \Delta 2 \text{H}^+; \text{ only } \sim1\text{ATP/NO}_2^-\]

Representative Genera:
- *Nitrobacter* sp.
- *Nitrococcus* sp.
- *Nitrospira* sp.
DISCUSSION QUESTIONS:

1. Describe the biochemical process by which inorganic nitrate ions enter biosynthetic metabolism?

2. According to the simple ecosystem model in the notes:
   i) What is the predominant mode by which nitrogen is removed from the terrestrial environment?
   ii) Is the terrestrial environment in N balance?

3. Why is nitrification often considered a detrimental process from a human perspective?

5. Why do nitrifying bacteria and nitrosofying bacteria have low growth rates and low growth yields?

6. What are the net reactions for the metabolic conversions of the nitrifying and nitrosofying bacteria?

7. For each process below, does it occur in:
   a. primarily aerobic environments
   b. primarily anaerobic environments
   c. either aerobic or anaerobic environments.

   Explain why this is the case.

   Denitrification (dissimilatory nitrate reduction)
   Assimilatory Nitrate Reduction
   Nitrification
   Nitrogen Fixation