From Chap. 4 "Ecology of denitrification and dissimilatory nitrate reduction to ammonium" by J.M. Tiedje *In* Biology of Anaerobic Microorganisms edited by A.J.B. Zhender. Wiley-Liss 1988.

Dissimilatory nitrate reduction - two forms

- Denitrification - $NO_3^-$ to $NO_2^-$ then to gaseous end products $N_2O$ or $N_2$
- Dissimilatory reduction of nitrate to ammonium - $NO_3^-$ to $NO_2^-$ to $NH_4^+$

Although the theoretical energy yield of the two processes appears similar,

$$2NO_3^- + 2H^+ + 5\,H_2 \rightarrow N_2 + 6H_2O$$

$\Delta G° = -1120.5$ kJ per reaction

vs

$$NO_3^- + 2H^+ + 4H_2 \rightarrow NH_4^+ + 3H_2O$$

$\Delta G° = -599.6$ kJ per reaction

the denitrification process yields much more ATP.

Dissimilatory vs assimilatory nitrate reduction

Dissimilatory

- oxidized nitrogen used as electron acceptor for cells' metabolic processes
- results in greater ATP yield/substrate oxidized than fermentation
- nitrogen not incorporated primarily into cells - inhibited by oxygen
- usually only occurs in anaerobic environments

Assimilatory

- nitrogen incorporated into cell mass
- represents energy loss
- usually repressed or quantitatively insignificant in anaerobic environments due to the usual presence in anaerobic environments of large concentrations of ammonia and organic nitrogen compounds
- not affected by oxygen status
- inhibited by reduced nitrogen compounds

**Respiratory denitrification** - reduction of oxidized nitrogen coupled to electron transport phosphorylation (ETP) - get more ATP formed than if oxidized nitrogen acting simply as electron acceptor

80% or more of nitrate or nitrite converted to nitrous oxide and nitrogen gas

rapid rate of reduction

Convenient preliminary id of most respiratory denitrifiers

acetylene block - near stoichiometric accumulation of $N_2O$

Acetylene inhibits nitrous oxide reductase - does not affect the amount of nitrous oxide produced from nonrespiratory denitrifiers or chemical processes

**Nonrespiratory denitrifiers** typically produce nitrous oxide not nitrogen gas as end product - several groups of organisms may carry out this process - typically relatively minor amount of nitrogen

The mechanism is unknown - in the Enterobacteriaceae possibly nitrate reductase acting on nitrite

**Dissimilatory nitrate reduction to ammonium**

- dissimilatory pathway regulated by oxygen - unaffected by ammonia
- assimilatory pathway regulated by ammonia, unaffected by oxygen

In anaerobic environments, availability of electron acceptors is often limiting step, reduction of nitrate to ammonia takes 8 electrons

Since both dissimilatory nitrate reduction to ammonia and assimilatory nitrate reduction both yield ammonia as the primary product - distinguished by production of ammonia in excess of that needed for cell growth by the dissimilatory pathway. Also, addition of 1 mM ammonium is sufficient to repress assimilatory pathways.

**Denitrifiers** cause losses of nitrogen fertilizers to the atmosphere often 20 to 30% but may be up to 70%

Beneficial in waste treatment can remove excess nitrogen

May contribute to ozone depletion by production of nitrous oxide

Denitrification produces nitrite, and small amount of nitric oxide which are toxic and may cause local hazards.

In addition nitrite can react with secondary amines to produce nitrosamines

Balances nitrogen cycle

Increase nitrogen load on an ecosystem often also accompanied by increased carbon load - this results in increased oxygen consumption creating conditions favorable for denitrifiers Organisms

Denitrifiers are usually aerobic bacteria which have alternative capacity to reduce nitrogen oxides when oxygen becomes limiting (don't require strict anaerobic conditions for culture)

Using an aerobic inoculum directly into strictly anaerobic medium may delay or prevent growth due to inability of organism to generate the energy necessary to synthesize the required denitrifying enzymes.

Most denitrifiers can use the other nitrogen compounds in the pathway, nitrite and nitrous oxide, as electron acceptor in media as substitute for nitrate. (concentration of nitrite in medium should be reduced - 2 to 3 mM nitrite will inhibit some denitrifiers).

Energy sources for denitrifiers can include organic, inorganic, or light.

Most common denitrifiers are Pseudomonas then Alcaligenes.

Denitrification is widely distributed in nature - large diversity of organisms

Easier to list those groups which do not have at least some strains which denitrify - three most notable are obligate anaerobes, gram-positive organisms other than Bacillus, and the Enterobacteriaceae.

Potential use for pollutant destruction

1. Denitrifiers have the highest growth yield and are easiest to grow of organisms capable of anoxic growth
2. genetic understanding and manipulation is advanced in the Pseudomonads
3. Nitrate is very soluble and cheap so it can be readily delivered to polluted areas

If nitrate-reducers can be used for degrading a pollutant under anaerobic conditions (1) construction costs lower since lower hydraulic volumes possible in nonaerated systems, (2) odor control, (3) high growth yields hence high reaction rates.

Stopping reaction at the stage of nitrite or nitrous oxide undesirable

1. may occur because of limited electron donor
2. too short incubation time
3. production of toxic products in static incubation such that metabolism halts before completion (stationary phase reached, pH shift, etc.).

## Dissimilatory nitrate reduction to ammonia

Not well characterized on a biochemical level, but recognized as being important in some habitats such as the rumen, anaerobic sludge, and anoxic sediments

Usually found in organisms having fermentative rather than oxidative metabolism

First step is nitrate reduction to nitrite and is coupled to energy production in most organisms (except Clostridium). This is referred to as nitrate respiration - this step occurs in many organisms including those which do not reduce nitrate to ammonia. Production of ammonia is the defining step.

Postulated benefits to cell

(1) detoxification of accumulated nitrite
(2) electron sink allow reoxidation of NADH to $NAD^+$
(3) energy by ETP

#2 is thought to be the most important

In Clostridium which has no ETP, if grown on glucose with nitrate reduction to ammonia vs glucose alone, growth yield increased 15.7%

This increase thought to be due to increase substrate level phosphorylation permitted by dumping electrons on oxidized nitrogen.

Since energy is not conserved in the nitrite to ammonia steps in most organisms but is conserved in the nitrate to nitrite step, possible explanation for accumulation of nitrite under carbon limited conditions.

Under nitrate limitation, the need for electron acceptor is predominant so reduction to ammonium is expected.

**Adaptive traits of denitrifiers**

Positive chemotaxis for nitrate, thought to be important in aqueous environments and microbial films

**Oxygen control of denitrification**

Evidence for a threshold oxygen concentration

Oxygen of microsite controlled by rate of oxygen supply to that site versus rate of oxygen consumption (respiration).

Oxygen transport heavily influenced by
- impermeable particles (clay, rock, etc)
- restriction of oxygen transport also occurs due to
  - biological surfaces (cell membranes, plant roots, organic debris, etc.).
- Water affects transport of oxygen

aerobic photosynthesis adds $O_2$

Interplay of oxygen diffusion and oxygen consumption shown by distance from oxygenated water to denitrifying zone 10 μm in biofilms to over 100 m in some low carbon open ocean areas.