Biological nutrient removal

Biological oxidation of nitrogen

- Necessity for NH_4-N & NO_2-N oxidation
  - The effect of ammonia on receiving water with respect to DO concentrations and fish toxicity
  - The need to provide nitrogen removal to control eutrophication
  - The need to provide nitrogen control for water-reuse applications

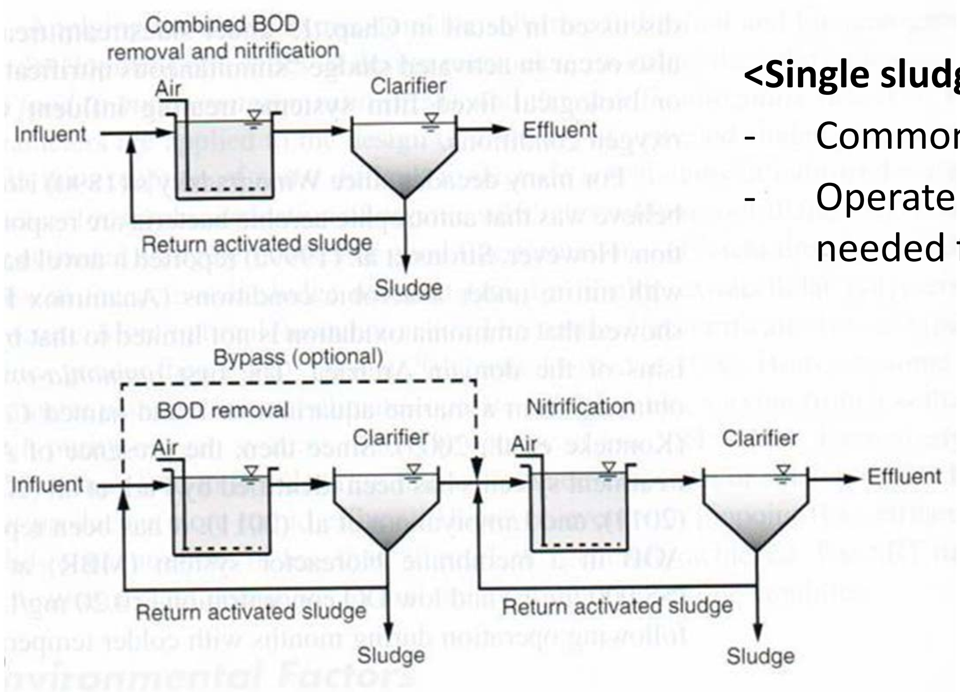
- **Nitrification**
  - Two-step biological process: NH_4-N → NO_2-N & NO_2-N → NO_3-N
  - The first step [NH_4-N → NO_2-N] is termed as "nitritation"
  - Different type of microorganisms are involved for each step

- **Anammox process**
  - **An**aerobic **Amm**onia **Ox**idation
  - Some bacteria can oxidize ammonia with nitrite under anaerobic conditions:

$$NH_4{}^+ + NO_2{}^- \rightarrow N_2 + 2H_2O$$

Nitrification processes

- Both suspended & attached growth applicable
- Suspended growth nitrification processes
  - Note nitrifying bacteria are less competent than aerobic heterotrophs → need maintaining low BOD conc. to activate them!



<Single sludge suspended growth system>

- Common
- Operate at high SRT than what's needed for BOD removal

<Two-sludge suspended growth system>

- Good for wastewater containing toxic substances
- 1st unit operated at short SRT for BOD removal (+toxic removal)
- 2nd unit for nitrification at low BOD

Microbiology of nitrification

- **Ammonia-oxidizing bacteria (AOB) & nitrite-oxidizing bacteria (NOB)**
- Aerobic chemoautotrophs
- Major AOB: *Nitrosomonas* (+*Nitrosospira*)
- Major NOB: *Nitrobacter* (+*Nitrococcus, Nitrospina, Nitrospira*)

Stoichiometry of nitrification

AOB (nitration): $2NH_4{}^{+} + 3O_2 \rightarrow 2NO_2{}^{-} + 4H^{+} + 2H_2O$

NOB: $2NO_2{}^{-} + O_2 \rightarrow 2NO_3{}^{-}$

Overall: $NH_4{}^{+} + 2O_2 \rightarrow NO_3{}^{-} + 2H^{+} + H_2O$

*Note: This is stoichiometry **NOT** considering biomass production*

- Oxygen requirement: 2 mole O_2/1 mole NH_4^+
  = 4.57 g O_2/g NH_4-N oxidized
- Alkalinity consumption: 2 eq alkalinity/1 mole NH_4^+
  = 7.14 g Alk as $CaCO_3$/g NH_4-N oxidized
- Nitrification cell yield: 0.10~0.15 for AOB & 0.04~0.07 for NOB
- Considering biomass production, the O_2 requirements and alkalinity consumption is slightly less than the calculated values above (**<u>why??</u>**)

Stoichiometry of nitrification

ex) Assuming Y=0.12 g VSS/g NH_4-N for AOB and Y=0.04 g VSS/g NO_2-N for NOB, the overall stoichiometry is:

$$NH_4(HCO_3) + 0.9852Na(HCO_3) + 0.0991CO_2 + 1.8675O_2 \rightarrow$$
$$0.01982C_5H_7O_2N + 0.9852NaNO_3 + 2.9232H_2O + 1.9852CO_2$$

→ 1.8675 mole O_2/1 mole NH_4^+
1.9852 eq Alk/1 mole NH_4^+

Environmental factors affecting nitrification

- **DO concentration**
  - Nitrifying bacteria are more sensitive to DO than heterotrophs
  - Monod equation is applicable to nitrification kinetics, and in most cases DO should be treated as one of the major limiting substrate

$$\mu_{AOB} = \mu_{max,AOB}\left(\frac{S_{NH}}{S_{NH} + K_{NH}}\right)\left(\frac{S_o}{S_o + K_{o,AOB}}\right) - b_{AOB}$$

$$\mu_{NOB} = \mu_{max,NOB}\left(\frac{S_{NO}}{S_{NO} + K_{NO}}\right)\left(\frac{S_o}{S_o + K_{o,NOB}}\right) - b_{NOB}$$

  - Nitrite oxidation inhibited more at low DO than ammonia oxidation: elevated NO_2-N concentration at low DO (K value 2~3 times greater for NOB)

Environmental factors affecting nitrification

- **pH**
  - Optimum at pH of 7.5~8.0
  - Ammonia oxidation rate reduces significantly at pH<7.0
  - Possibly due to the reduction of free ammonia (NH_3) concentration
  - **Sufficient alkalinity is needed!**
  - For wastewater with high NH_4^+ concentrations and low alkalinity, addition of alkalinity may be needed (lime, soda ash, $NaHCO_3$, …)
- **Toxicity**
  - AOB is sensitive to a wide range of organic & inorganic compounds
  - Show significantly reduced ammonia oxidation rate in the presence of toxic substances
- **Free ammonia & nitrous acid inhibition**
  - NH_3-N & HNO_2
  - High pH: NH_3-N ↑ / low pH: HNO_2 ↑

Denitrification

- **Biological reduction of nitrate (NO_3^-) or nitrite (NO_2^-) to nitrogen gas (N_2)**

- **Denitrification required**
  - To complete the biological nitrogen removal process
  - Otherwise, accumulation of NO_3-N: health threats!
  - "Blue baby syndrome"
  - Korean regulation: < 10 mg NO_3-N/L

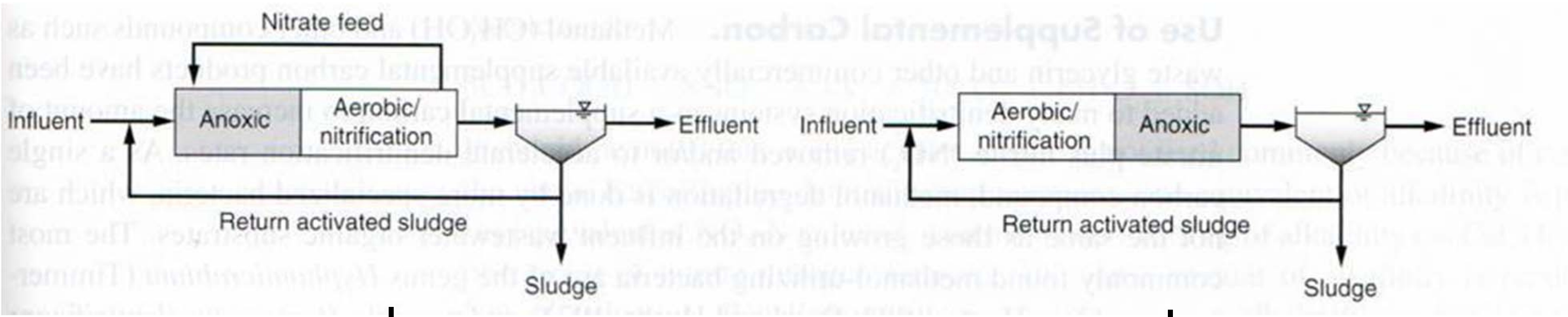
- **Usually by heterotrophic bacteria**
  - Wide range of heterotrophs – mostly facultative aerobes
  - Some autotrophs are capable of nitrate/nitrite reduction
    - Use Fe^0, Fe^{2+}, S^{2-}, S^0, …, or NH_4^+

Denitrification

- Two modes of nitrate removal in biological processes
  - **Assimilatory nitrate reduction**
    - Reduction of NO_3-N to NH_4-N for use in cell synthesis when NH_4-N is not available
    - Independent of DO concentration
  - **Dissimilatory nitrate reduction**: much more significant!
    - Nitrate/nitrite serves as an electron acceptor
    - When DO is absent or limited
    - Mostly facultative bacteria
    - Nitrate reduction proceeds through a series of intermediate products:

$$NO_3^{-} \rightarrow NO_2^{-} \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Denitrification processes



- **Preanoxic denitrification**
  - Electron donor provided by influent
  - MLE (Modified Ludzak-Ettinger) process: most common for biological nitrogen removal in municipal wastewater treatment
- **Postanoxic denitrification**
  - BOD not available in anoxic reactor: denitrification by endogenous decay
  - Much slower rate than preanoxic
  - Often external carbon source is added (e.g. methanol, acetate)

Stoichiometry

Wastewater: $C_{10}H_{19}O_3N + 10NO_3{}^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^-$

Methanol: $5CH_3OH + 6NO_3{}^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^-$

Acetic acid: $5CH_3COOH + 8NO_3{}^- \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^-$

- **Production of alkalinity**
  - 3.57 g Alk as $CaCO_3$ produced per g NO_3-N (or NO_2-N) reduced
  - 50% of alkalinity consumed by nitrification can be recovered

Organic substrate requirements

- A sufficient amount of organic substrate (e^- donor) should be available
  - **bsCOD or BOD as an important design parameter**
  - **Sources of e^- donor for denitrification**
    1) bsCOD in the influent
    2) bsCOD produced during biological hydrolysis
    3) bsCOD produced during endogenous decay
    4) External source such as methanol or acetate
  - ~4 g BOD required per g NO_3-N reduced
    - actual requirement depending on operating conditions and the type of e^- donor
  - Especially important to determine the BOD requirements when external carbon source is provided

Simultaneous nitrification and denitrification

- In activated sludge floc (suspended growth) or biofilm (attached growth)
- Local conditions in the floc or biofilm may be different from bulk liquid
- **High DO at the exterior and low DO inside** → conditions for nitrification and denitrification may develop in a single floc or biofilm
- Can be significant if optimal conditions are developed

Anammox process

- **Anaerobic oxidation of ammonia to produce nitrogen gas**

$$NH_4{}^+ + NO_2{}^- \rightarrow N_2 + H_2O$$

e^- donor *e^- acceptor*

- **Requires aerobic nitritation of ammonia to NO_2^-** for the process to occur (~55% conversion of NH_4-N to NO_2-N)

- **By autotrophic bacteria**
  → No organic carbon consumption during the process

Anammox process

$$NH_4{}^{+} + NO_2{}^{-} \rightarrow N_2 + H_2O$$

- **Proposed metabolic model** (Van de Graaf et al., 1997)
  1) Reduction of nitrite to hydroxylamine (NH_2OH)
  2) Condensation of hydroxylamine with ammonium to hydrazine (N_2H_4)
  3) Oxidation of hydrazine to nitrogen gas

- **Some formation of NO_3-N from NO_2-N**
  – To provide the reducing power to fix CO_2

$$CO_2 + 2NO_2{}^{-} + H_2O \rightarrow CH_2O + 2NO_3{}^{-}$$

- **Overall reaction** (Strous et al., 1999)

$$1.0NH_4{}^{+} + 1.32NO_2{}^{-} + 0.066HCO_3{}^{-} + 0.13H^{+}$$
$$\rightarrow 1.02N_2 + 0.26NO_3{}^{-} + 0.066CH_2O_{0.5}N_{0.021} + 2.03H_2O$$

Anammox process

- **Microorganisms for Anammox**
  - Forms dense granular flocs
  - Slow-growing bacteria – better seed the reactor with the dense granular flocs
  - High bacterial concentration (10^{10}~10^{11} cells/mL) should be maintained for good anammox activity

GHG from biological N removal

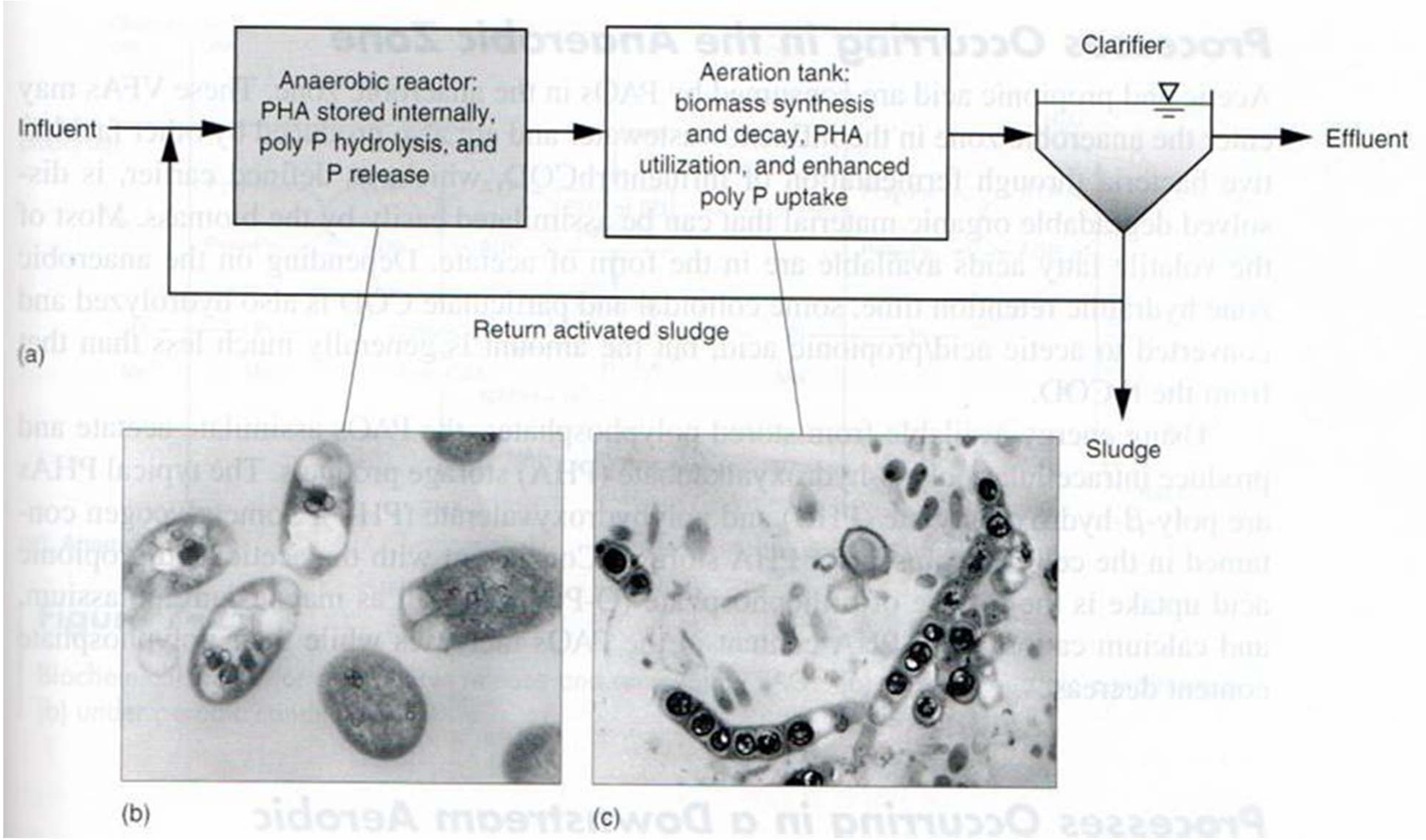
- **Nitrous oxide (N_2O)**
  - A potent greenhouse gas (GHG): 300 times greater potency than CO_2
  - Agriculture is the major source of N_2O emission

- N_2O emissions by wastewater treatment
  - Contributes 3% of total global emissions
  - N_2O emissions greater in the aerobic zones than the anoxic zones
  - **From heterotrophic denitrification**
    - Not produced significantly at steady-state operations, but can be significant at transient state
  - **From ammonia oxidation (AOBs)**
    - By hydroxylamine oxidation: $NH_2OH \rightarrow NOH\cdot \rightarrow NO \rightarrow N_2O$
    - By nitrite reduction: AOBs can use hydroxylamine, H_2, and NH_4^+ as e^- donors for NO_2^- reduction

Enhanced biological P removal

- Involves incorporation of P in the biomass produced in the treatment system and subsequent removal of the biomass as waste sludge
- Biomass of heterotrophic bacteria contains ~0.015 g P/g VSS
  - Insufficient to remove P from influent wastewater (only 10~20% of total)
- Use **phosphorus accumulating organisms (PAOs)** for ***enhanced*** **biological phosphorus removal (EBPR)**
- Reduced chemical costs and less sludge production compared to chemical precipitation

Enhanced biological P removal



Enhanced biological P removal

- **Process description**
  - Place an anaerobic tank ahead of the aeration tank
    - Provide selectivity for growth of PAOs
  - In the anaerobic tank, PAOs consume energy stored in the form of polyphosphates
    - The energy generated is used to convert volatile fatty acids into carbohydrate storage products (PHA)
  - In the aerobic tank, PAOs consume COD & stored PAH for biomass growth
    - Use some of the energy for enhanced P uptake to store polyphosphates
  - So:
    - Anaerobic tank: PHA accumulation & P release
    - Aerobic tank: excessive P uptake & PHA utilization
  - PAOs form very dense floc with good settleability – additional benefit

Enhanced biological P removal

- **Process occurring in the anaerobic zone**
  - Volatile fatty acids (VFAs) are produced by fermentation
  - VFAs are assimilated by PAOs into PHAs by energy available from stored polyphosphates
    - Typical PHAs: poly-β-hydroxybutyrate (PHB) & polyhydroxyvalerate (PHV)
    - Some glycogen contained in the cell is also used
- **Processes occurring in the aerobic/anoxic zone**
  - Stored PHA is metabolized to provide energy for cell growth
  - Some glycogen is produced from PHA metabolism
  - Soluble orthophosphate in solution in taken up by PAOs to form polyphosphates in the existing cells and the new cells
  - Portion of the biomass is wasted → P removal
  - The process can occur in the anoxic zone as well (NO_3^- or NO_2^- as e^- acceptors)

Enhanced biological P removal

- **Environmental factors**
  - Competition with GAOs
  - Glycogen accumulating organism (GAO): glycogen storage under aerobic condition & VFA uptake in the anaerobic tank to store PHA under anaerobic condition
  - Higher GAO population results in reduced P removal efficiency
  - Factors affecting the competition between PAOs & GAOs
    - pH > 7.0 favorable for PAO growth over GAOs (pH~7.5 optimum)
    - PAOs dominate GAOs below 15°C & above 30°C
    - Low aerobic tank SRT favorable for PAOs
    - Alternating VFA feed between acetate and propionate can eliminate GAOs