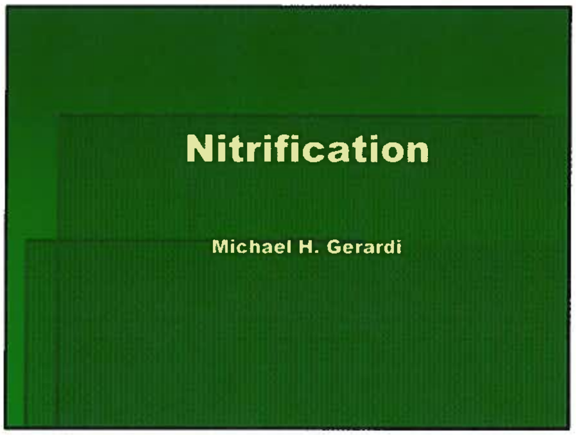
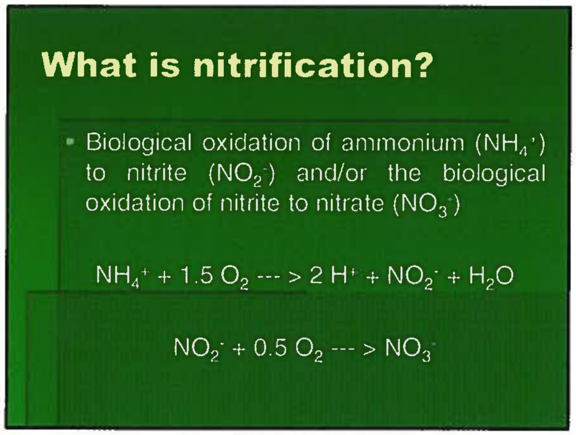


Nitrification

Michael H. Gerardi

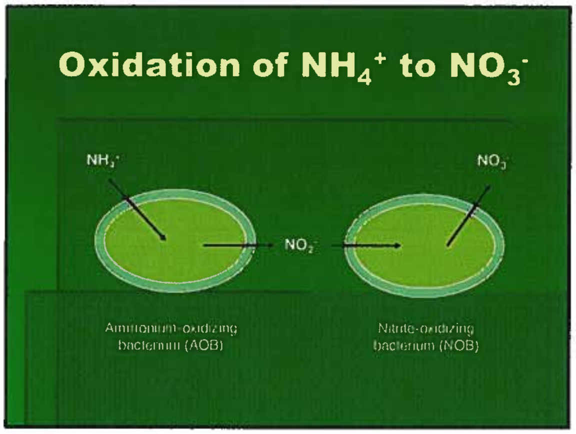


What is nitrification?

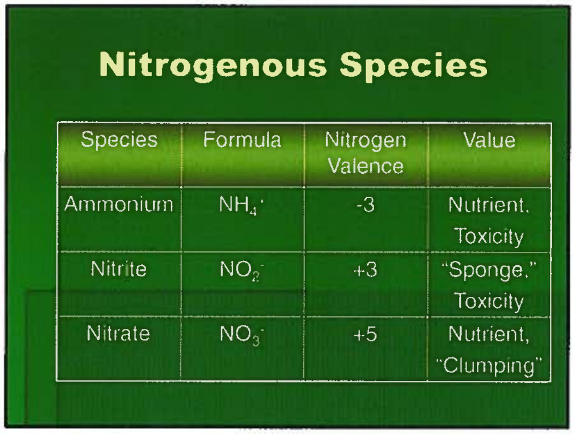
- Biological oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and/or the biological oxidation of nitrite to nitrate (NO_3^-)

$$NH_4^+ + 1.5\ O_2 \text{ --- > } 2\ H^+ + NO_2^- + H_2O$$

$$NO_2^- + 0.5\ O_2 \text{ --- > } NO_3^-$$

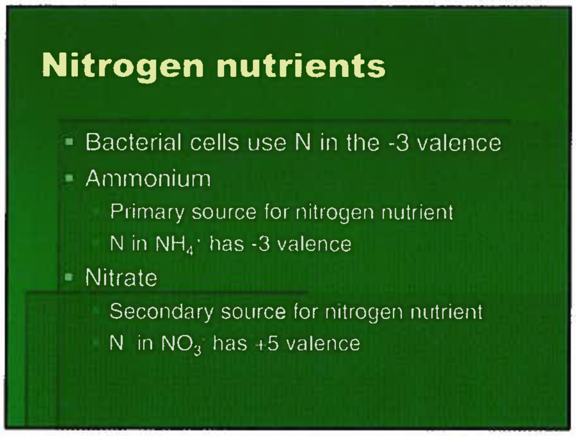


Oxidation of NH_4^+ to NO_3^-



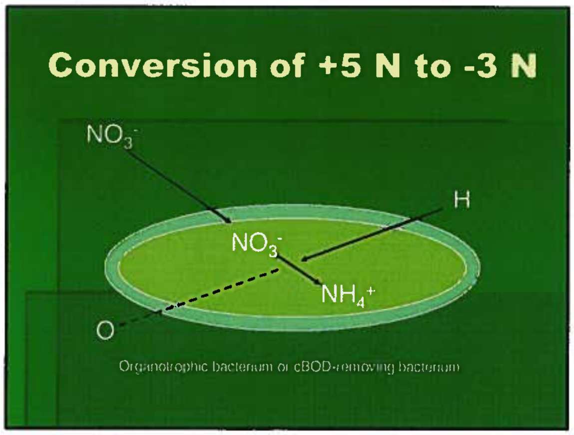
Nitrogenous Species

Species	Formula	Nitrogen Valence	Value
Ammonium	NH_4^+	-3	Nutrient, Toxicity
Nitrite	NO_2^-	+3	"Sponge," Toxicity
Nitrate	NO_3^-	+5	Nutrient, "Clumping"



Nitrogen nutrients

- Bacterial cells use N in the -3 valence
- Ammonium
  - Primary source for nitrogen nutrient
  - N in NH_4^+ has -3 valence
- Nitrate
  - Secondary source for nitrogen nutrient
  - N in NO_3^- has +5 valence



Conversion of +5 N to -3 N

"Clumping"

- Clumping is denitrification or "rising sludge" in the secondary clarifier
  - Occurs in the absence of free molecular oxygen (O_2) or presence of an oxygen gradient
  - Occurs in the presence of soluble cBOD and nitrate (NO_3^-)
  - Results in the release of three gases, molecular nitrogen ($N_2\uparrow$), nitrous oxide ($N_2O\uparrow$) and carbon dioxide ($CO_2\uparrow$)

Clumping/Denitrification

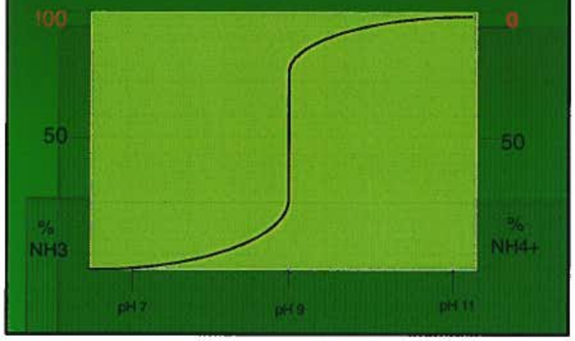
$cBOD + NO_3^-$ --- >

cells (sludge) + $H_2O + CO_2\uparrow + N_2\uparrow + N_2O\uparrow + OH^-$

Oxygen Gradient

X-sectional view of floc particle with oxic (O_2 use) and anoxic (NO_3^- use) zones

pH: Ammonia/Ammonium



Sources of ammonium

Domestic wastewater
  - Amino groups ($-NH_2$) in amino acids
  - Amino groups ($-NH_2$) in proteins
  - Urea (H_2NCONH_2)
- Amino groups are deaminated
- Urea is hydrolyzed
- Influent ammonium: ~ 30 – 45 mg/L

Deamination of Amino Groups

$-NH_2$ --- > NH_3 --- > NH_4^+

< 9 pH

Hydrolysis of Urea

$NH_2CONH_2 + H_2O \text{ --- > } 2\ NH_3\uparrow + CO_2$

$\geq$ 9 pH

Step 1,

if < 9 pH, then Step 2

$2\ NH_3 \text{ --- > } 2\ NH_4^+$

< 9 pH

Step 2

Sources of ammonium

- Industrial discharges
  - Automotive facilities
  - Chemical manufacturing
  - Food processing facilities
  - Landfill leachate
  - Ordnance sites
  - Metal industries
  - Refineries
  - Steel manufacturing
  - Tanneries

Sources of ammonium

- Industrial discharges & high ammonium
  - Coal gasification
  - Fertilizer manufacturing
  - Landfill leachate
  - Livestock maintenance
  - Meat processing
  - Petrochemical

Sources of nitrite

- Corrosion inhibitors
- Leachate (biologically pretreated)
- Meat processing (preservatives)
- Meat processing (biologically pretreated)

Sources of nitrate

- Leachate (biologically pretreated)
- Meat processing (flavor additives)
- Meat processing (biologically pretreated)
- Steel mill wastewater

Influent NO_2^- and NO_3^-

- Typically not found in sewers
- If found in sewer
  - Industrial discharge
  - Reduces the influent cBOD concentration
- Malodor control compounds
  - Sodium nitrate ($NaNO_3$)
  - Calcium nitrate ($Ca(NO_3)_2$)
- Nitrification does not occur in sewers

No nitrification in sewers

- Low dissolved oxygen concentration
- Small population size of nitrifying bacteria
- Relatively short retention time in sewers
- Presence of inhibitory compounds
  - Hydrogen sulfide (H_2S)
  - Recognizable soluble cBOD

Production of H_2S

$\text{cBOD} + SO_4^{2-}$ --- >
cells (biofilm) + $H_2S\uparrow/HS^- + CO_2\uparrow + H_2O$

SO_4^{2-} found in urine and groundwater

H_2S dominant at < 7 pH
HS^- dominant at $\geq$ 7 pH

Production of Recognizable soluble cBOD

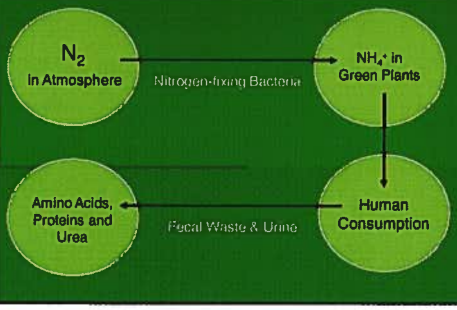
$\text{cBOD} + CH_2O$ --- >
cells (biofilm) + acids↑ + alcohols + aldehydes↑ + ketones↑
$+ CO_2\uparrow + H_2O$

Fermentation (mixed acid production)
produces recognizable soluble cBOD

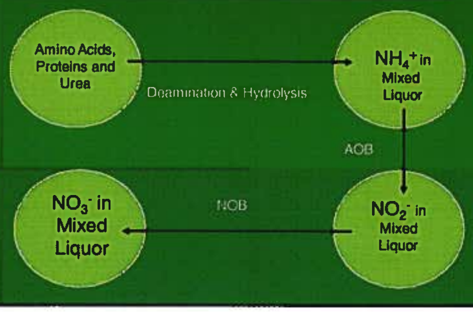
Recognizable soluble cBOD

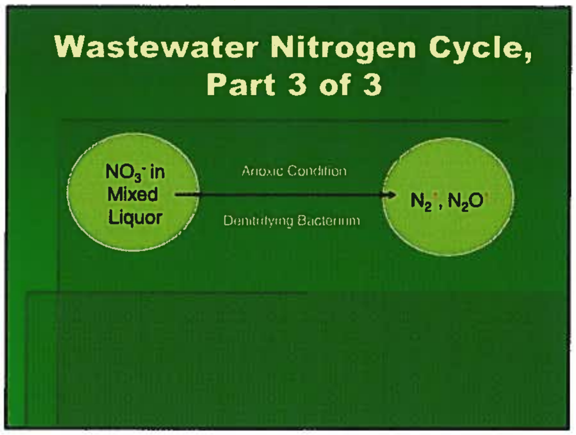
Compound	Formula	Carbon Units
Methanol	CH_3OH	1
Methylamine	CH_3NH_2	1
Ethanol	CH_3CH_2OH	2
n-propanol	$CH_3CH_2CH_2OH$	3
Ethyl acetate	$CH_3CO_2C_2H_5$	4

Wastewater Nitrogen Cycle, Part 1 of 3

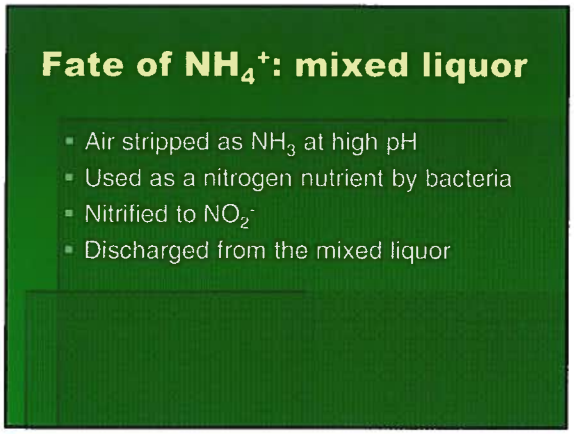


Wastewater Nitrogen Cycle, Part 2 of 3



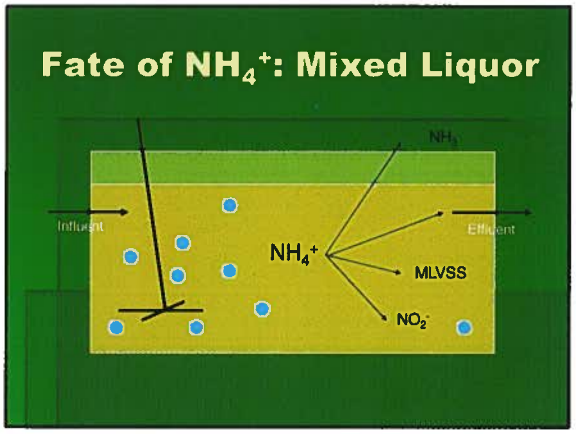


Wastewater Nitrogen Cycle, Part 3 of 3

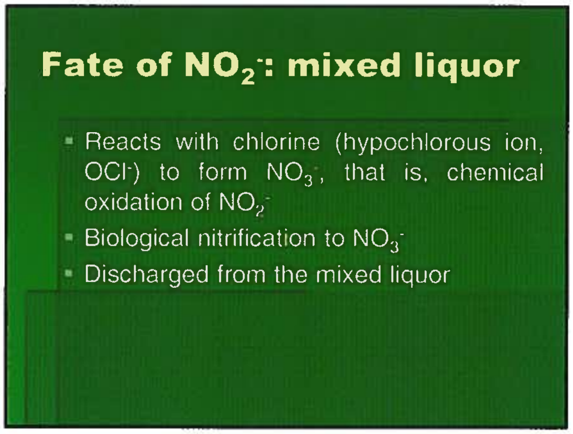


Fate of NH_4^+: mixed liquor

- Air stripped as NH_3 at high pH
- Used as a nitrogen nutrient by bacteria
- Nitrified to NO_2^-
- Discharged from the mixed liquor

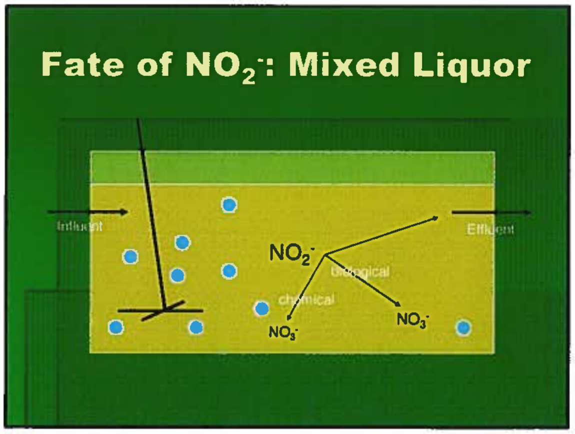


Fate of NH_4^+: Mixed Liquor

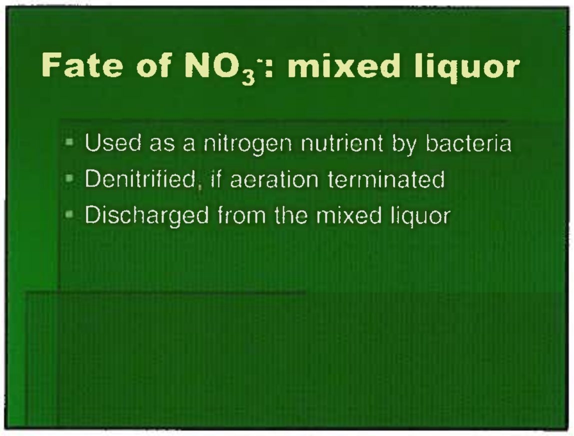


Fate of NO_2^-: mixed liquor

- Reacts with chlorine (hypochlorous ion, OCl^-) to form NO_3^-, that is, chemical oxidation of NO_2^-
- Biological nitrification to NO_3^-
- Discharged from the mixed liquor



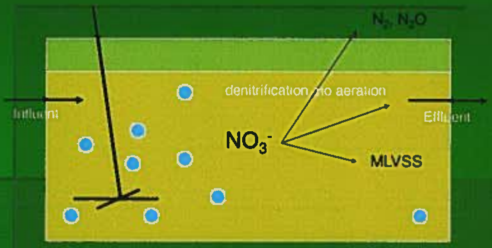
Fate of NO_2^-: Mixed Liquor



Fate of NO_3^-: mixed liquor

- Used as a nitrogen nutrient by bacteria
- Denitrified, if aeration terminated
- Discharged from the mixed liquor

Fate of NO_3^-: Mixed Liquor



Fate of organic-nitrogen

- Adsorbed to floc particles in mixed liquor
  - Solublized and degraded if adequate time
  - Wasted to digester for additional treatment
    - Aerobic digester
    - Anaerobic digester

Fate of organic-nitrogen: aerobic digester

- Deamination of amino groups ($-NH_2$)
- Production of ammonium (NH_4^+)
- Nitrification of NH_4^+ to nitrate (NO_3^-)
- Operational concerns
  - Decrease in alkalinity and pH in digester
  - Discharge of NO_3^- to treatment process
  - Check decant/recycle streams (NO_3^-)

Fate of organic-nitrogen: anaerobic digester

- Deamination of amino groups ($-NH_2$)
- Production of ammonium (NH_4^+)
- If pH of digester increases
  - NH_4^+ is converted to NH_3
  - NH_3 is toxic to anaerobic digesters
- Operational concerns
  - Toxicity to anaerobic digesters
  - Check decant/recycle streams (NH_4^+)

Purpose of nitrification

- Protect the health of the community
- Protect the quality of the receiving water

Health and Environmental Concerns

Nitrogenous Species	Undesired Impact
NH_4^+	Eutrophication, Oxygen Depletion, Toxicity
NO_2^-	Oxygen Depletion, Toxicity
NO_3^-	Eutrophication, Methemoglobinemia, Toxicity

Nitrifying bacteria

- Nitrifying bacteria/chemolithoautotrophs
- Genera of nitrifying bacteria
  - Oxidize only NH_4^+ to NO_2^-
    - Ammonia-oxidizing bacteria (AOB)
    - *Nitrosomonas* and *Nitrosospira*
  - Oxidize only NO_2^- to NO_3^-
    - Nitrite-oxidizing bacteria (NOB)
    - *Nitrobacter* and *Nitrospira*

Significant Characteristics of Nitrifying Bacteria

Characteristic	AOB	NOB
Carbon source	Inorganic	Inorganic
Habitat	Soil & Water	Soil & Water
O_2 requirement	Strict Aerobe	Strict Aerobe
pH growth	5.8 to 8.5	6.5 to 8.5
Max generation	8 hours (lab)	10 hours (lab)
Temp growth	5 to 30 °C	5 to 40 °C
Sludge yield	0.04# / # NH_4^+	0.02# / # NH_4^+

Sludge Yield

$NH_4^+ + 1.5\ O_2$ – AOB --->

$NO_2^- + 2H^+ + H_2O$ + cells (MLVSS)

Equation 1

$NO_2^- + 0.5\ O_2$ – NOB --->

NO_3^- + cells (MLVSS)

Equation 2

Growth of AOB and NOB

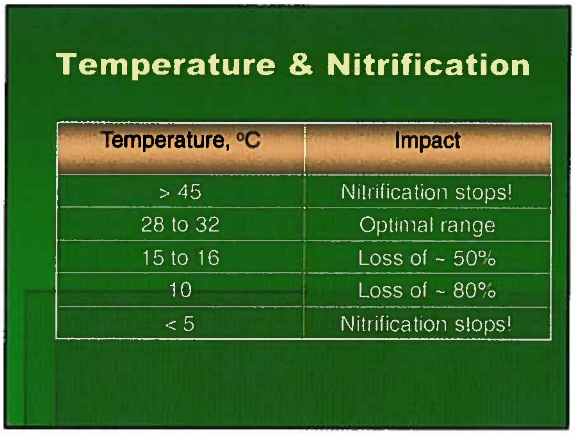
Hours	AOB	NOB	MCRT (days)
0	1	1	0
8	2	1	0
24	8	4	1
96	4,096	512	4
144	262,144	16,384	6
192	16,777,216	524,288	8

Factors affecting population size of nitrifying bacteria and nitrification

- Substrate concentration
  - NH_4^+
  - NO_2^-
- Operational conditions
  - Alkalinity/pH
  - Dissolved oxygen concentration
  - Inhibition/toxicity
  - MCRT/Temperature

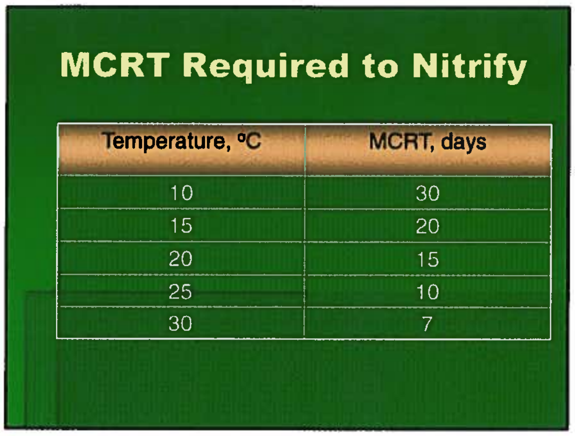
Factors affecting population size of nitrifying bacteria and nitrification

- With increasing temperature bacteria become more active and less bacteria are needed to successfully nitrify
- With decreasing temperature bacteria become less active and more bacteria are needed to successfully nitrify
- Therefore, temperature and MCRT are most critical factors



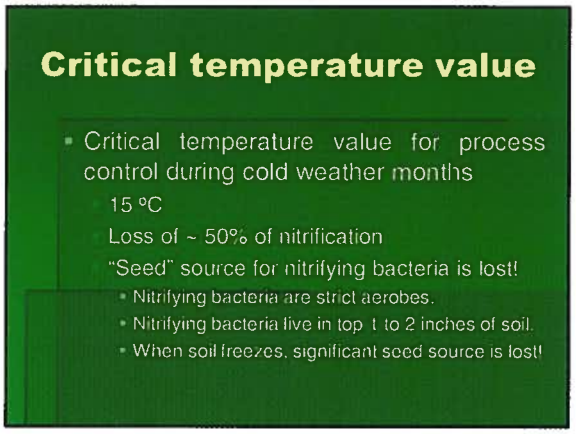
Temperature & Nitrification

Temperature, °C	Impact
> 45	Nitrification stops!
28 to 32	Optimal range
15 to 16	Loss of ~ 50%
10	Loss of ~ 80%
< 5	Nitrification stops!



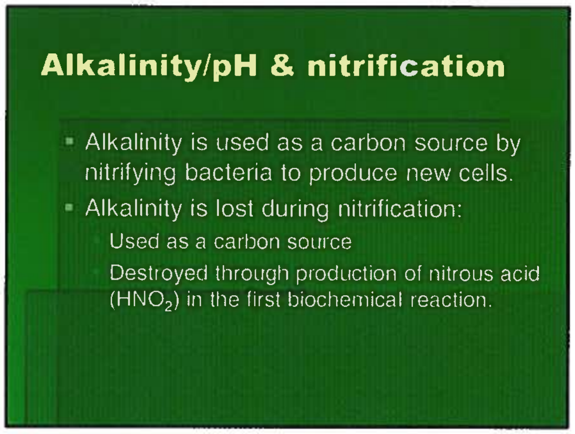
MCRT Required to Nitrify

Temperature, °C	MCRT, days
10	30
15	20
20	15
25	10
30	7



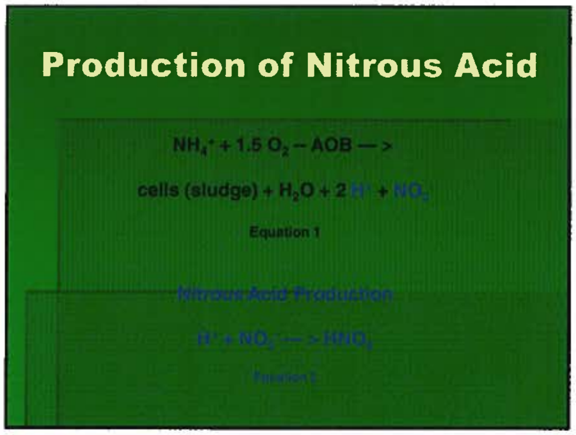
Critical temperature value

- Critical temperature value for process control during cold weather months
  - 15 °C
  - Loss of ~ 50% of nitrification
  - "Seed" source for nitrifying bacteria is lost!
    - Nitrifying bacteria are strict aerobes.
    - Nitrifying bacteria live in top 1 to 2 inches of soil.
    - When soil freezes, significant seed source is lost!



Alkalinity/pH & nitrification

- Alkalinity is used as a carbon source by nitrifying bacteria to produce new cells.
- Alkalinity is lost during nitrification:
  - Used as a carbon source
  - Destroyed through production of nitrous acid (HNO_2) in the first biochemical reaction.



Production of Nitrous Acid

$NH_4^+ + 1.5\ O_2$ – AOB — >

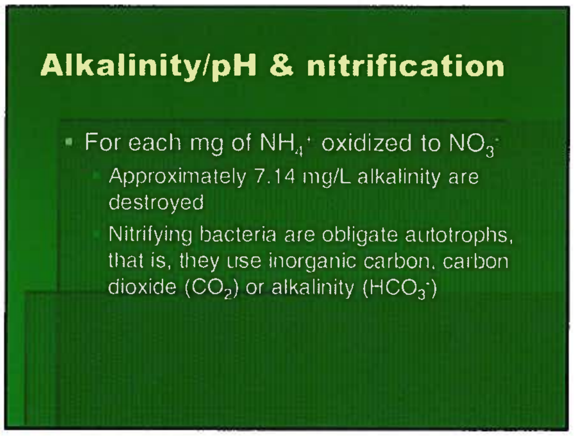
cells (sludge) + $H_2O + 2\ H^+ + NO_2^-$

Equation 1

Nitrous Acid Production

$H^+ + NO_2^-$ — > HNO_2

Equation 2



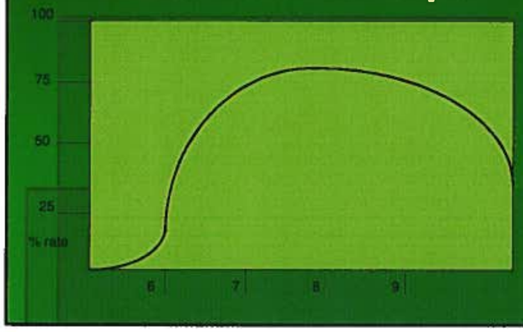
Alkalinity/pH & nitrification

- For each mg of NH_4^+ oxidized to NO_3^-
  - Approximately 7.14 mg/L alkalinity are destroyed
  - Nitrifying bacteria are obligate autotrophs, that is, they use inorganic carbon, carbon dioxide (CO_2) or alkalinity (HCO_3^-)

pH and Nitrification

pH range	Impact
4.0 to 4.9	Nitrifying bacteria present; Organotrophic nitrification occurs.
5.0 to 6.7	Nitrification by nitrifying bacteria; Nitrification is sluggish.
6.7 to 7.2	Nitrification by nitrifying bacteria; Nitrification rate increases. Most plants nitrify at near neutral pH.
7.3 to 8.5	Nitrification by nitrifying bacteria; Optimal pH range for nitrification.

% Max Nitrification Rate as a Function or pH



Dissolved oxygen & nitrification

- Nitrifying bacteria are strict aerobes
- Biochemical reactions are aerobic

$NH_4^+ + 1.5\ O_2$ -- AOB --- > $2\ H^+ + NO_2^- + H_2O$
Equation 1

$NO_2^- + 0.5\ O_2$ – NOB --- > NO_3^-
Equation 2

Dissolved oxygen & nitrification

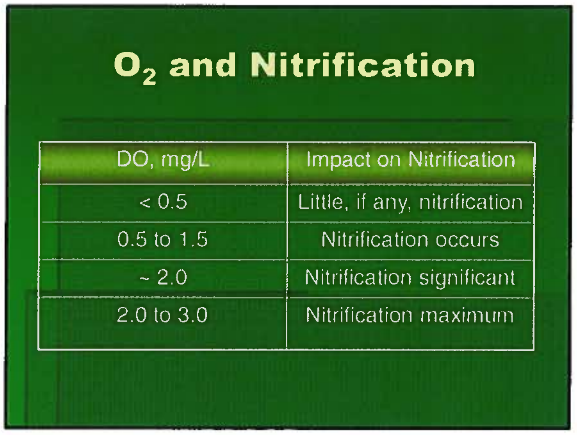
- Approximately 4.6 mg of O_2 are consumed per mg NH_4^+ oxidized to NO_3^-.
- Note: approximately 30 mg/L NH_4^+ enter the treatment process and additional NH_4^+ is produced in the mixed liquor when organic-nitrogen compounds are degraded.

Dissolved oxygen & nitrification

- Significant organic-nitrogen compounds that produced NH_4^+ when they are degraded
  - Amino acids
  - Proteins
  - Cationic polymers
  - Chloramines
  - Polyelectrolytes
  - Surfactants

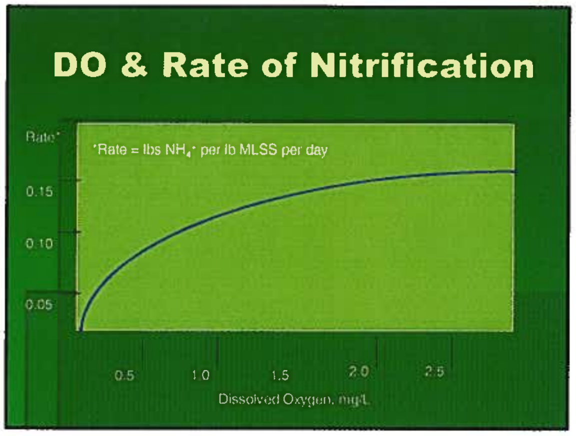
O_2 Consumed during Nitrification

Reaction	O_2, lb	O_2, kg
1 kg NH_4^+ to 1 kg NO_2^-		1.56
1 lb NH_4^+ to 1 lb NO_2^-	3.43	
1 kg NO_2^- to 1 kg NO_3^-		0.52
1 lb NO_2^- to 1 lb NO_3^-	1.14	
1 kg NH_4^+ to 1 kg NO_3^-		2.08
1 lb NH_4^+ to 1 lb NO_3^-	4.57	



O_2 and Nitrification

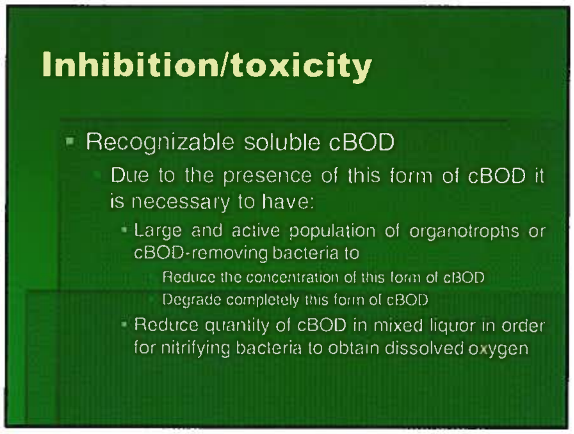
DO, mg/L	Impact on Nitrification
< 0.5	Little, if any, nitrification
0.5 to 1.5	Nitrification occurs
~ 2.0	Nitrification significant
2.0 to 3.0	Nitrification maximum



DO & Rate of Nitrification

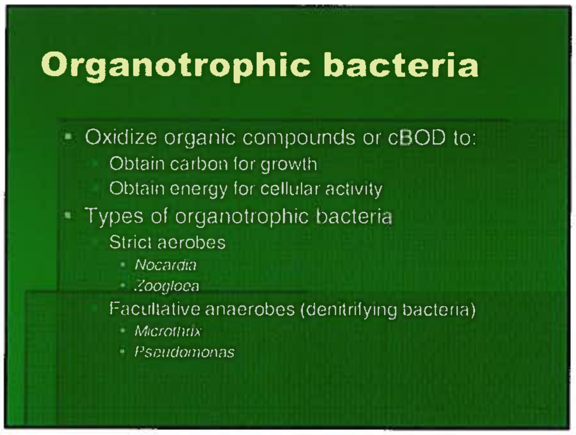
Inhibition/toxicity

- Forms or inhibition/toxicity
  - Free chlorine residual
  - Inorganic
    - Hydrogen sulfide (H_2S)
    - Hydrogen cyanide (HCN)
    - Heavy metals
  - Organic
    - Industrial wastes, for example, phenol
    - Recognizable soluble cBOD
  - Substrate
    - Free ammonia (NH_3)
    - Free nitrous acid (HNO_2)



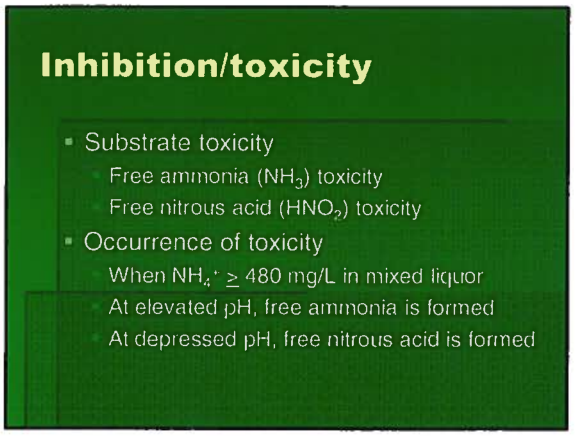
Inhibition/toxicity

- Recognizable soluble cBOD
  - Due to the presence of this form of cBOD it is necessary to have:
    - Large and active population of organotrophs or cBOD-removing bacteria to
      - Reduce the concentration of this form of cBOD
      - Degrade completely this form of cBOD
    - Reduce quantity of cBOD in mixed liquor in order for nitrifying bacteria to obtain dissolved oxygen



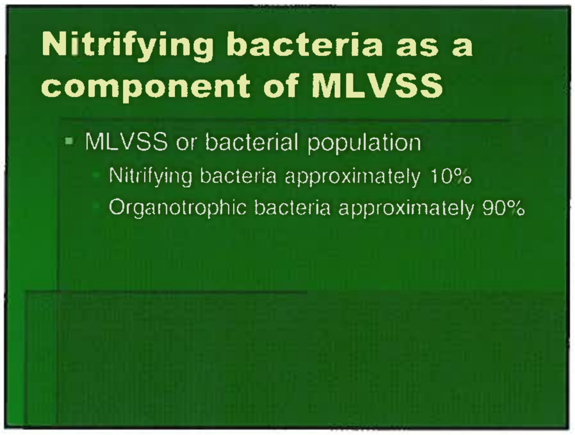
Organotrophic bacteria

- Oxidize organic compounds or cBOD to:
  - Obtain carbon for growth
  - Obtain energy for cellular activity
- Types of organotrophic bacteria
  - Strict aerobes
    - *Nocardia*
    - *Zoogloea*
  - Facultative anaerobes (denitrifying bacteria)
    - *Microthrix*
    - *Pseudomonas*



Inhibition/toxicity

- Substrate toxicity
  - Free ammonia (NH_3) toxicity
  - Free nitrous acid (HNO_2) toxicity
- Occurrence of toxicity
  - When $NH_4^+ \geq 480$ mg/L in mixed liquor
  - At elevated pH, free ammonia is formed
  - At depressed pH, free nitrous acid is formed

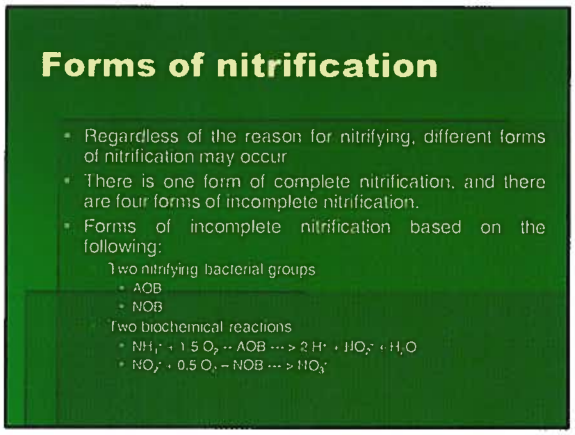


Nitrifying bacteria as a component of MLVSS

- MLVSS or bacterial population
  - Nitrifying bacteria approximately 10%
  - Organotrophic bacteria approximately 90%

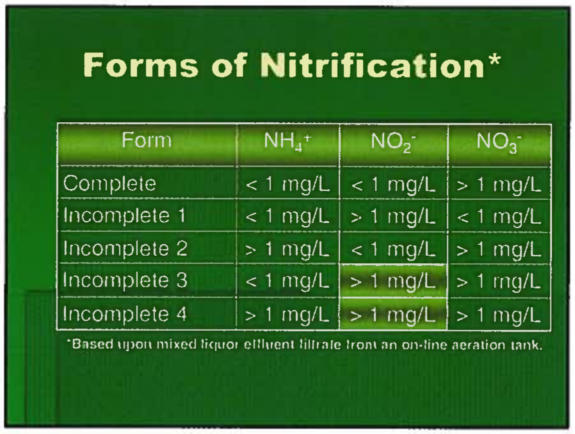
Forms of nitrification

- Why do wastewater treatment plants nitrify?
  - Must nitrify: regulatory requirement
    - Satisfy an ammonia (NH$_3$) discharge limit
    - Satisfy a total nitrogen discharge limit
  - Desired nitrification
    - Produce NO_3^- for anoxic control of undesired filamentous organism growth
    - Produce small quantity (2 to 4 mg/L) NO_3^- to indicate a "healthy" biomass
  - "Slip" into nitrification
    - Operational conditions are acceptable for nitrification
    - Monitoring is not performed for nitrification



Forms of nitrification

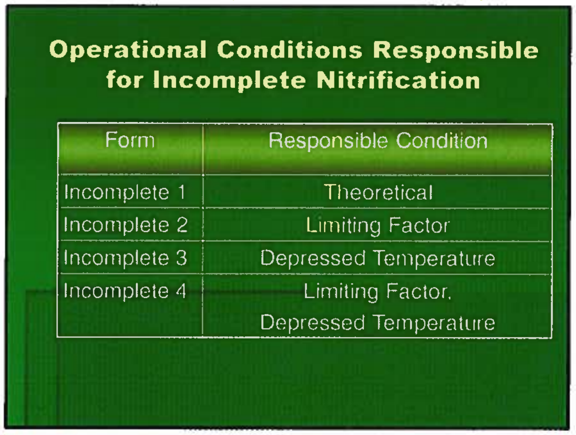
- Regardless of the reason for nitrifying, different forms of nitrification may occur
- There is one form of complete nitrification, and there are four forms of incomplete nitrification.
- Forms of incomplete nitrification based on the following:
  - Two nitrifying bacterial groups
    - AOB
    - NOB
  - Two biochemical reactions
    - $NH_4^+ + 1.5\ O_2$ -- AOB ---> $2\ H^+ + NO_2^- + H_2O$
    - $NO_2^- + 0.5\ O_2$ – NOB ---> NO_3^-



Forms of Nitrification*

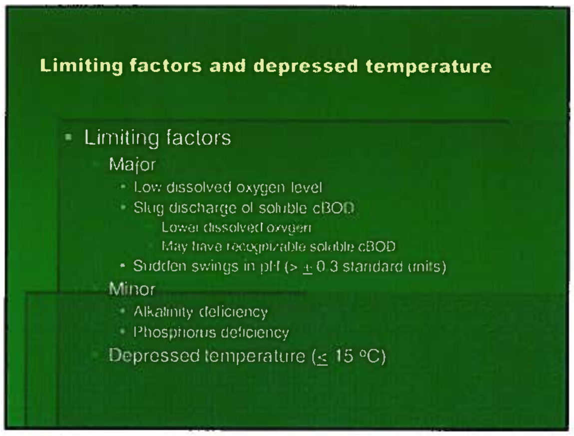
Form	NH_4^+	NO_2^-	NO_3^-
Complete	< 1 mg/L	< 1 mg/L	> 1 mg/L
Incomplete 1	< 1 mg/L	> 1 mg/L	< 1 mg/L
Incomplete 2	> 1 mg/L	< 1 mg/L	> 1 mg/L
Incomplete 3	< 1 mg/L	> 1 mg/L	> 1 mg/L
Incomplete 4	> 1 mg/L	> 1 mg/L	> 1 mg/L

*Based upon mixed liquor effluent filtrate from an on-line aeration tank.



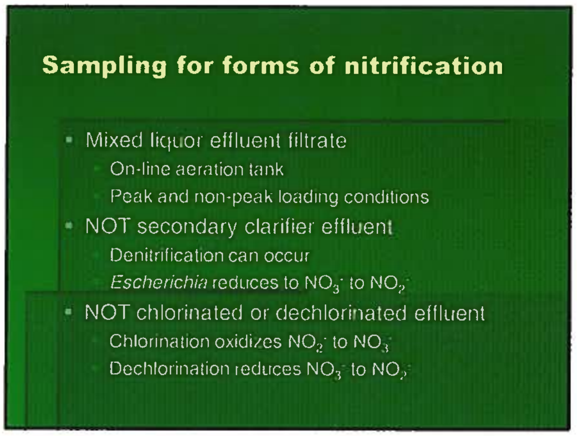
Operational Conditions Responsible for Incomplete Nitrification

Form	Responsible Condition
Incomplete 1	Theoretical
Incomplete 2	Limiting Factor
Incomplete 3	Depressed Temperature
Incomplete 4	Limiting Factor, Depressed Temperature



Limiting factors and depressed temperature

- Limiting factors
  - Major
    - Low dissolved oxygen level
    - Slug discharge of soluble cBOD
      - Lower dissolved oxygen
      - May have recognizable soluble cBOD
    - Sudden swings in pH (> ± 0.3 standard units)
  - Minor
    - Alkalinity deficiency
    - Phosphorus deficiency
  - Depressed temperature (≤ 15 °C)

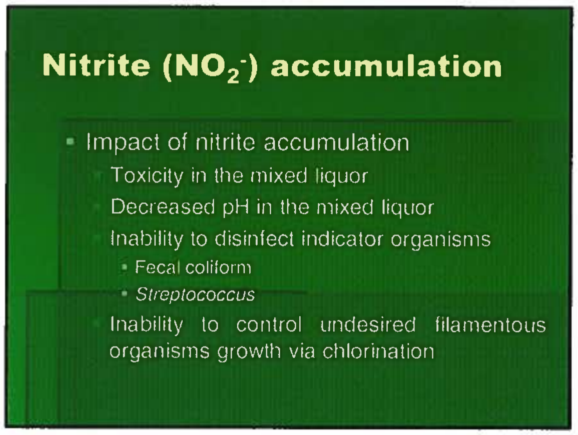


Sampling for forms of nitrification

- Mixed liquor effluent filtrate
  - On-line aeration tank
  - Peak and non-peak loading conditions
- NOT secondary clarifier effluent
  - Denitrification can occur
  - *Escherichia* reduces NO_3^- to NO_2^-
- NOT chlorinated or dechlorinated effluent
  - Chlorination oxidizes NO_2^- to NO_3^-
  - Dechlorination reduces NO_3^- to NO_2^-

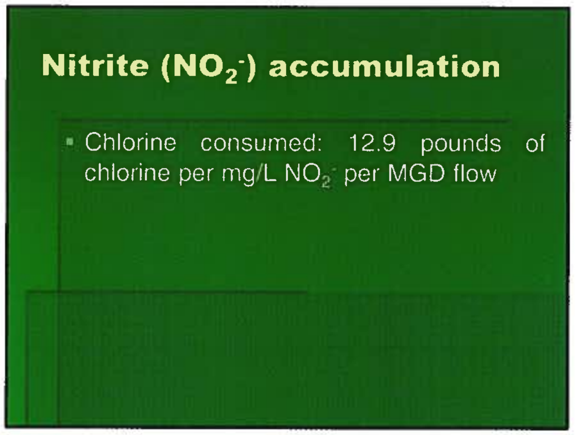
Nitrite (NO_2^-) accumulation

- Also known as
  - "Chlorine sponge"
  - "Nitrite kick"
  - "Nitrite lock"
- Nitrite accumulation occurs during
  - Incomplete 3
  - Incomplete 4



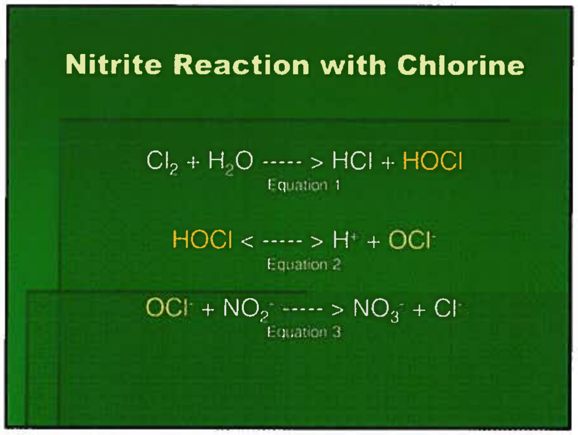
Nitrite (NO_2^-) accumulation

- Impact of nitrite accumulation
  - Toxicity in the mixed liquor
  - Decreased pH in the mixed liquor
  - Inability to disinfect indicator organisms
    - Fecal coliform
    - *Streptococcus*
  - Inability to control undesired filamentous organisms growth via chlorination



Nitrite (NO_2^-) accumulation

- Chlorine consumed: 12.9 pounds of chlorine per mg/L NO_2^- per MGD flow



Nitrite Reaction with Chlorine

$$Cl_2 + H_2O \text{ ----- > } HCl + HOCl$$

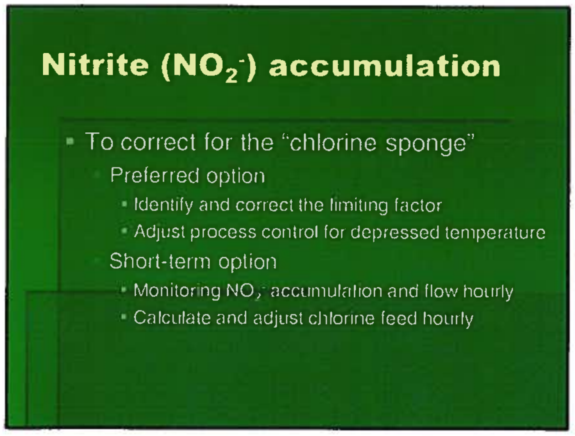
Equation 1

$$HOCl \text{ < ----- > } H^+ + OCl^-$$

Equation 2

$$OCl^- + NO_2^- \text{ ----- > } NO_3^- + Cl^-$$

Equation 3



Nitrite (NO_2^-) accumulation

- To correct for the "chlorine sponge"
  - Preferred option
    - Identify and correct the limiting factor
    - Adjust process control for depressed temperature
  - Short-term option
    - Monitoring NO_2^- accumulation and flow hourly
    - Calculate and adjust chlorine feed hourly

Nitrite (NO_2^-) accumulation

- Biological factors responsible for NO_2^- accumulation under adverse operational condition
  - Smaller number of NOB than AOB; therefore NH_4^+ is oxidized more quickly to NO_2^- than NO_2^- is oxidized to NO_3^-
  - NOB obtain less energy from oxidizing NO_2^- than AOB obtain from oxidizing NH_4^+; therefore AOB can tolerate an adverse operational condition more easily than NOB

Demonstrating nitrification

- The occurrence of nitrification in a biological wastewater treatment process can be demonstrated only through the production of NO_2^- or NO_3^-, not by the removal or decrease in NH_4^+

Demonstrating nitrification

- Biological indicators of possible nitrification
  - Algal growth in secondary clarifier
  - Duckweed growth in secondary clarifier
  - Increase in mixed liquor dissolved oxygen demand

Demonstrating nitrification

- Chemical indicators of possible nitrification
  - Decrease in mixed liquor alkalinity and possibly pH
  - Increase in chlorine demand for indicator organism "kill" in chlorine contact tank
  - Increase in chlorine demand for control of undesired filamentous organism growth
  - Increase in secondary clarifier effluent alkalinity and possibly pH

Demonstrating nitrification

- Physical indicators of possible nitrification
  - "Clumping:" rising clumps of solids in the secondary clarifier
  - "Clumping:" rising bubbles in the secondary clarifier

Depressed temperature

- Monitor and anticipate depressed temperature
- Take appropriate operational measures to maintain effective nitrification during cold weather months
- Implement operational measures at temperature value > 15 °C

Depressed temperature

- Reasons for implementing appropriate process control measures
  - Bacterial populations are sluggish in activity
    - Nitrifying bacterial population
    - Organotrophic (cBOD-removing) bacterial population
  - With frozen soil "seed" source of nitrifying bacteria is lost or reduced in quantity

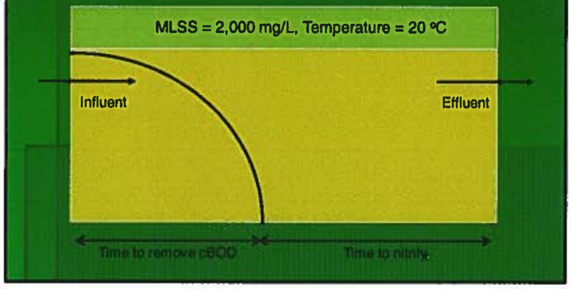
Depressed temperature

- Appropriate process control measures
  - Increase MLSS concentration
  - Ensure adequate alkalinity
  - Increase primary clarifier efficiency to remove particulate and colloid BOD
  - Increase HRT in aeration tank
  - Increase dissolved oxygen concentration in aeration tank
  - Thicken sludge blanket in secondary clarifier and decrease RAS rate
  - Add bioaugmentation products
  - Use integrated fixed film media

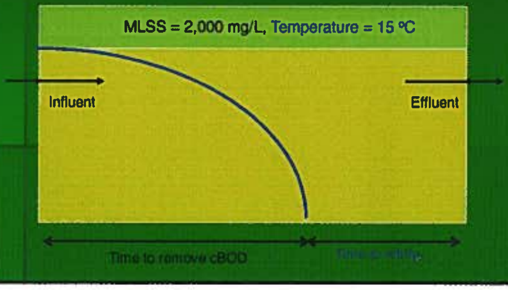
Depressed temperature

- Increase MLSS
  - Increases population size of nitrifying bacteria
  - Increases population size of organotrophic (cBOD-removing) bacteria

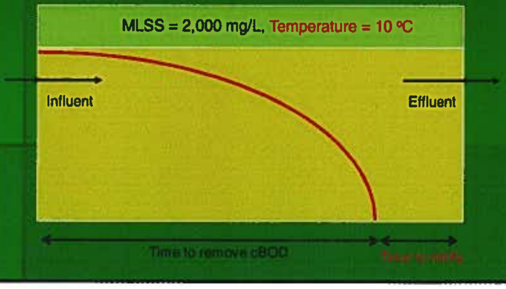
Loss of Effective Nitrification Time (with no increase in MLSS)

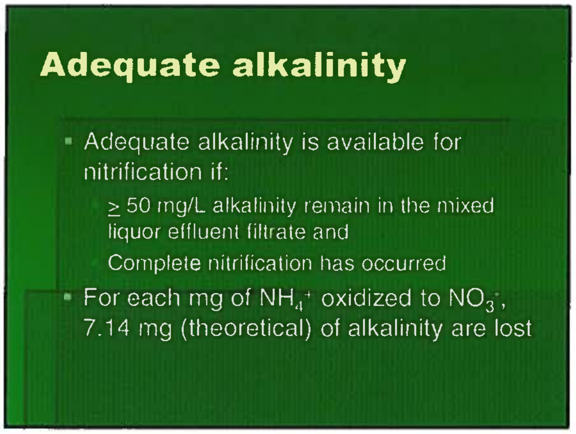


Loss of Effective Nitrification Time (with no increase in MLSS)



Loss of Effective Nitrification Time (with no increase in MLSS)



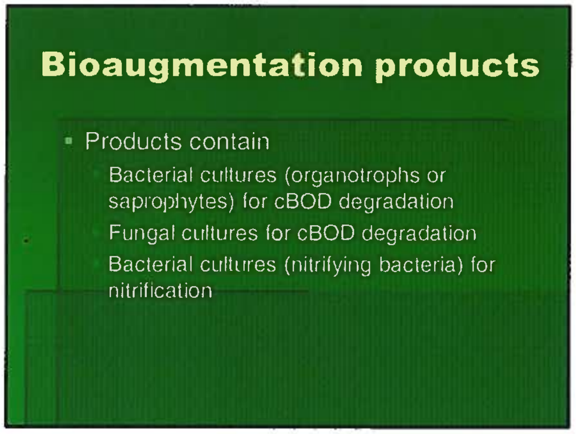


Adequate alkalinity

- Adequate alkalinity is available for nitrification if:
  - $\geq$ 50 mg/L alkalinity remain in the mixed liquor effluent filtrate and
  - Complete nitrification has occurred
- For each mg of NH_4^+ oxidized to NO_3^-, 7.14 mg (theoretical) of alkalinity are lost

Compounds Suitable for Alkalinity Addition

Compound	Formula	Name
Sodium bicarbonate	$NaHCO_3$	Baking soda
Calcium carbonate	$CaCO_3$	Calcite
Sodium carbonate	Na_2CO_3	Soda ash
Calcium hydroxide	$Ca(OH)_2$	Lime
Magnesium hydroxide	$Mg(OH)_2$	Magnesia
Sodium hydroxide	NaOH	Caustic soda

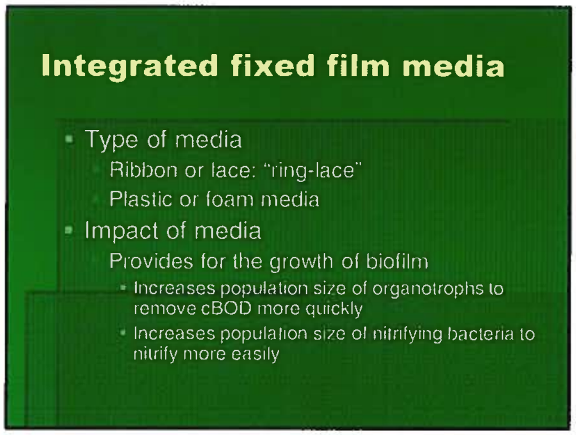


Bioaugmentation products

- Products contain
  - Bacterial cultures (organotrophs or saprophytes) for cBOD degradation
  - Fungal cultures for cBOD degradation
  - Bacterial cultures (nitrifying bacteria) for nitrification

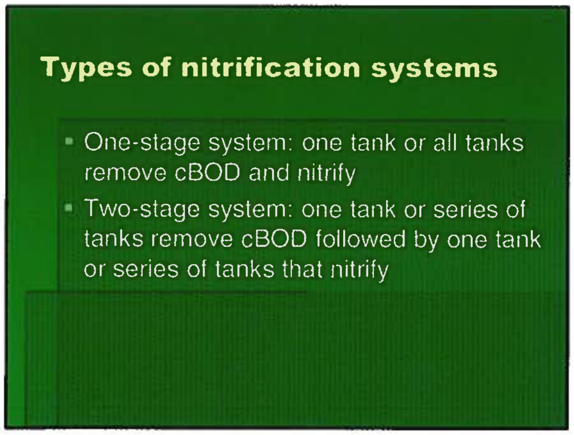
Bioaugmentation products

- If treatment process is nitrifying
  - Nitrifying bacteria are present
    - Add only saprophytes first
    - Add nitrifying bacteria if needed
- If treatment process in not nitrifying
    - Add saprophytes to remove cBOD more quickly to provide time for nitrifying bacteria to work
    - Add nitrifying bacteria



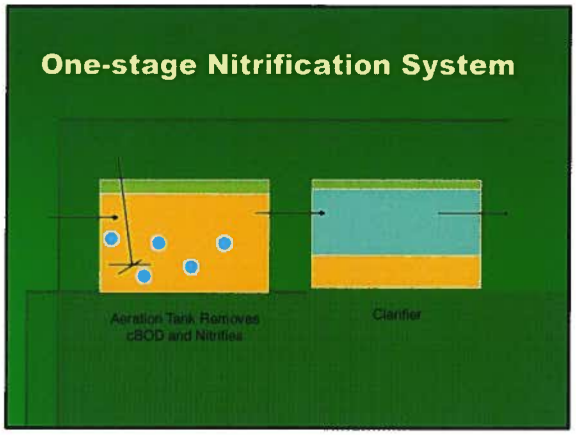
Integrated fixed film media

- Type of media
  - Ribbon or lace: "ring-lace"
  - Plastic or foam media
- Impact of media
  - Provides for the growth of biofilm
    - Increases population size of organotrophs to remove cBOD more quickly
    - Increases population size of nitrifying bacteria to nitrify more easily

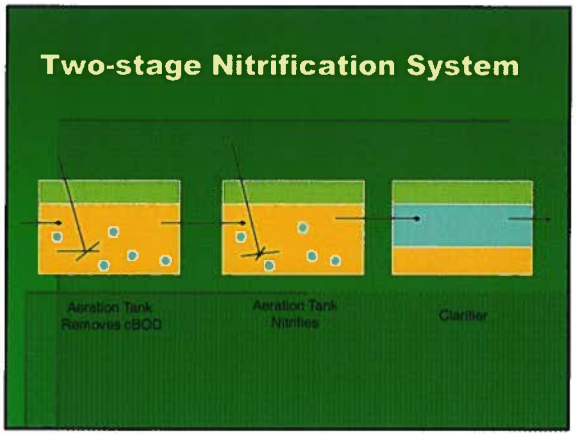


Types of nitrification systems

- One-stage system: one tank or all tanks remove cBOD and nitrify
- Two-stage system: one tank or series of tanks remove cBOD followed by one tank or series of tanks that nitrify



One-stage Nitrification System



Two-stage Nitrification System