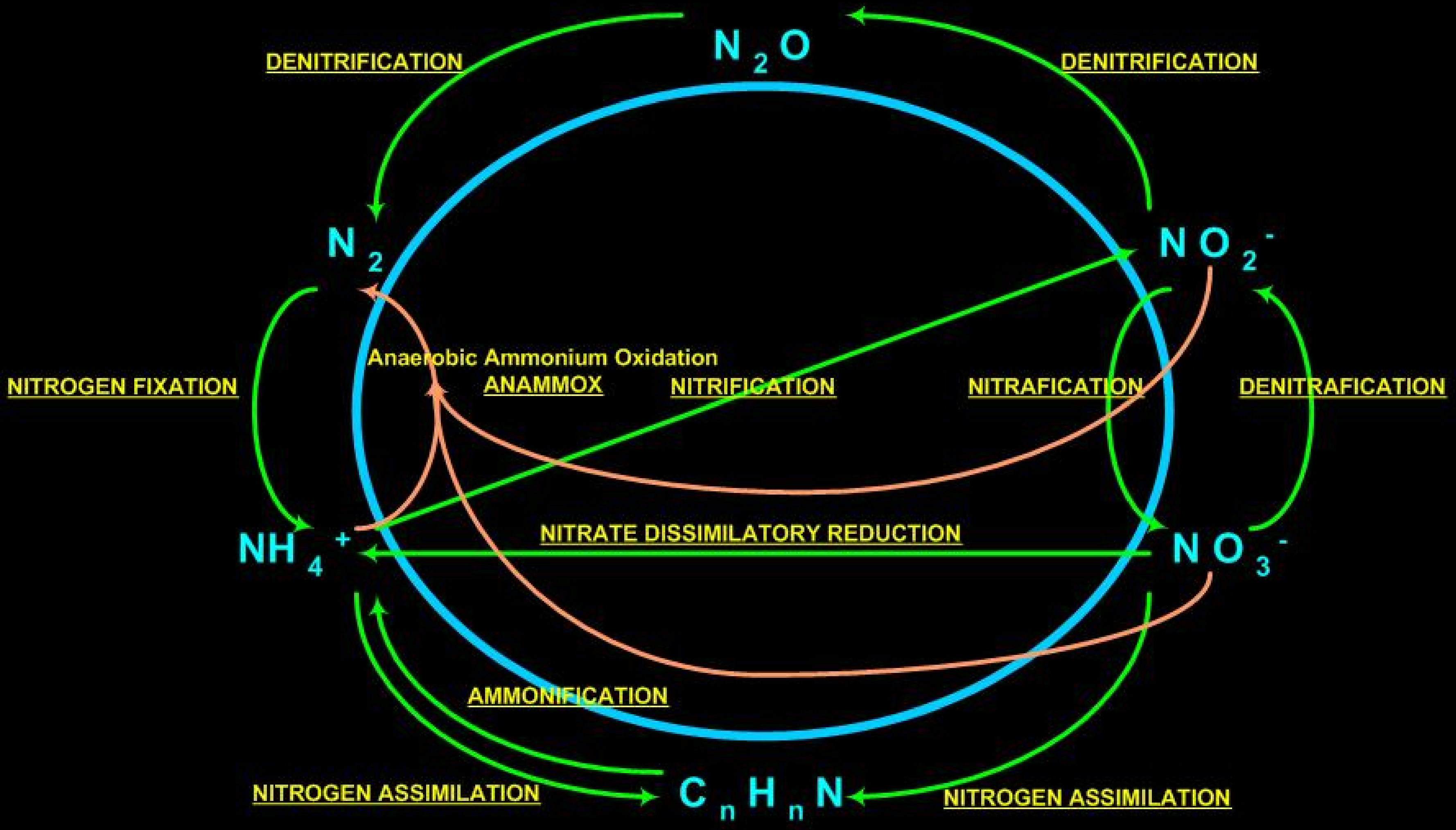
Microbiology and Biochemistry of the Nitrogen Cycle
Process Applications: InNitri

m²t
technologies

Mixing and Mass Transfer Technologies LLC
LOTEPRO Environmental Systems and Services

THE NITROGEN CYCLE



N_2O

DENITRIFICATION

DENITRIFICATION

N_2

NO_2^-

Anaerobic Ammonium Oxidation

ANAMMOX

NITROGEN FIXATION

NITRIFICATION

NITRAFICATION

DENITRAFICATION

NITRATE DISSIMILATORY REDUCTION

NH_4^+

NO_3^-

AMMONIFICATION

NITROGEN ASSIMILATION

C_nH_nN

NITROGEN ASSIMILATION

THERMODYNAMICS, energy for reactions

$\Delta H = \Delta G + T\Delta S$

H is enthalpy energy
G is free energy
S is entropy energy

$\Delta G = \Delta H - T\Delta S$

If ΔG is – the reaction is spontaneous

If ΔG is + the reaction needs an energy input to occur

Nitrification (Nitrosifying Bacteria)

$NH_3 + O_2 + 2e^- + 2H^+ \rightarrow NH_2OH + H_2O$

$NH_2OH + 1.5O_2 \rightarrow NO_2^- + H^+ + H_2O$

$\Delta G^{O'} = - 287$ kJ/mole

Nitrafication (Nitrifying Bacteria)

$NO_2^- + 0.5O_2 \rightarrow NO_3^-$

$\Delta G^{O'} = -76$ kJ/mole

Denitrafication (Many Bacteria)

NO_3 +2e- + 2H+ → NO_2^- + H_2O
$\Delta G^{O'}$ = - 81.2 kJ/mole

Denitrification (Many Bacteria)

NO_2^- $2H^+$ + e^- → NO + H_2O
$\Delta G^{O'}$ = - 36.1 kJ/mole

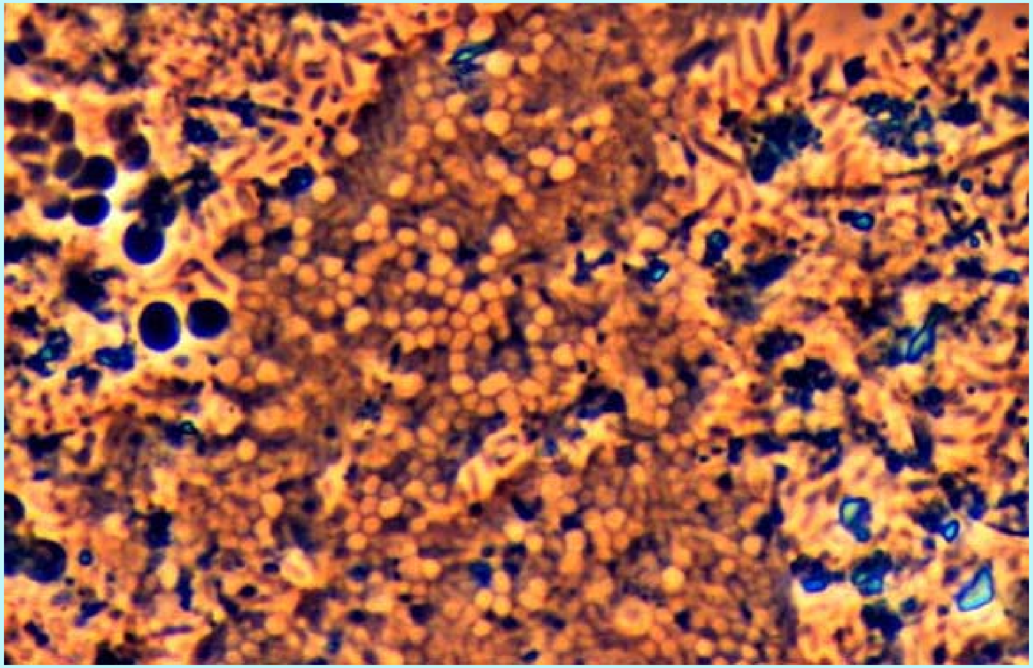
2NO + $2H^+$ + $2e^-$ → N_2O + H_2O
$\Delta G^{O'}$ = -227 kJ/mole

N_2O + $2H^+$ + $2e^-$ → N_2 + H_2O
$\Delta G^{O'}$ = -260.9 kJ/mole

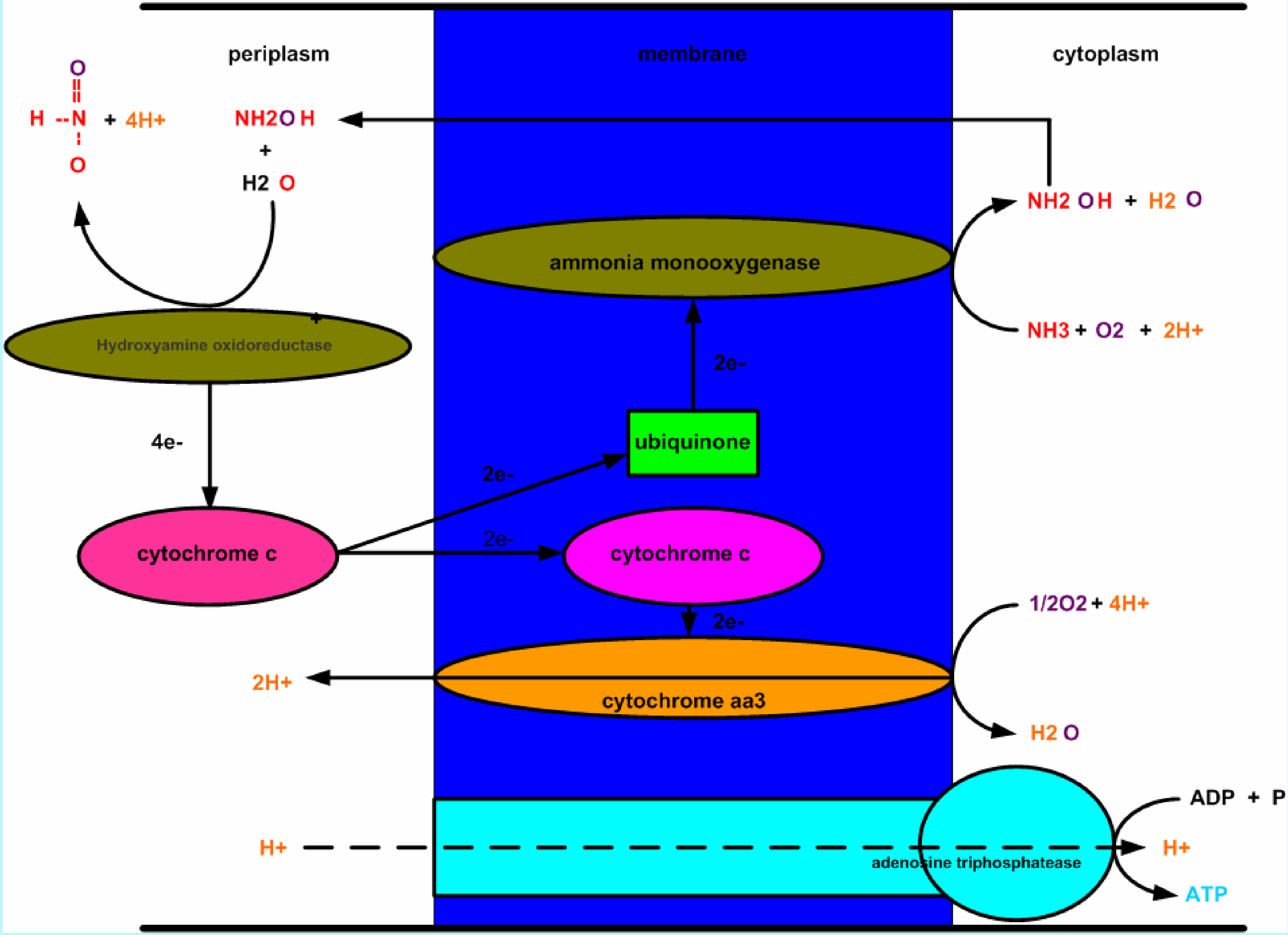
THE MICRO-ORGANISMS INVOLVED IN NITRIFICATION / DENITRIFICATION and NITRAFICATION / DENITRAFICATION

Nitrogen Compound Reactions	Bacteria
Ammonia Oxidizers (nitrifying bacteria) $NH_4 \rightarrow NO_2$	Nitrosomonas, Nitrosococcus, Nitrosopira, Nitrosolobus, and Nitrosovibro
Nitrate Oxidizers (nitrafying bacteria) $NO_2 \rightarrow NO_3$	Nitrobactor, Nitrospina, Nitrococcus, and Nitrospira
Nitrate Nitrite Reducers (denitra and denitrifying bacteria) $NO_3^- \rightarrow NO_2^-$ $NO_2^- \rightarrow NO$ $NO \rightarrow N_2O$ $N_2O \rightarrow N_2$	Achromobacter, Acinetobacter, Agrobacterium, Alcaligenes, Arthrobacter, Baccillus, Chromobacterium,Coryenbacterium Flavobacterium, Hypomicrobium Moraxella, Neisseria, Paracoccus, Propionbacterium, Pseudomonas, Rhizobium, Rhodopseudomonas, Spirillum, and Vibrio

Nitrosomonas



METABOLIC PATHWAY NITRIFYING BACTERIA



periplasm

membrane

cytoplasm

O
||
H --N + $4H^+$
|
O

NH_2OH
+
H_2O

ammonia monooxygenase

NH_2OH + H_2O

NH_3 + O_2 + $2H^+$

Hydroxyamine oxidoreductase

4e-

2e-

ubiquinone

2e-

cytochrome c

2e-

cytochrome c

2e-

cytochrome aa3

$2H^+$

1/2O_2 + $4H^+$

H_2O

H^+

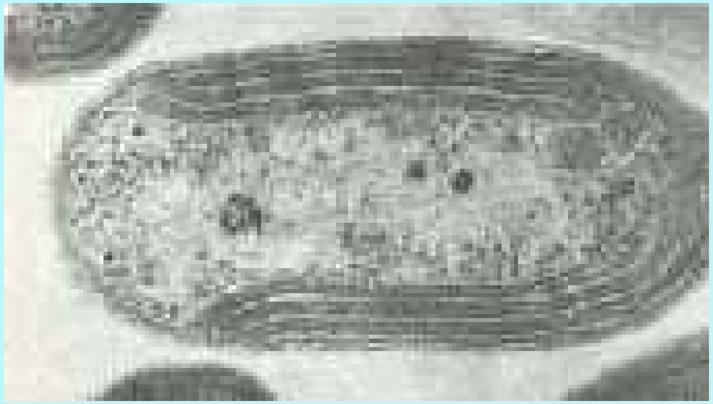
adenosine triphosphatease

ADP + P

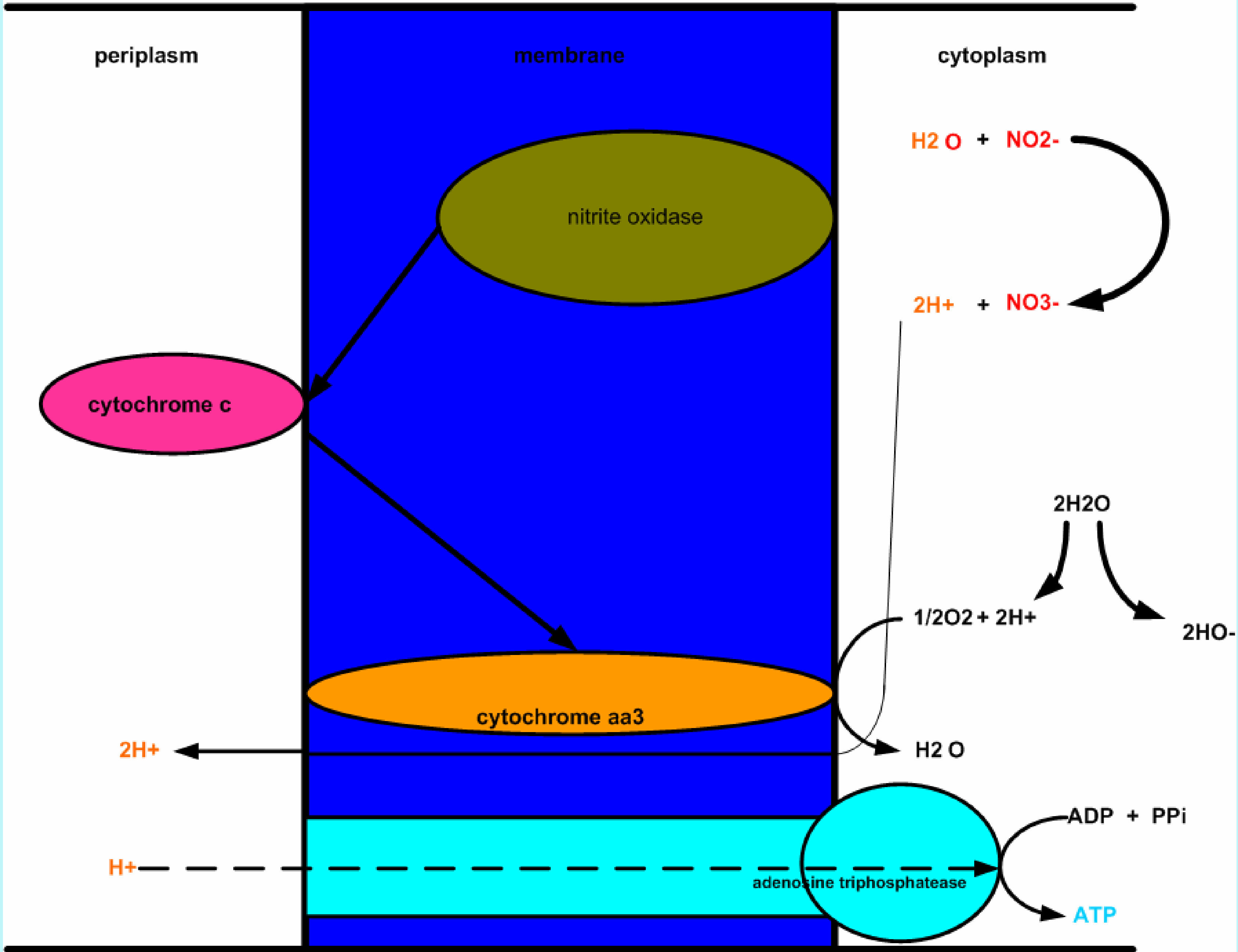
H^+

ATP

Nitrobactor



METABOLIC PATHWAY NITRAFYING BACTERIA



periplasm

membrane

cytoplasm

H_2O + NO_2^-

nitrite oxidase

$2H^+$ + NO_3^-

cytochrome c

$2H_2O$

$1/2O_2 + 2H^+$

$2HO^-$

cytochrome aa3

$2H^+$

H_2O

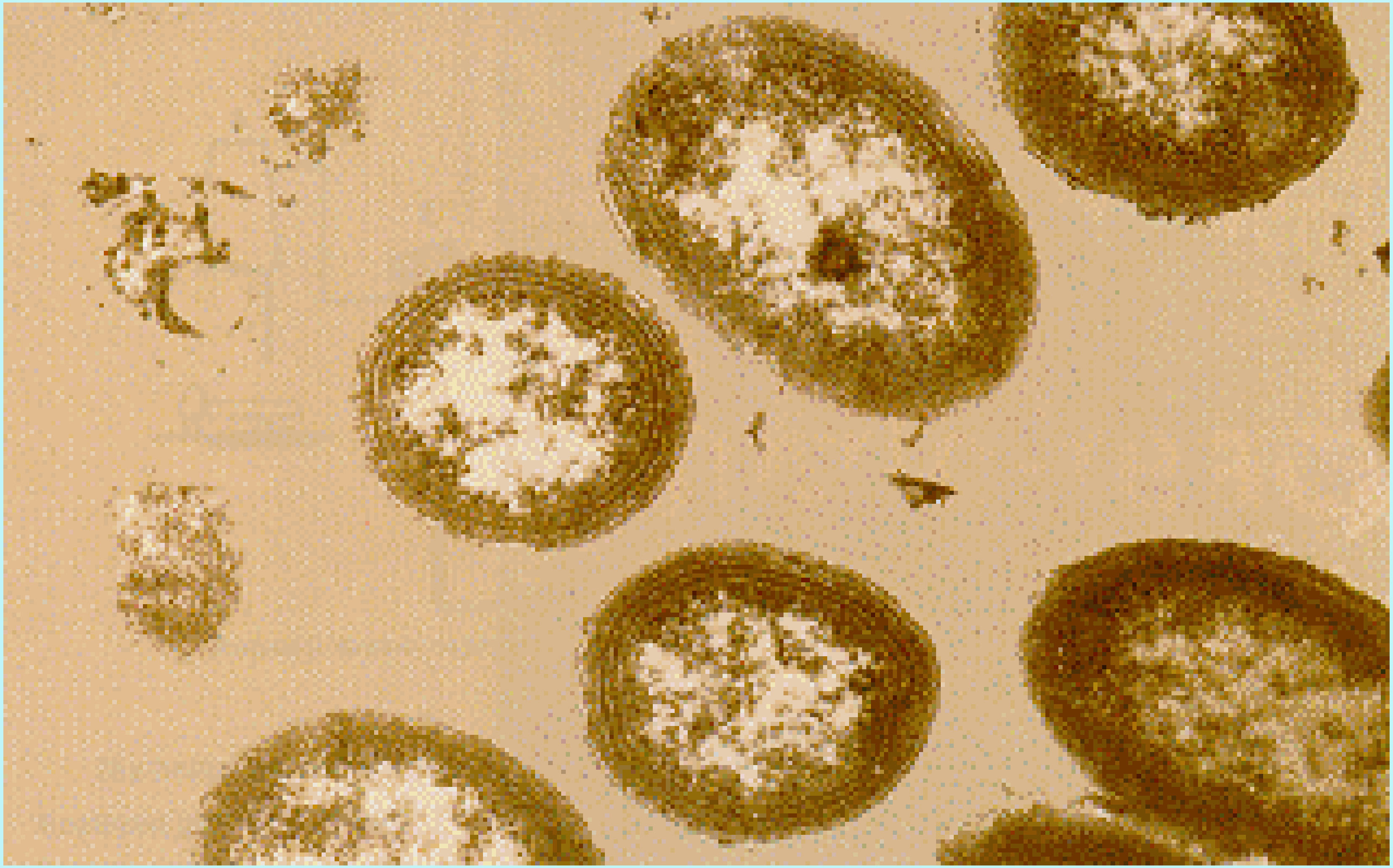
ADP + PPi

H^+

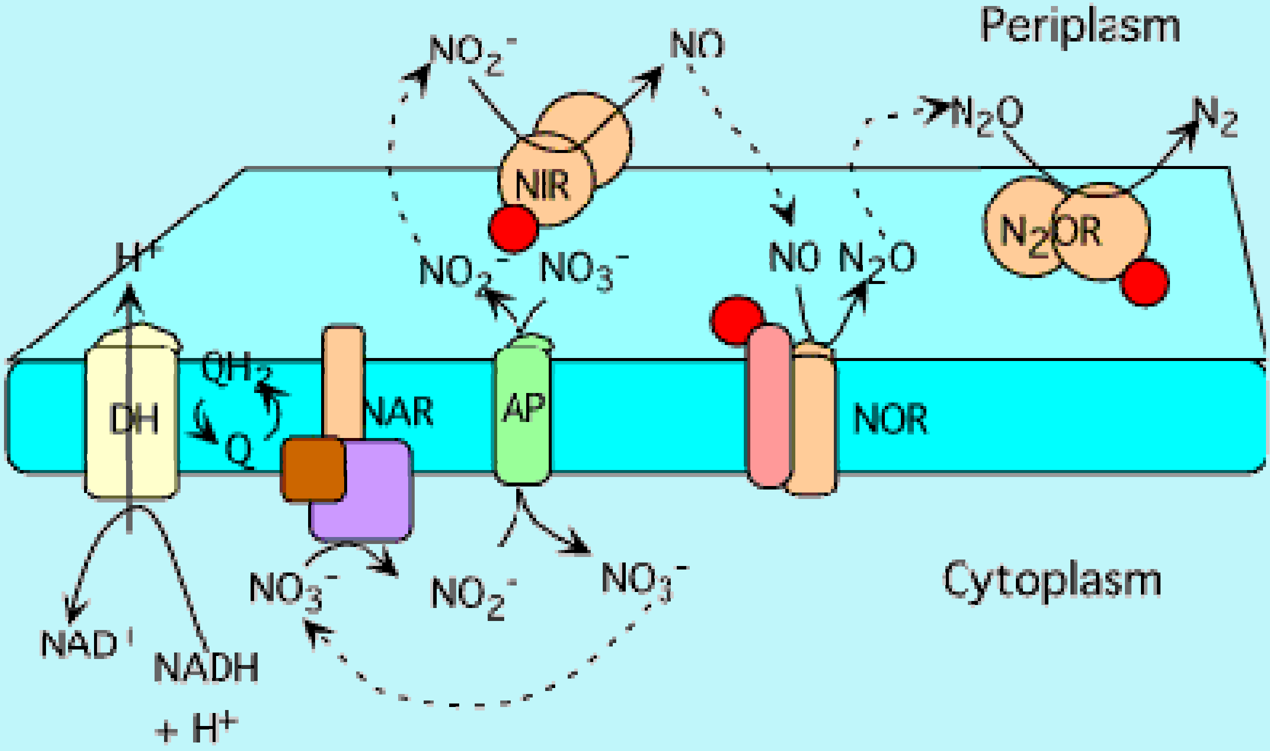
adenosine triphosphatease

ATP

Denitri- and Denitra- fying Bacteria

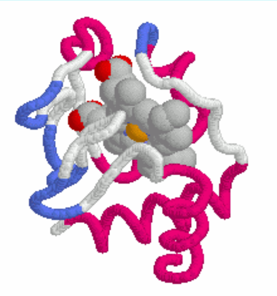


TYPICAL METABOLIC PATHWAY
for
DENITRAFYING and DENITRIFYING BACTERIA



ENZYMES

IMPORTANCE OF **IRON** IN ELECTRON TRANSPORT



FE is present in all cytochrome c molecules

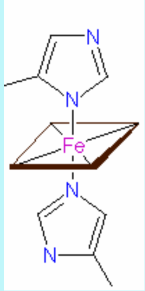
NH_2OH

+ H_2O

↓

HAO $4e^-$ → 2 cyt c_{554} ↓ $4e^-$ → 4 cyt c_{552} $4e^-$ → terminal oxidase **(1)**

↓



→ → $4e^-$ → ↑

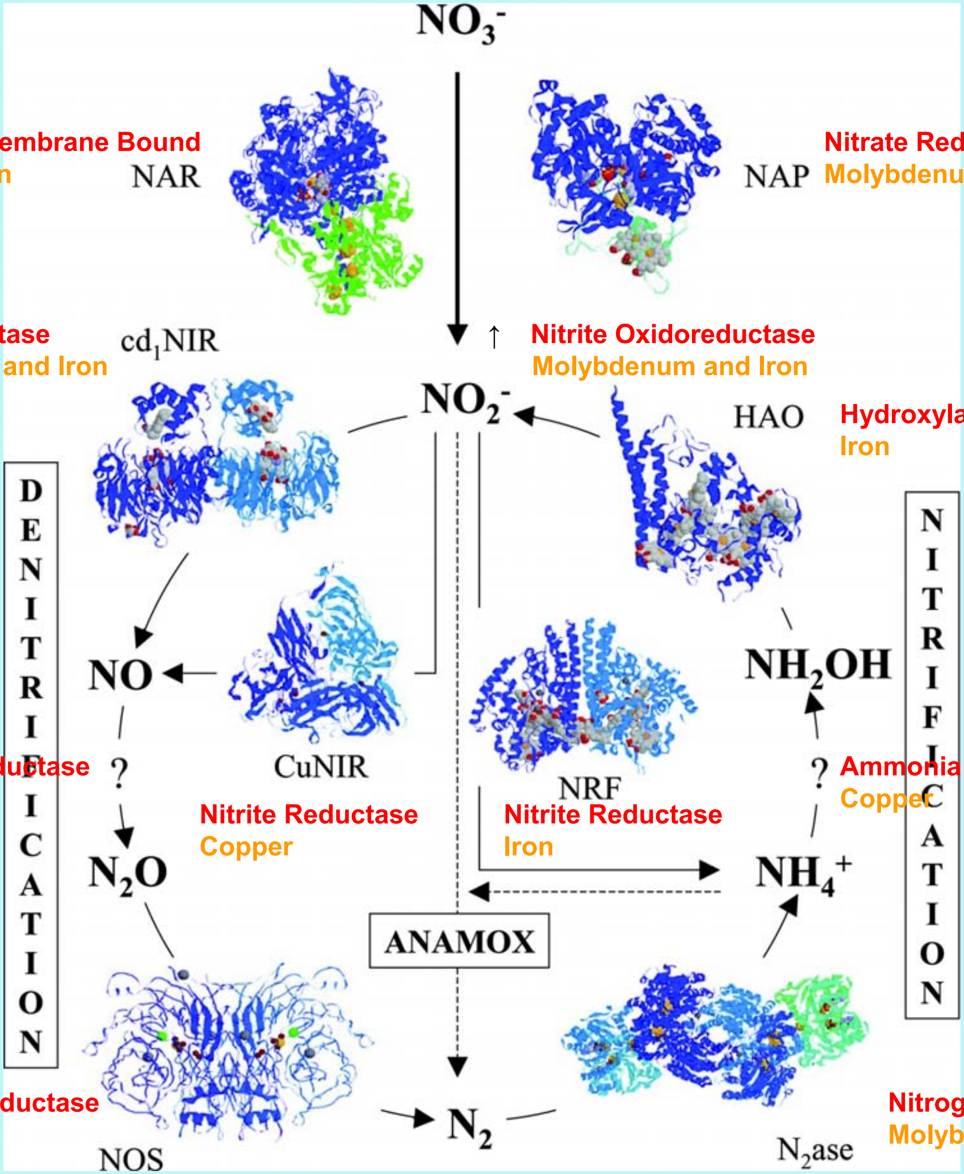
NO_2^-

+ $5H^+$

(2)

ENZYMES

X-ray structures of the enzymes of the bacterial nitrogen cycle
illustrating **the metals** necessary for successful bacterial metabolism



Nitrate Reductase, Membrane Bound
Molybdenum and Iron

Nitrate Reductase, Periplasmic
Molybdenum

Nitrite Reductase
Molybdenum and Iron

Nitrite Oxidoreductase
Molybdenum and Iron

Hydroxylamine Oxidoreductase
Iron

Nitric Oxide Reductase
Iron

Ammonia Mono-Oxygenase
Copper

Nitrite Reductase
Copper

Nitrite Reductase
Iron

Nitrous Oxide Reductase
Copper

Nitrogenase
Molybdenum and Iron

Biochemical Society Transactions (2005) 33, 113-118 Butler and Richardson

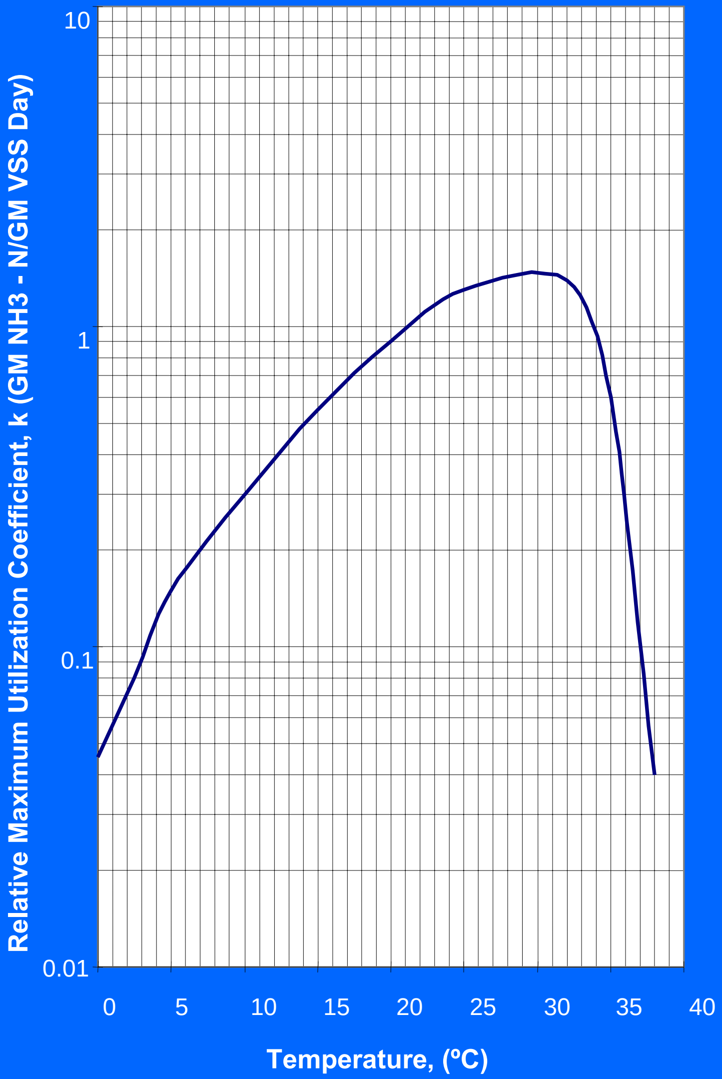
Biological Nitrification and Denitrification Reactor Process Design Parameters

1. TEMPERATURE
2. DISSOLVED OXYGEN LEVEL
3. pH AND ALKALINITY
4. SUBSTRATE LEVELS:

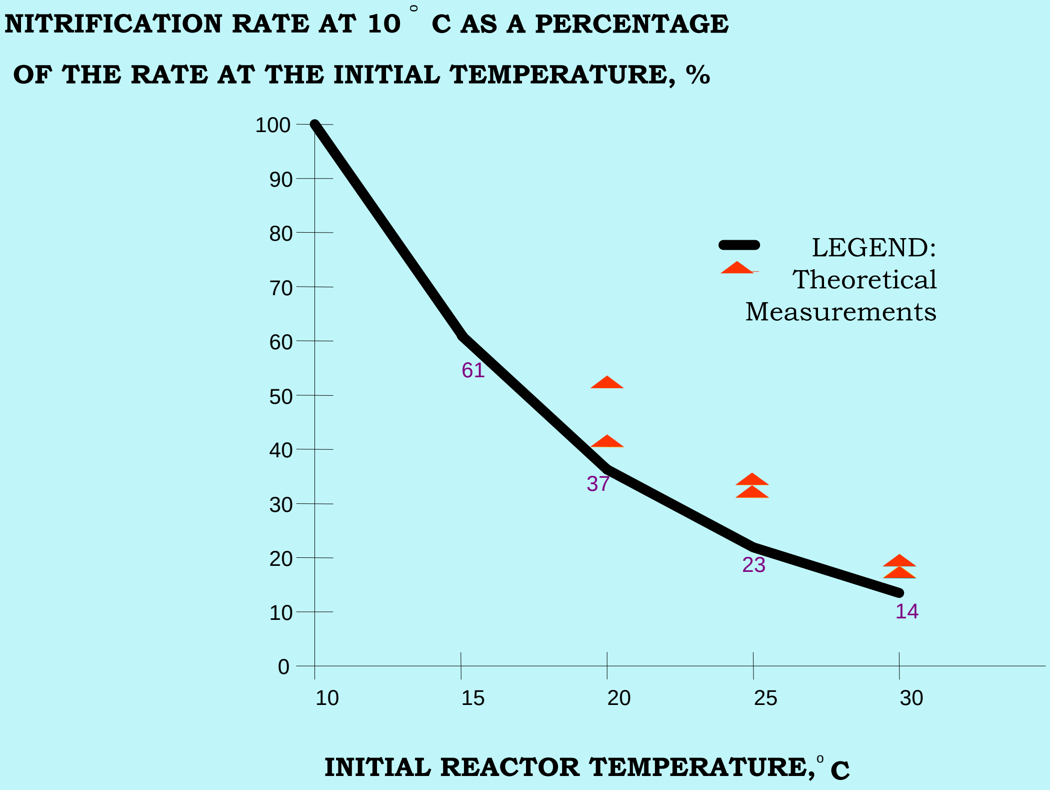
   NH_4^+, NO_3^-, NO_2^-, C
5. GROWTH RATE OF MICRO-ORGANISMS
6. INHIBITORY SUBSTANCES

Effect of Temperature

Maximum Ammonia Nitrogen Utilization Coefficient, k, (Nitrosomonas)

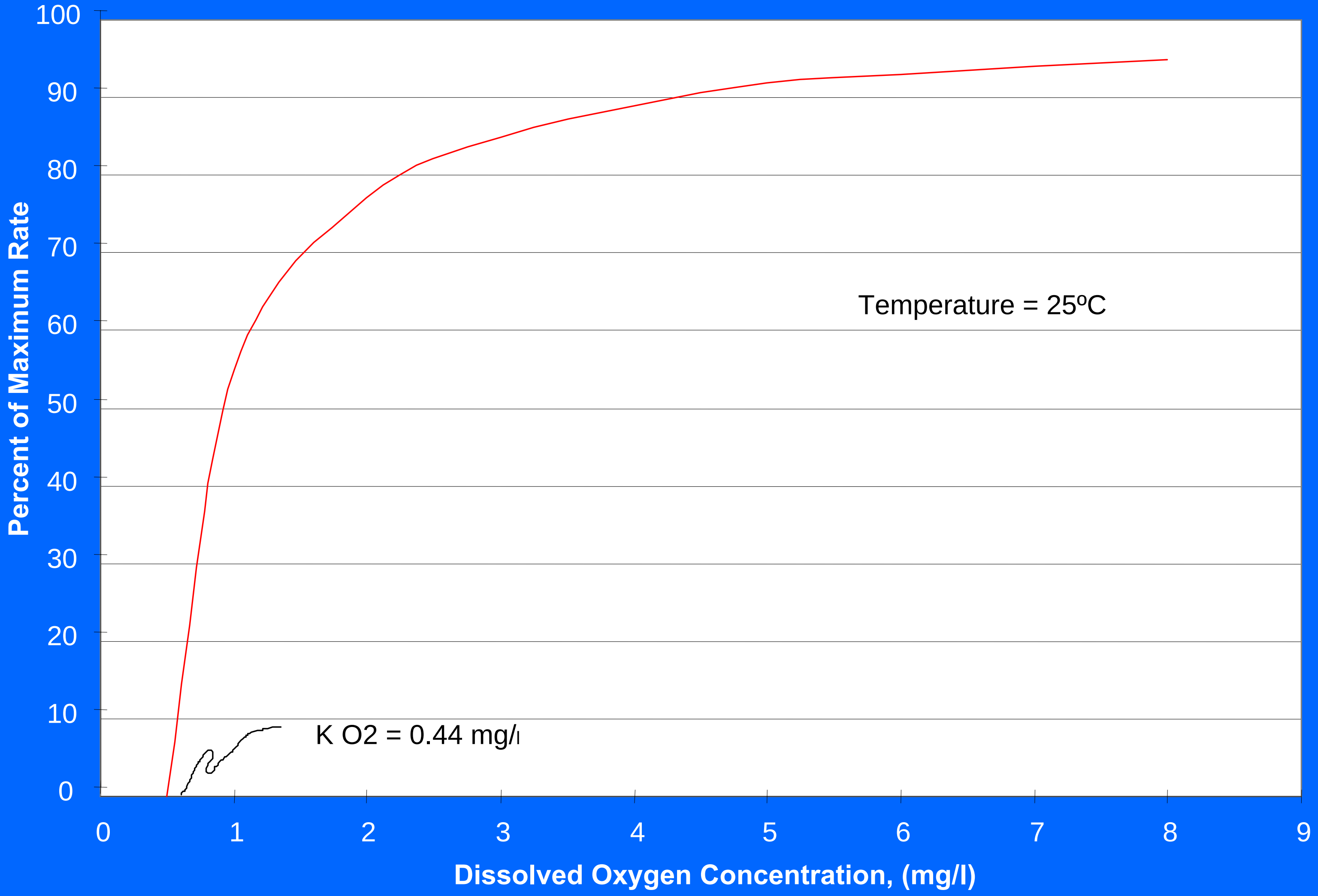


RESEARCH ON “SHOCK” EFFECT OF TEMPERATURE ON NITRIFICATION RATES

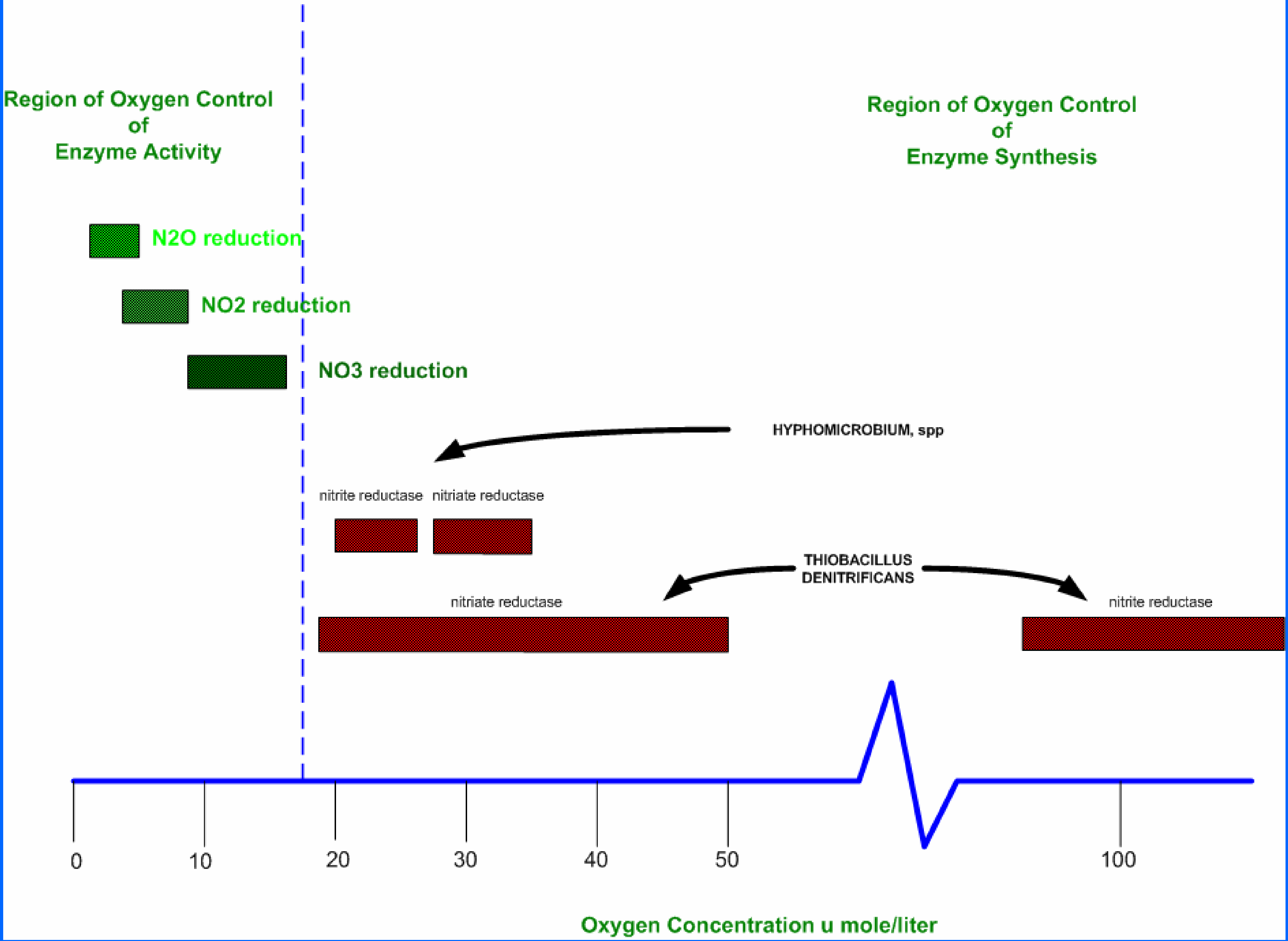


Work done @ University of Manitoba in Canada by Melanie Head[2]

Effect of Dissolved Oxygen on the Growth Rate of Nitrifiers



INFLUENCE of OXYGEN LEVEL on DENITRIFICATION and DENITRAFICATION



Region of Oxygen Control
of
Enzyme Activity

Region of Oxygen Control
of
Enzyme Synthesis

N2O reduction

NO2 reduction

NO3 reduction

HYPHOMICROBIUM, spp

nitrite reductase nitriate reductase

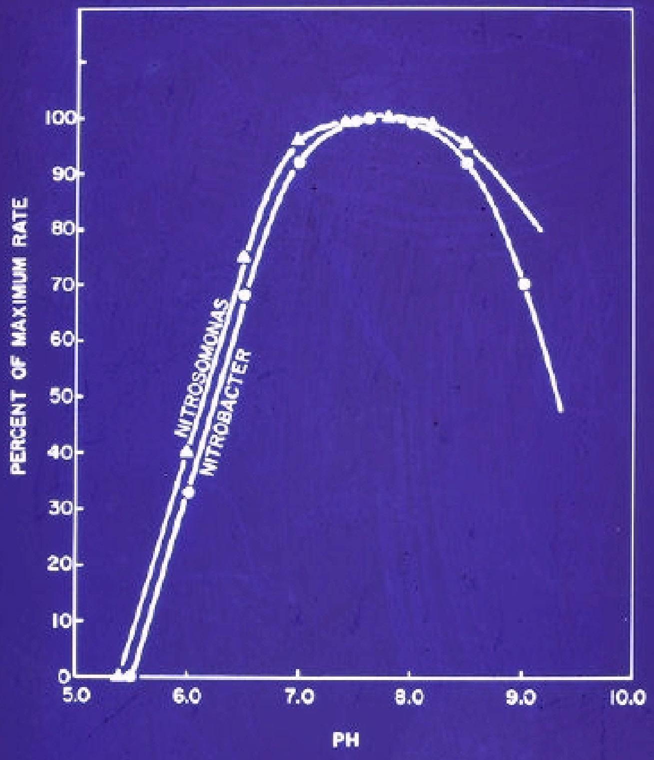
THIOBACILLUS
DENITRIFICANS

nitriate reductase

nitrite reductase

Oxygen Concentration u mole/liter

The Effect of pH
on the growth rate of Nitrifiers and Nitrafiers

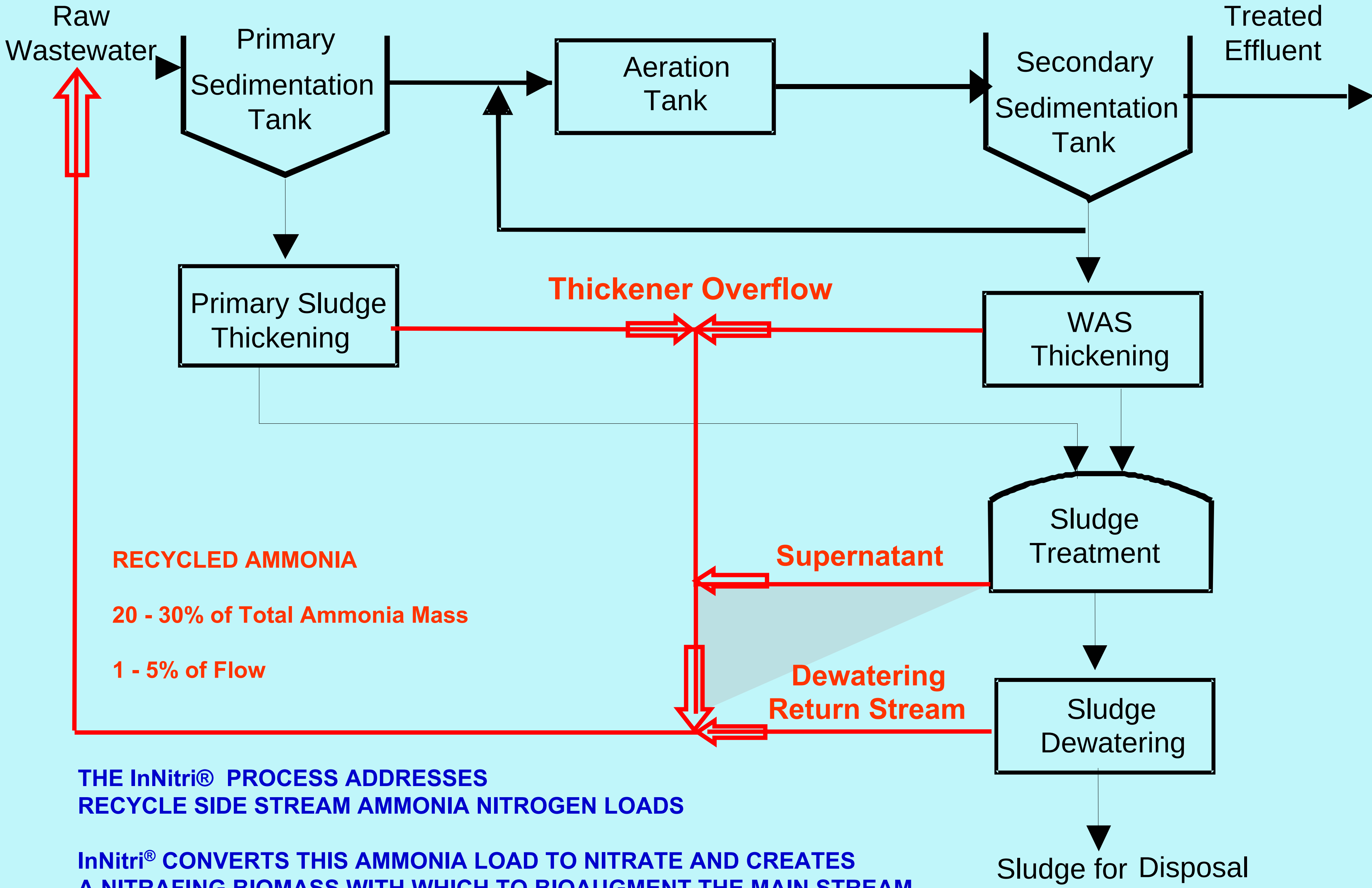


EFFECT OF PH ON THE GROWTH RATE
OF NITRIFYING ORGANISMS
(CULTURE ACCLIMATED TO PH 7.5-8.0)

InNitri®

Inexpensive **Nitri**fication

Peter Kos PhD
Dorr-Oliver
Malcolm Pirnie
Private Consultant



Raw Wastewater

Primary Sedimentation Tank

Aeration Tank

Secondary Sedimentation Tank

Treated Effluent

Primary Sludge Thickening

Thickener Overflow

WAS Thickening

Sludge Treatment

Supernatant

RECYCLED AMMONIA

20 - 30% of Total Ammonia Mass

1 - 5% of Flow

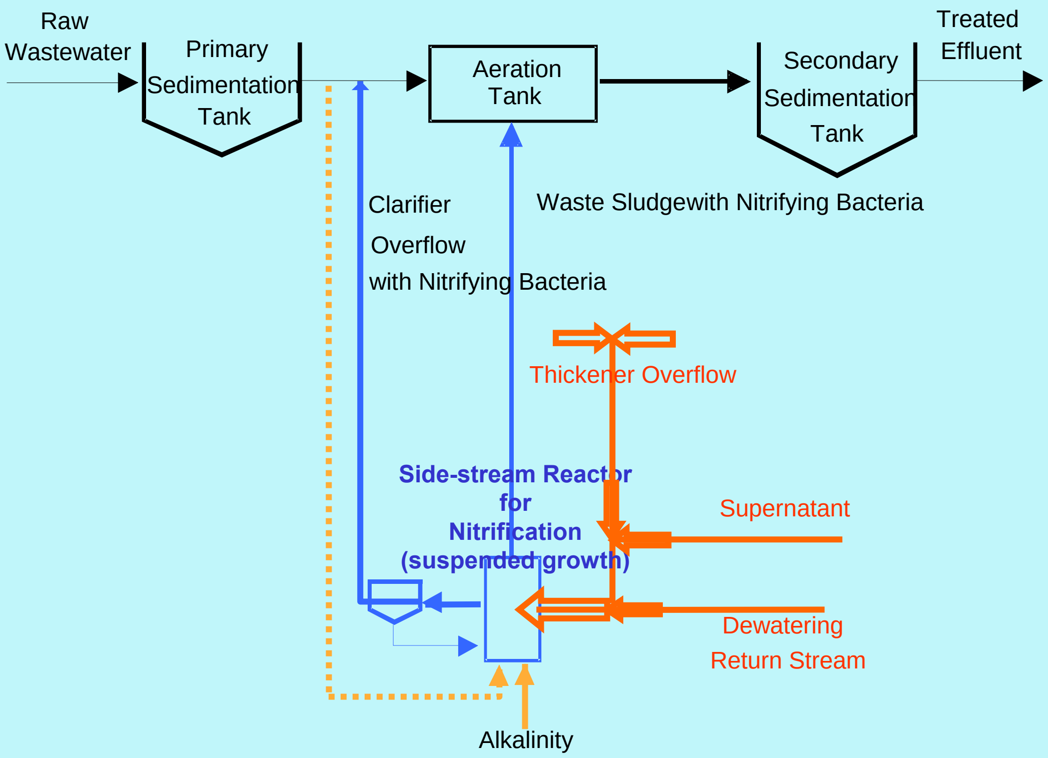
Dewatering Return Stream

Sludge Dewatering

Sludge for Disposal

THE InNitri® PROCESS ADDRESSES RECYCLE SIDE STREAM AMMONIA NITROGEN LOADS

InNitri® CONVERTS THIS AMMONIA LOAD TO NITRATE AND CREATES A NITRAFING BIOMASS WITH WHICH TO BIOAUGMENT THE MAIN STREAM REACTOR REDUCING THE SRT NEEDED TO NITRAFY IN THE MAIN STREAM



Raw Wastewater

Primary Sedimentation Tank

Aeration Tank

Secondary Sedimentation Tank

Treated Effluent

Clarifier Overflow with Nitrifying Bacteria

Waste Sludgewith Nitrifying Bacteria

Thickener Overflow

Side-stream Reactor for Nitrification (suspended growth)

Supernatant

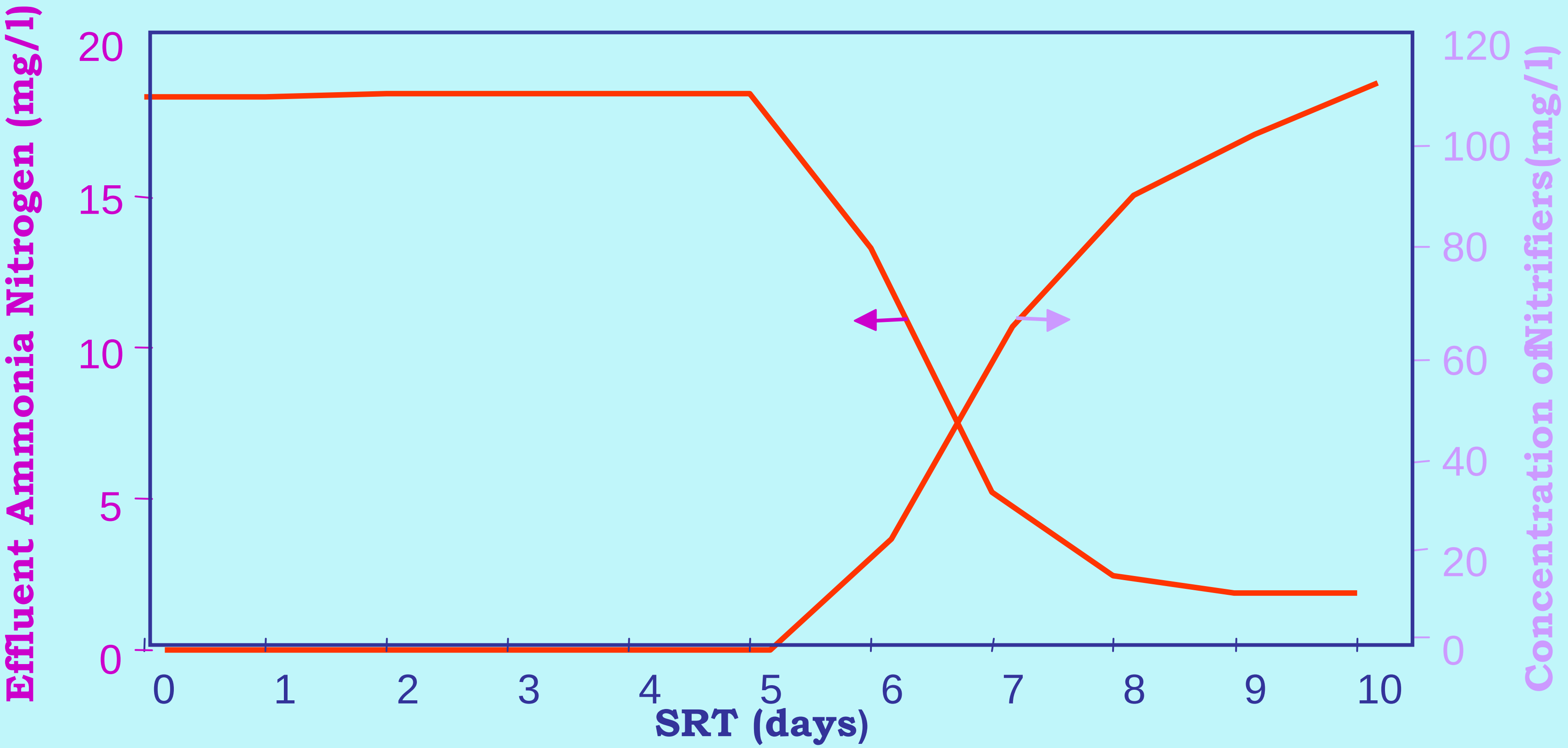
Dewatering Return Stream

Alkalinity

InNitri® Reactor

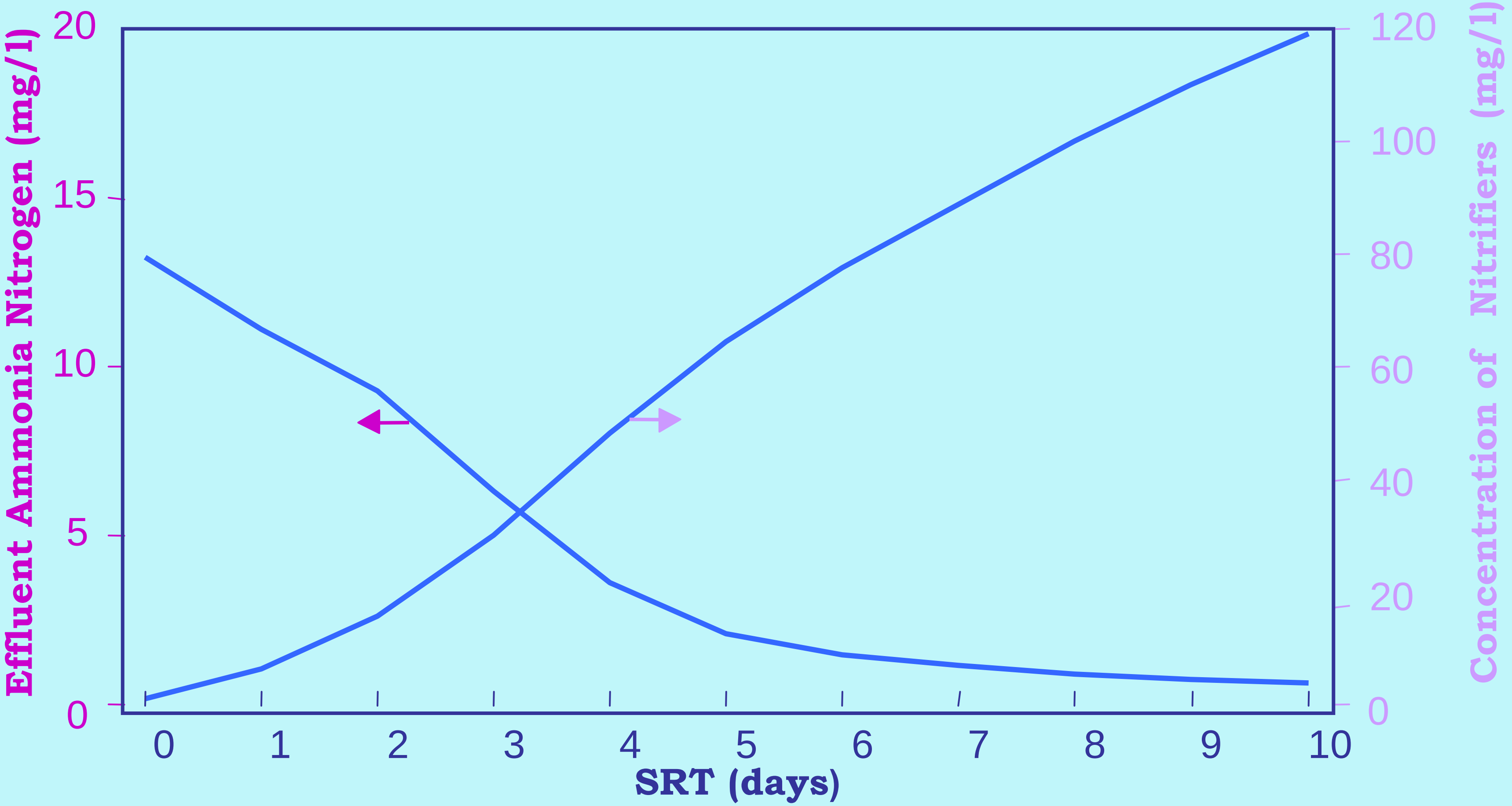
Buildup of Nitrifiers and the Effect on Effluent @ 10 ^{o}C

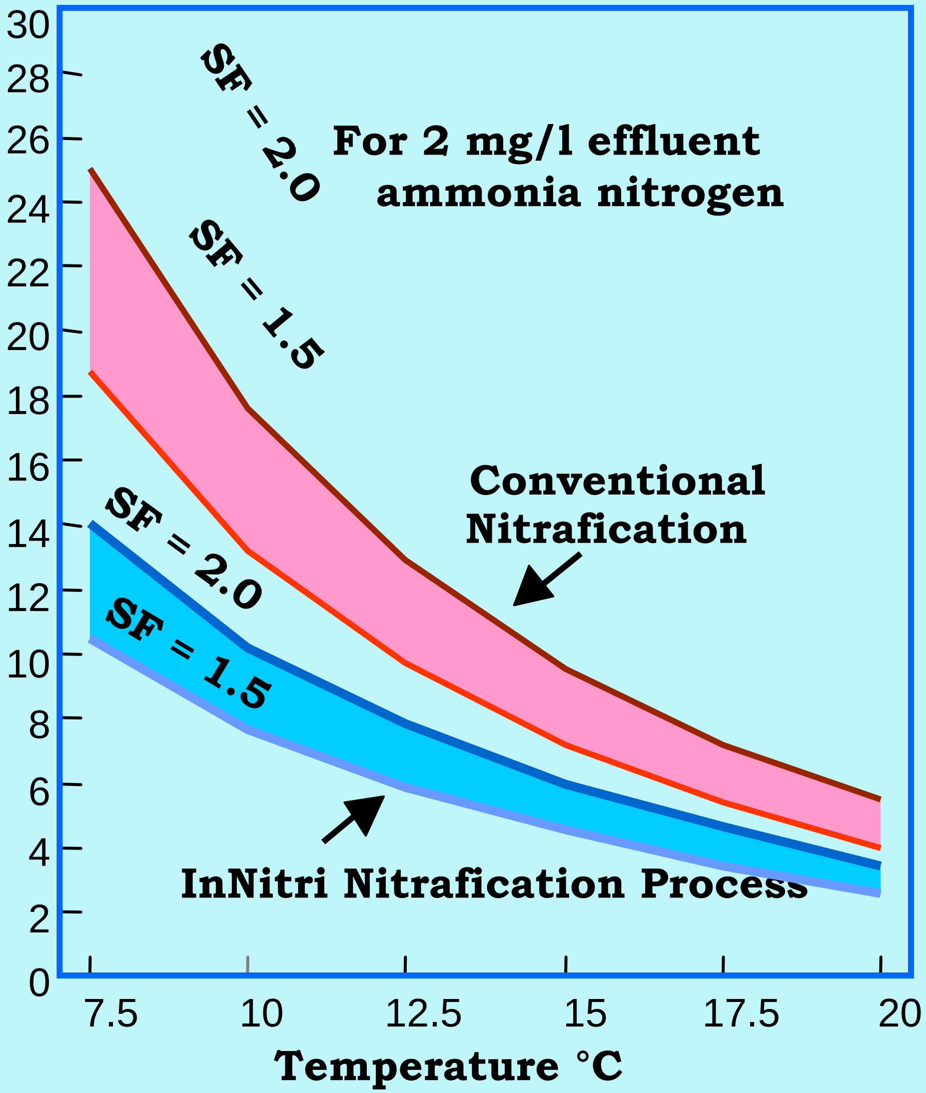
Legend — **Conventional Nitrification Process**



Buildup of Nitrifiers and the Effect on Effluent @10^{O} C

Legend — **Sidestream Nitrification Process**





For 2 mg/l effluent ammonia nitrogen
SF = 2.0
SF = 1.5
Conventional Nitrafication
SF = 2.0
SF = 1.5
InNitri Nitrafication Process
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
7.5
10
12.5
15
17.5
20
Temperature °C

COMMERCIAL DEVELOPMENT

Projects in North America

LOCATION	NITROGEN LOAD lbs/day	COMMISIONING DATE	STATUS
Tucson, AZ	2043	Demonstration Program	(P)
Richmond, VA	2852	Designed Ready for Bid	(D)

(P) in demonstration (D) in design; (C) in construction; (O) in operation

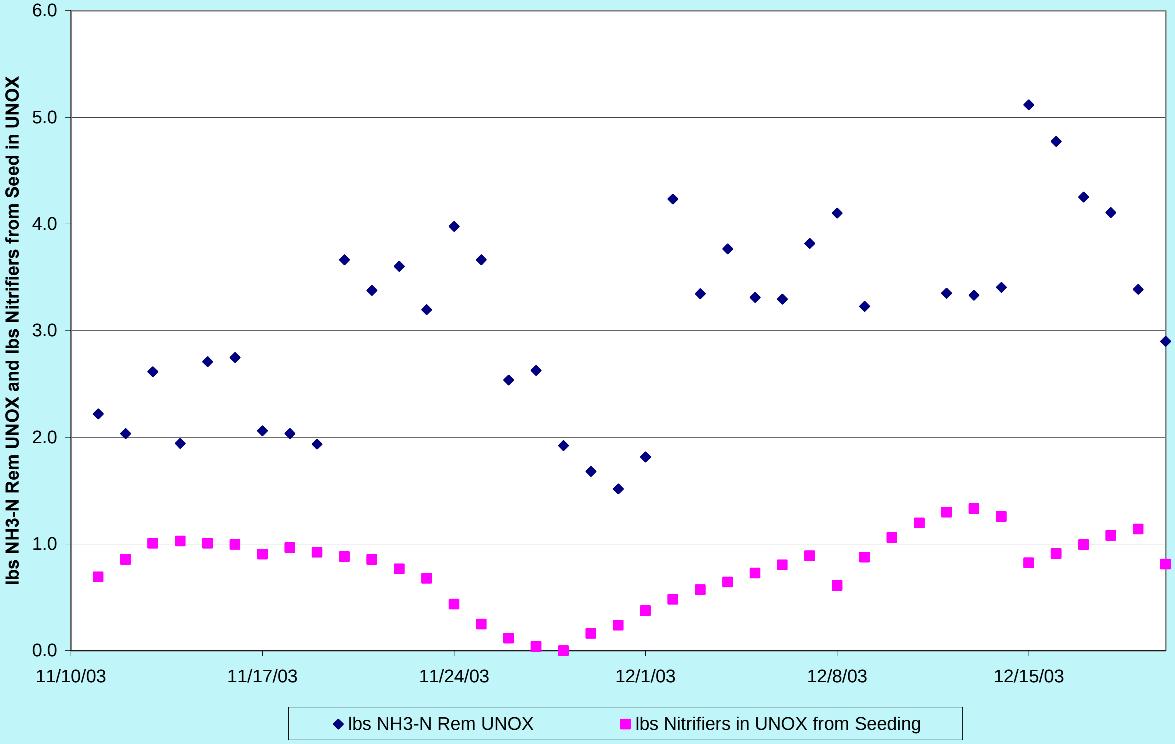
Tuscon, AZ, USA, Ina Road, Pilot Plant

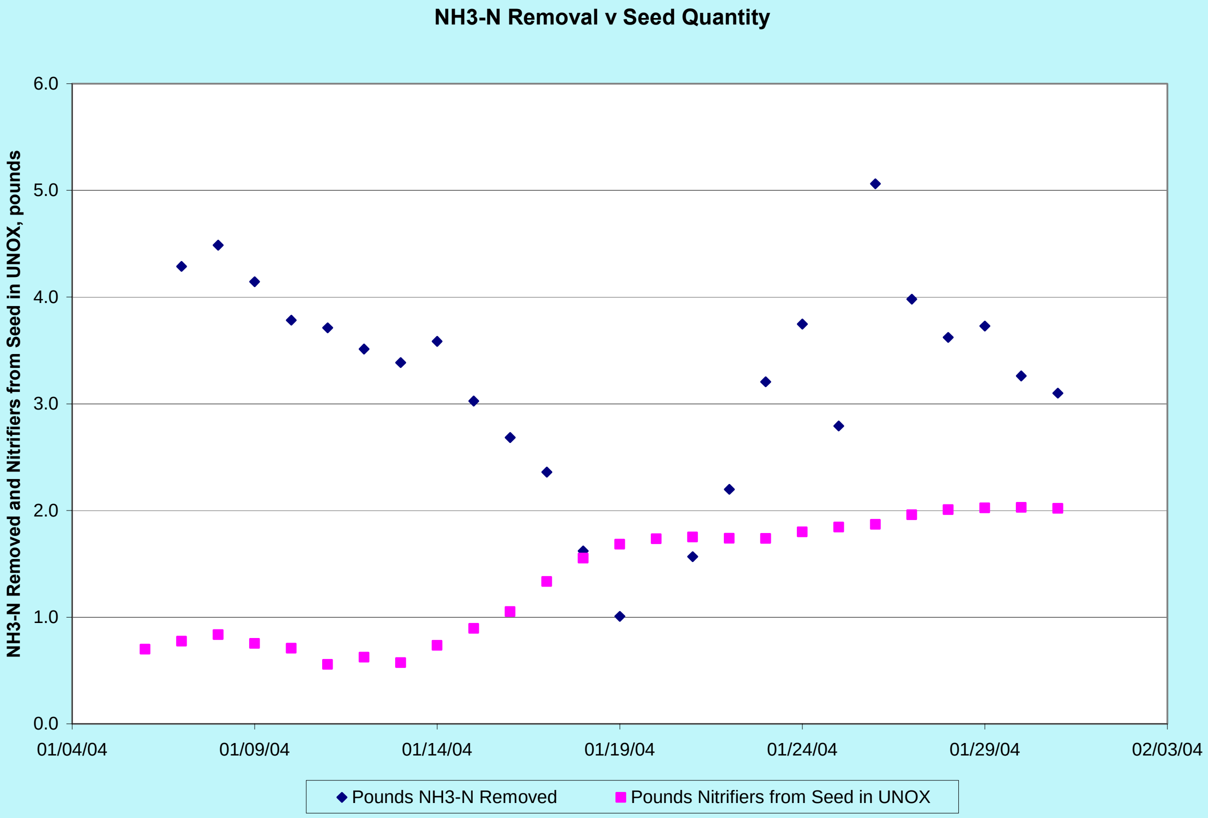
The InNitri Reactor

CENTRATE TREATMENT SYSTEM SUMMARY PERFORMANCE

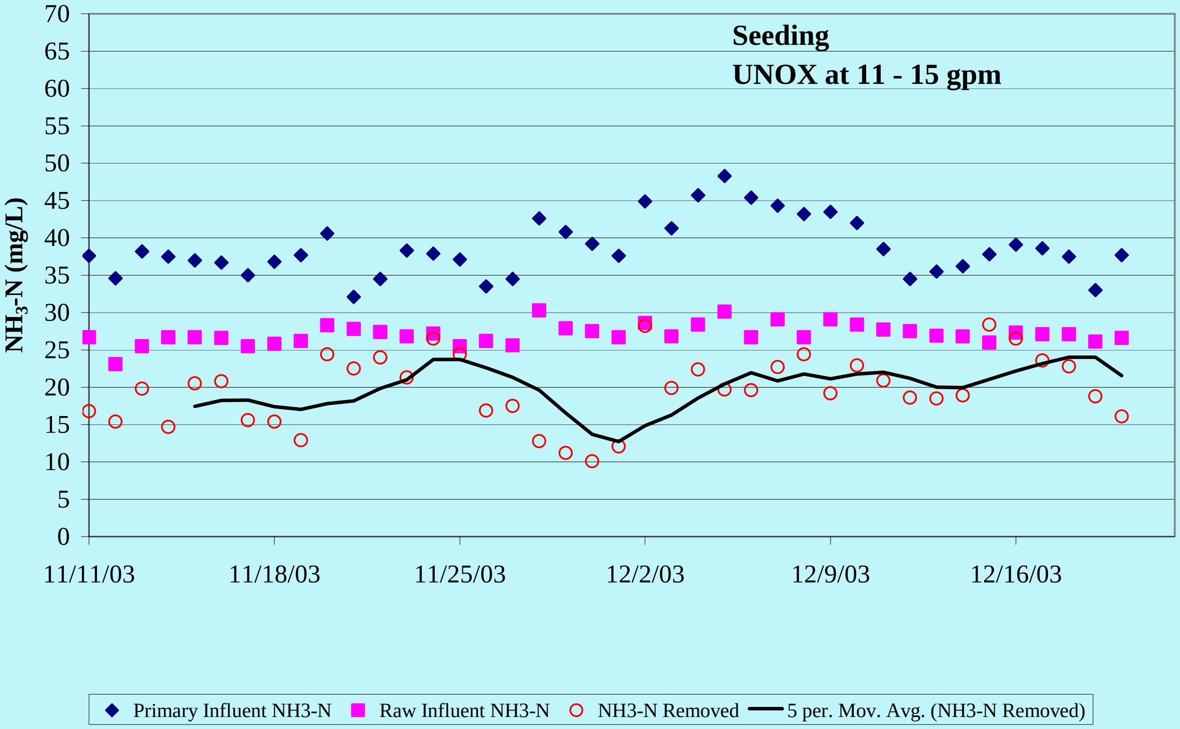
Operating Conditions	Phase 1	Phase 2
Duration, days	39	25
Average SRT, days	3.78	4.75
Average Feed NH3-N, mg/l	767	940
Average Effluent NH3-N, mg/l	12	4
Average Temperature, °C	27	29
Average Dissolved Oxygen, mg/l	3.1	3.1
Average / Maximum NH3-N loading, lbs/1000ft3/day	26 / 38	35 / 42
Average Percent of Seed produced transferred to UNOX™ System, %	60%	80%
Equivalent Centrate Flow for 25 MGD UNOX™ Flow, gpd (Based on observed seed transfer, assuming a 50 mg/l TSS concentration in the final clarifier effluent of the centrate nitrification system.)	200,000	250,000

UNOX System
NH3-N Removal vs Seed Quantity




NH3-N Removal v Seed Quantity
NH3-N Removed and Nitrifiers from Seed in UNOX, pounds
6.0
5.0
4.0
3.0
2.0
1.0
0.0
01/04/04
01/09/04
01/14/04
01/19/04
01/24/04
01/29/04
02/03/04
◆ Pounds NH3-N Removed
■ Pounds Nitrifiers from Seed in UNOX

UNOX Reactor



Richmond, Virginia *InNitri*® System

80 MGD Activated Sludge Plant

Design Engineer Greeley & Hansen

Bid Tender 3rd OTR 2009
Construction 4th QTR 20 09

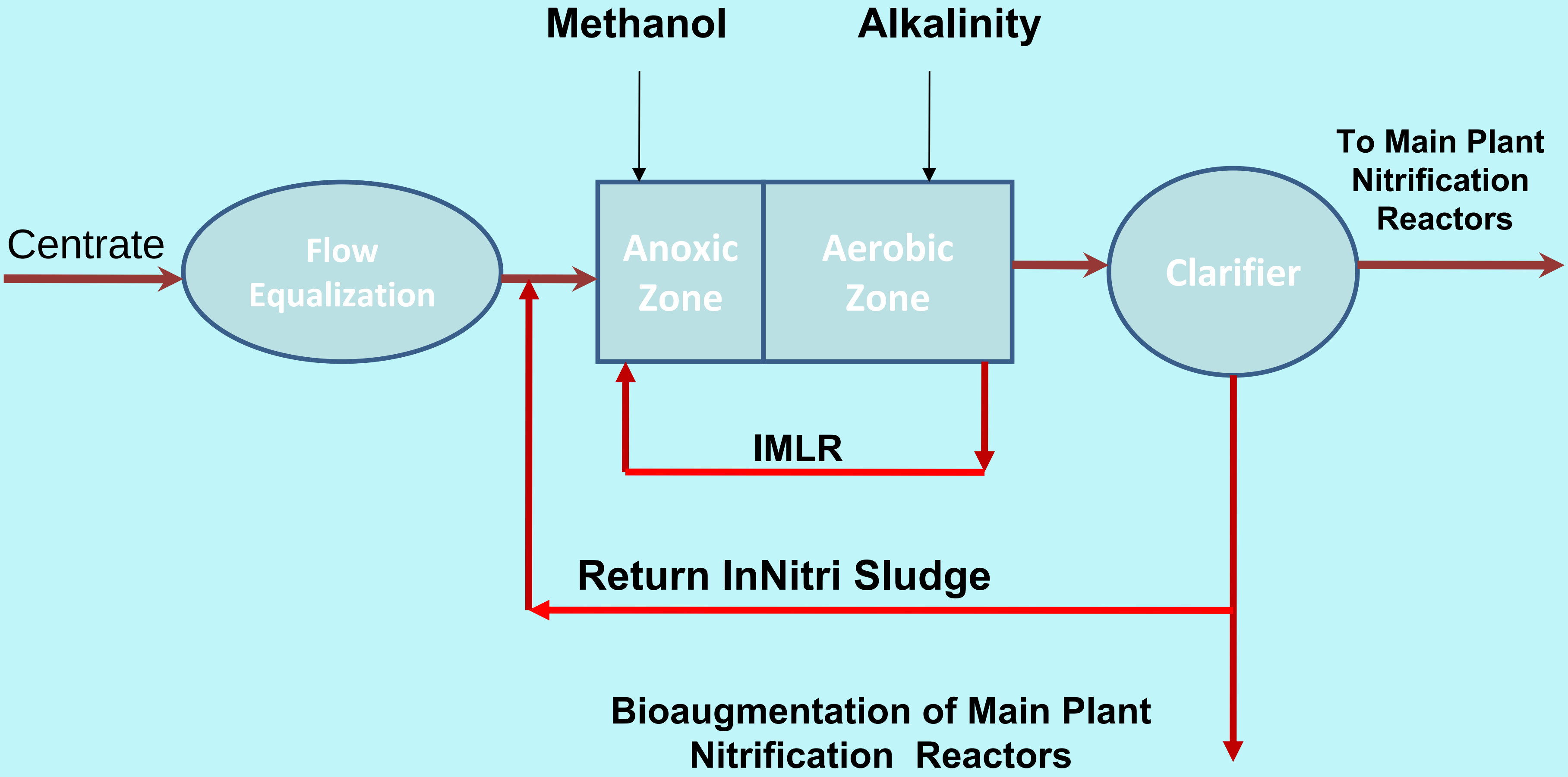
Reason for Bioaugmentation

Inability to achieve nitrification in winter

InNitri System Process Design Parameters
Richmond, Virginia

Process	Average	Maximum
Flow, MGD	0.3	0.36
CBOD,mg/l	830	830
TSS, mg/l	380	380
TKN, mg/l	1000	1000
NH3, mg/l	850	850
Alkalinity, mg/l	3500	3500
Temperature, c	20 - 27	20 - 27
IMLR Ratio to Flow	5	4.2
IMLR flow, MGD	1.5	1.5
Alkalinity Addition, mg/l	2100	2100
Methanol Addition (30%), gpd	2950	2950
RAS, MGD	0.3	0.3

InNitri System Process Schematic
Richmond, Virginia



Methanol

Alkalinity

Centrate

Flow Equalization

Anoxic Zone

Aerobic Zone

Clarifier

To Main Plant Nitrification Reactors

IMLR

Return InNitri Sludge

Bioaugmentation of Main Plant Nitrification Reactors

m2t technologies

Dr Peter Kos

WE, THANK YOU FOR YOUR TIME
ARE THERE ANY QUESTIONS