CE 421/521 Environmental Biotechnology - Fall 2008

Biogeochemcial Cycles (C, N, P, & S)

- composition of b_______ cell (molar formula: $C_5H_7O_2N$ with P 1/5 of the N requirement)
- limiting nutrients are _____ and _____

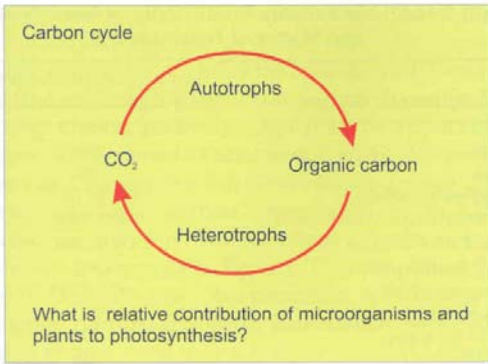


FIGURE 14.1 The carbon cycle is dependent on autotrophic organisms that fix carbon dioxide into organic carbon and heterotrophic organisms that respire organic carbon to carbon dioxide.

TABLE 14.1 Chemical Composition of an *E. coli* Cell

Elemental breakdown	% dry mass of an *E. coli* cell
Major elements	
Carbon	50
Oxygen	20
Hydrogen	8
Nitrogen	14
Sulfur	1
Phosphorus	3
Minor elements	
Potassium	2
Calcium	0.05
Magnesium	0.05
Chlorine	0.05
Iron	0.2
Trace elements	
Manganese	all trace elements combined comprise 0.3% of dry weight of cell
Molybdenum	
Cobalt	
Copper	
Zinc	

Adapted from Neidhardt *et al.* (1990).

TABLE 14.3 Global Carbon Reservoirs

Carbon reservoir	Metric tons carbon	Actively cycled
Atmosphere		
CO_2	6.7×10^{11}	Yes
Ocean		
Biomass	4.0×10^{9}	No
Carbonates	3.8×10^{13}	No
Dissolved and particulate organics	2.1×10^{12}	Yes
Land		
Biota	5.0×10^{11}	Yes
Humus	1.2×10^{12}	Yes
Fossil fuel	1.0×10^{13}	Yes
Earth's crust[a]	1.2×10^{17}	No

[a] This reservoir includes the entire lithosphere found in either terrestrial or ocean environments. (Data from Dobrovolsky, 1994.)

TABLE 14.4 Net Carbon Flux between Selected Carbon Reservoirs

Carbon source	Flux (metric tons carbon/year)
Release by fossil fuel combustion	7×10^{9}
Land clearing	3×10^{9}
Forest harvest and decay	6×10^{9}
Forest regrowth	-4×10^{9}
Net uptake by oceans (diffusion)	-3×10^{9}
Annual flux	9×10^{9}

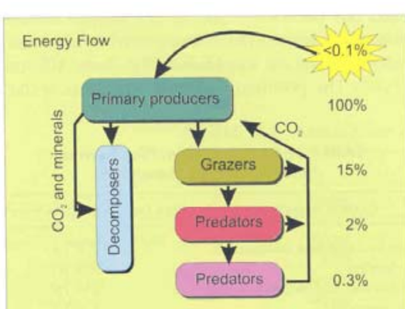


FIGURE 14.2 Diagram of the efficiency of sunlight energy flow from primary producers to consumers.

Table 14.6 Major Types of Organic Components of Plants

Plant component	% dry mass of plant
Cellulose	15–60
Hemicellulose	10–30
Lignin	5–30
Protein and nucleic acids	2–15

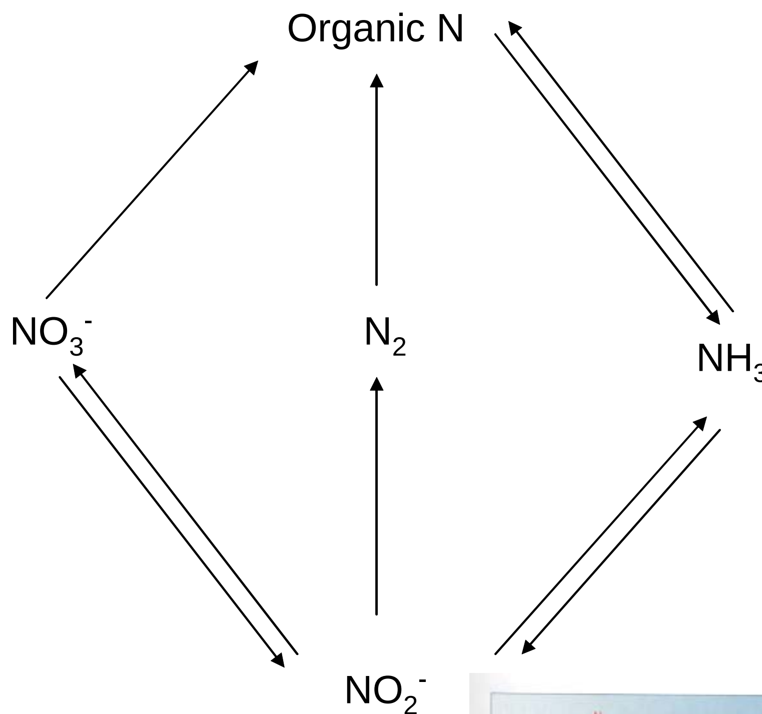
NITROGEN

- Atmosphere is _____% nitrogen, yet nitrogen is considered a l__________ n__________
- required in p____________

TABLE 14.10 Global Nitrogen Reservoirs

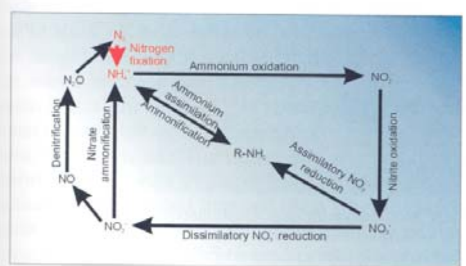
Nitrogen reservoir	Metric tons nitrogen	Actively cycled
Atmosphere		
N_2	3.9×10^{15}	No
Ocean		
Biomass	5.2×10^{8}	Yes
Dissolved and particulate organics	3.0×10^{11}	Yes
Soluble salts (NO_3^-, NO_2^-, NH_4^+)	6.9×10^{11}	Yes
Dissolved N_2	2.0×10^{13}	No
Land		
Biota	2.5×10^{10}	Yes
Organic matter	1.1×10^{11}	Slow
Earth's crust[a]	7.7×10^{14}	No

[a] This reservoir includes the entire lithosphere found in either terrestrial or ocean environments. (Adapted from Dobrovolsky, 1994.)



Fixation

- _____________ metric tons/y compared to 2.5×10^{10} metric tons C/y
- c__________________ & few others
  - non-s_________ – *Clostridia*
  - symbiotic – *Rhizobium*
- n_______________ – requires Mg^{2+} & ATP (15 to 20 ATP/N_2)



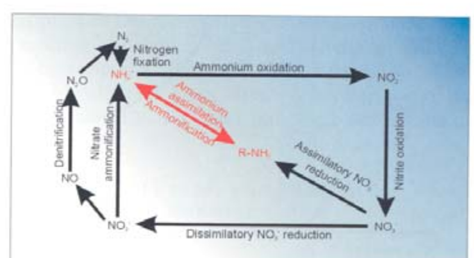
Summary:
N_2 fixation is energy intensive
End product of N_2 fixation is ammonia
N_2 fixation is inhibited by ammonia
Nitrogenase is O_2 sensitive, some free-living N_2 fixers require reduced O_2 tension

Assimilation – NH_3 (NH_4^+) preferred, will use NO_3^- but has to be reduced to NH_4^+

- C/N ratio is approximately ______:1 for aerobes
- C/N ratio is approximately ______:1 for anaerobes
- C/N ratio is approximately ______:1 for anaerobes in highly loaded (high rate) system
- cell c_____________ is characterized by the empirical formula: C H O N with the P requirement as _________the N requirement (alternatively $C_{60}H_{87}O_{23}N_{12}P$)
- in general cell composition is
  - * 50%
  - * 20%
  - * 10-15%
  - * 8-10%
  - * 1-3%
  - * 0.5-1.5%

Ammonification: breakdown of o_________ N to inorganic nitrogen

ex. urea:



Summary:
Assimilation and ammonification cycles ammonia between its organic and inorganic forms
Assimilation predominates at C:N ratios > 20
ammonification predominates at C:N ratios < 20

proteins: proteins ➜ amino acids

Nitrification: two step process

1.

2.

- requires _____________ mg O_2/mg NH_4^+-N converted to NO_3^- – N

TABLE 14.13 Chemoautotrophic Nitrifying Bacteria

Genus	Species
Ammonium oxidizers	
Nitrosomonas	*europaea* *eutrophus* *marina*
Nitrosococcus	*nitrosus* *mobilis* *oceanus*
Nitrosospira	*briensis*
Nitrosolobus	*multiformis*
Nitrosovibrio	*tenuis*
Nitrite oxidizers	
Nitrobacter	*winogradskyi* *hamburgensis* *vulgaris*
Nitrospina	*gracilis*
Nitrococcus	*mobilis*
Nitrospira	*marina*

Nitrification Kinetics

$$\mu = \frac{\mu_{max} S_{NH4}}{K_S + S_{NH4}} \cdot \frac{S_{O2}}{K_O + S_{O2}}$$

(double M__________, pronounced muh nō′)

where
μ_{max} = maximum specific g__________ rate, h^{-1}
K_S = half s________________ coefficient for ammonia, mg/L as NH_4-N
K_O = half saturation coefficient, mg/L as O_2
Yield = mg b____________ formed/mg a________________ utilized

	Nitrosomonas		Nitrobacter	
parameter	range	typical (@ 20°C)	range	typical (@ 20°C)
μ_{max}	0.014 - 0.092	0.032	0.006 - 0.06	0.034
K_S	0.06 - 5.6	1.0	0.06 - 8.4	1.3
K_O	0.3 - 1.3	0.5	0.3 - 1.3	0.68
Yield	0.04 - 0.13	0.1	0.02 - 0.07	0.05

Optimum pH for nitrifiers is around 8.0, range 7.5 - 8.5 (higher than for most other biological processes).

Nitrifiers are sensitive to

- d__________________ o____________________
- t________________
- p___
- i_________________________

$$\mu = \frac{\mu_{\max} S_{NH4}}{K_S + S_{NH4}} \cdot \frac{K_I}{K_I + I}$$

where I = concentration of inhibitor, mg/L
K_I = inhibition coeficient, mg/L

Effects of Temperature

derivation of the A________________ equation $k = Ae^{\frac{-\mu}{RT}}$

$$k_2 = k_1 \theta^{(T_2 - T_1)}$$

where $k_{1,2}$ = reaction rate coefficient at temperature $T_{1,2}$
θ = t_______________ c_______________________________-

theta values

	Nitrosomonas	Nitrobacter
μ_{max}	1.098 - 1.118	1.068 - 1.112
K_S	1.125	1.157
k_d	1.029 - 1.104	1.029 - 1.104

given the following measured data, calculate the theta value

T, °C	b, h^{-1}
10	0.0037
20	0.0095
30	0.0229
40	0.0372

DENITRIFICATION

1. A_____________________ nitrate reduction: NO_3^- ➜ NH_4^+ nitrate is incorporated into cell material and reduced inside the cell

2. D___________________ nitrate reduction (denitrification)

NO_3^- serves as the t____________ e_______________ a_________________ (TEA) in an anoxic (anaerobic) environment

nitrate reductase, nitrite r., nitric oxide r., nitrous oxide r.

NO_3^- ➜ NO_2^- ➜ NO ➜ N_2O ➜ N_2

summarized as:

NO_3^- ➜ NO_2^- ➜ N_2

requires o______________ m________________(example: methanol)

$$6\,NO_3^- + 5CH_3OH \rightarrow 3N_2 + 5\,CO_2 + 7\,H_2O + 6\,OH^-$$

$$\mu = \frac{\mu_{max} S}{K_S + S} \cdot \frac{NO_3^-}{K_{NO3} + NO_3^-}$$

calculate COD of methanol:

calculate alkalinity:

kinetics for denitrification similar to those for heterotrophic aerobic growth

Nitrogen Removal in Wastewater Treatment Plants

Total Kjeldahl Nitrogen (TKN) = o___________ n___________ + a_____________
(measured by digesting sample with sulfuric acid to convert all nitrogen to ammonia)

- TKN ~ 35 mg/L in influent
- p____________ t____________ removes approximately 15%
- additional removal with biomass w______________

3 Methods for Nitrogen Removal

1. Biological

   - n_______________
   - d________________
   - ANAMMOX: ammonium is the electron donor, nitrite is the TEA

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

2. Chemical/Physical

   - air s_______________
   - breakpoint c__________________
   - ion e_____________________
   - reverse o___________________

Concerns for nitrogen discharge:
1. T________________
2. D________________ of DO
3. E__________________________
4. Nitrate in d________________ water – causes methemoglobinemia (blue baby) oxidizes hemoglobin to methemoglobin

PHOSPHORUS

— limiting n___________________ in algae (at approximately 1/5 the nitrogen requirement)
— 15% of population in US discharges to l_________________
— wastewater discharge contains approximately 7- 10 mg/L as P
— o__________________
— i______________: orthophosphate

Removal of Phosphorus

1. Chemical precipitation:

   a. traditional p___________________________ reactions

   $Al^{+3} + PO_4^{-3} \rightarrow AlPO_4$

   $Fe^{+3} + PO_4^{-3} \rightarrow FePO_4$

   b. as s__________________ (magnesium ammonium phopshate, MAP)

Mg^{+2} + NH_4^+ + PO_4^{-3} ➜ $Mg\ NH_4PO_4$

2. Enhanced Biological Phosphorus Removal (EBPR) see handout

SULFUR

— inorganic: SO_4^{-2} S° H_2S

— organic: R — O — SO_3^{-2}

— four key reactions:

1. H_2S o__________________ — can occur aerobically or anaerobically to elemental sulfur (S°)

a. a___________________ : *Thiobaccilus thioparus* oxidizes S^{-2} to S°

S^{-2} + ½ O_2 + $2H^+$ ➜ S° + H_2O

b. a_______________________: — phototrophs use H_2S as electron donor

— filamentous sulfur bacteria oxidize H_2S to S° in sulfur granules: *Beggiatoa, Thiothrix*

2. Oxidation of E_______________ Sulfur (Thiobacillus thiooxidans at low pH)

2S° + 3 O_2 + 2 H_2O ➜ 2 H_2SO_4

3. A_______________________ sulfate reduction: proteolytic bacteria breakdown organic matter containing sulfur (e.g. amino acids: methionine, cysteine, cystine)

TABLE 14.15 Genera of Denitrifying Bacteria

Genus	Interesting characteristics
Organotrophs	
Alcaligenes	Common soil bacterium
Agrobacterium	Some species are plant pathogens
Aquaspirillum	Some are magnetotactic, oligotrophic
Azospirillum	Associative N_2 fixer, fermentative
Bacillus	Spore former, fermentative, some species thermophilic
Blastobacter	Budding bacterium, phylogenetically related to *Rhizobium*
Bradyrhizobium	Symbiotic N_2 fixer with legumes
Branhamella	Animal pathogen
Chromobacterium	Purple pigmentation
Cytophaga	Gliding bacterium; cellulose degrader
Flavobacterium	Common soil bacterium
Flexibacter	Gliding bacterium
Halobacterium	Halophilic
Hyphomicrobium	Grows on one-C substrates, oligotrophic
Kingella	Animal pathogen
Neisseria	Animal pathogen
Paracoccus	Halophilic, also lithotrophic
Propionibacterium	Fermentative
Pseudomonas	Commonly isolated from soil, very diverse genus
Rhizobium	Symbiotic N_2 fixer with legumes
Wolinella	Animal pathogen
Phototrophs	
Rhodopseudomonas	Anaerobic, sulfate reducer
Lithotrophs	
Alcaligenes	Uses H_2, also heterotrophic, common soil isolate
Bradyrhizobium	Uses H_2, also heterotrophic, symbiotic N_2 fixer with legumes
Nitrosomonas	NH_3 oxidizer
Paracoccus	Uses H_2, also heterotrophic, halophilic
Pseudomonas	Uses H_2, also heterotrophic, common soil isolate
Thiobacillus	S-oxidizer
Thiomicrospira	S-oxidizer
Thiosphaera	S-oxidizer, heterotrophic nitrifier, aerobic denitrification

From Myrold, 1998.

4. D_______________________ sulfate reduction: under anaerobic conditions — s_____________ r________________ b_________________ (SRB)

SO_4^{-2} + Organics ➜ S^{-2} + H_2O + CO_2

S^{-2} + $2H^+$ ➜ H_2S

— *Desulvibrio* and others

— Sulfate is used as a TEA & l_____ m______________ w___________ organics serve as the electron donors

— Low cell y____________________

— P____________________ of SRB depends on COD:S ratio, particularly readily degradable (e.g., VFA) COD

— SRB compete with m______________________ for substrate: high COD:S favors methanogens, low COD:S favors SRB

Crown Sewer Corrosion

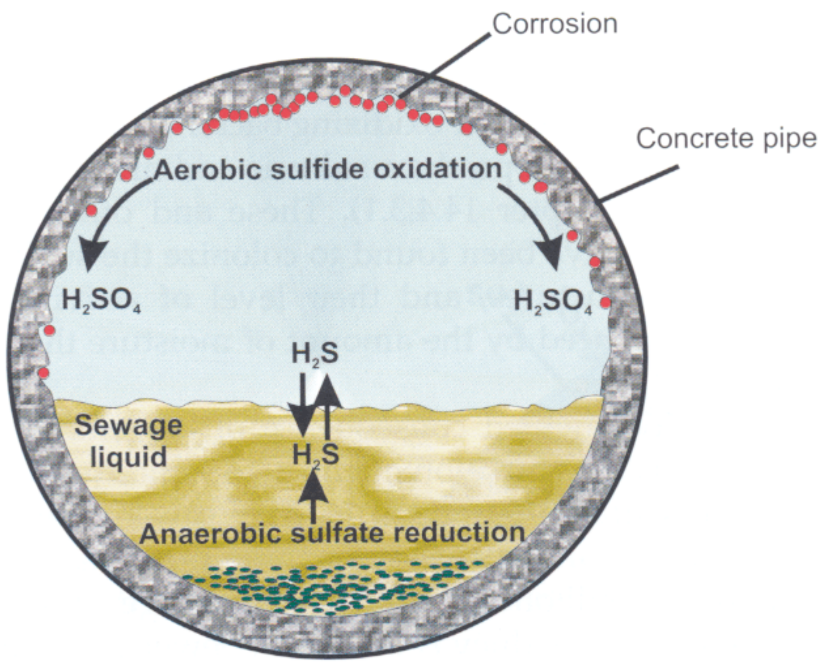


FIGURE 15.3 Cross section showing microbial involvement in the corrosion of a concrete sewer pipe. (Adapted from Sydney *et al.*, 1996.)