Tema 2 – Microbiología y Ciclos Biogeoquímicos
Microbiology and Biogeochemical Cycles

CI7115 – Biotecnología Ambiental
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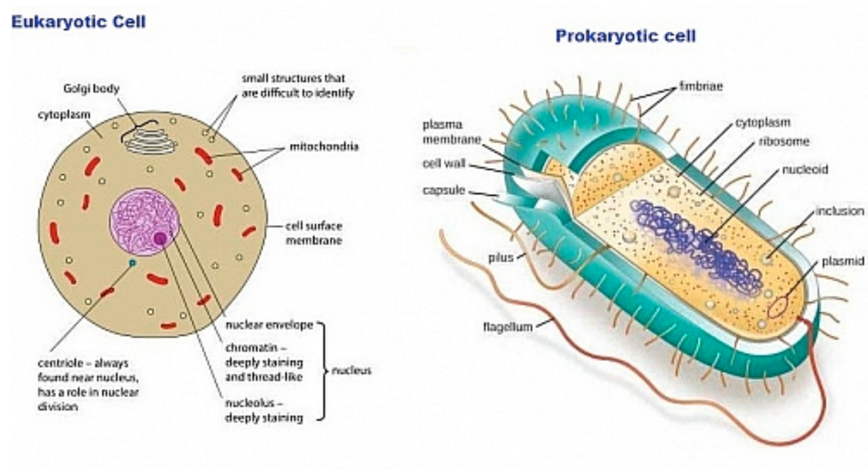
Clasificaciones

- Taxonomía – Taxonomy (science of classification)
  - Depends on observable physical properties (phenotype)
    - Morphology (appearance)
    - Response to staining or dying (gram +, gram -)
    - Ability to use or convert chemicals
- Filogenia - Phylogeny
  - Classifies according to evolutionary history; detects differences based on genetic characteristics
    - DNA (deoxyribonucleic acid) contains herditary information
    - RNA (ribonucleic acid) is involved in protein synthesis
      - 16S ribosomal RNA (16S rRNA) commonly used for identification
- Table 2.2 - Rittmann

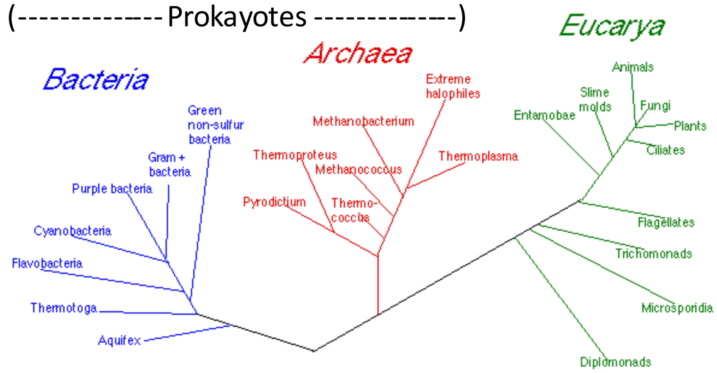
Estructura celular



Clasificaciones

- See Figure 1.1 (B&M)

(-------------- Prokayotes --------------)



http://www.sp.uconn.edu/~terry/229sp03/lectures/lect1.html

Clasificaciones

- **Eucariotas - Eukaryotes**
  - Higher multi-cellular organisms (e.g., plants and animals)
  - Fungi
    - *Eumycota* (true fungi)
    - *Mycophycomycota* (slime molds)
    - *Myxophycomycota* (lichens = fungus + alga)
    - EnvBiotech significance = composting, bioremediation
  - Algae
    - Phytoplanktons, all contain chlorophyll *a*
    - Cyanobacteria (blue green algae), which is actually a prokaryote, is often classified with algae.
    - EnvBiotech significance = phytoremediation, H_2 production, bioproducts (e.g., for antioxidants for nutraceuticals).
  - Protozoa
  - Rotifers, worms, nematodes, crustacea

Clasificaciones

- **Procariotas - Prokaryotes**
  - **Bacteria**
    - Cyanobacteria (blue green algae)
    - *E. coli*
  - **Archaea**
    - e.g., methanogens
- **Viruses**
  - Are they alive?
  - Submicroscopic genetic elements containing DNA/RNA and proteins
  - Cannot replace parts or carry out metabolism
  - At the lower limit of light microscope, requires electron microscope to view.
  - Bacteriophage (phage) – virus that infect prokaryotic cells
    - May play a significant role in biological WWT plants, cause changes in microbial populations, not well documented or understood.

Patógenos

- General term for microorganisms that cause infectious diseases
  - **Viruses** (e.g., Norwalk, hepatitus A, polio), **bacteria** (*E. coli, Legionella pneumophilia, Salmonella typhi, Shigella dysenteriae, Vibrio cholerae*), **algae** (Dinoflagellate), **protozoa** (*Giardia lamblia*, *Cryptosporidium parvum*), multicellular parasites (*Schistosoma mansoni*)

Rittmann &McCarty (2020)

Nomenclatura

- Kingdom, Phylum, Class, Order, Family, Genus, Species, (strains)
- "King Philip Came Over For Good Soup."
- Reino, Filo, Clase, Orden, Familia, Género, Especie – Reina Filomena come…
- *Genus species* or Genus species
  - Never capitalize species name
  - Examples:
    - *Escherichia coli*, Escherichia coli
    - *E. coli*

Transferencia de energía en células y ciclos biogeoquímicos

- Microbes need **energy** and **carbon**!

Crecimiento Microbiano

- Increase in activity
  - Measure Substrate utilization kinetics
  - Measure enzyme activity
- Increase in mass (biomass)
  - Measure volatile (organic) suspended solids
- Increase in numbers
  - Enumeration
    - Plate count or molecular techniques

Requisitos de Crecimiento

Bacterial stoichiometry (empirical formula)

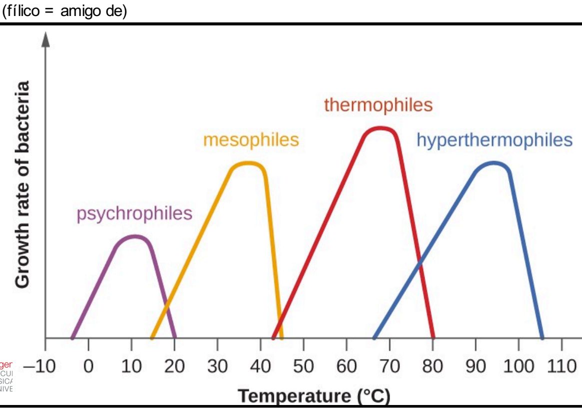
- **$C_5H_7O_2N$**
- 75-80% water (bound water vs. free water)
- 20-25% solids (dry matter), organic + inorganic
  - Organic matter ➔ N/P = 5/1

  VSS = 90% of dry weight
  - N = 12.4% (w/w) of organics
  - P = 2.5% (w/w) of organics
  - Trace nutrients: S, K, Na, Mg, Ca, Fe, Mn, Mo, Co, Zn, etc.

Chemical composition	
Constituents	Percentage
Water	75
Dry matter	25
Organic	90
C	45-55
H	22-28
O	5-7
N	8-13
Inorganic	10
P_2O_5	50
K_2O	6.5
Na_2O	10
MgO	8.5
CaO	10
SO_3	15

Agrupamiento microbiano: Temperatura

Temperature Class	Normal Temperature Range for Growth	
	(°C)	(°F)
Psychrophile	-5 to 20	23 to 68
Mesophile (e.g., methanogens)	8 to 45	46 to 113
Thermophile	40 to 70	104 to 158
Hyperthermophile	65 to 110	149 to 230
<u>Recall</u>: -phile = liking (fílico = amigo de)		



Nomenclatura

- **- gen** (produces) / geno o génico
  - Ex. **Methanogens** produce methane, **acetogens** produce acetate
- **- troph** (utilizes or consumes) / trofo
  - Ex., **Methanotrophs** utilize methane, **hydrogenotrophic methanogens** utilize hydrogen (and CO_2) and produce methane, **acetotrophic methanogens** utilize acetate to produce methane (and CO_2).

Reacciones de Oxido-Reducción (REDOX)

Recuerden:

Oxidación es pérdida de electrones

Reducción es ganancia de electrones

Un **reductor (electron donor o donador de electrones)**, dona o entrega electrones y se convierte en agente oxidado

Ex. H_2, H_2O, NH_3, Fe^{2+}, VFAs (acetate, propionate, etc.)

Un **oxidante (electron acceptor – receptor de electrones)**, acepta electrones y se convierte en agente reducido.

Ex. H^+ O_2, NO_3^-, Fe^{3+}, CO_2

Element	Most reduced form of element	Intermediate forms			Most oxidized form of element
C	CH_4 Alkanes	CH_3OH, many organics	CH_2O, many organics	HCOOH, CO	CO_2
H	H_2				H^+
O	H_2O				O_2
N	NH_3	N_2	N_2O	NO_2^-	NO_3^-
S	H_2S		S	SO_2	SO_4^{2-}
Fe	Fe(II)				Fe (III)
Mn	Mn(II)				Mn(IV)
Cl	Cl^-		Cl_2		ClO_4^-
Hg	Hg				Hg (II)
Cr	Cr (III)				Cr (VI)
	Electron donors (reductants)				Electron acceptors (oxidants)

Ejemplo de reacción REDOX: agua

$H_2 \rightarrow 2e^- + 2H^+$

Semi-reacción para el donante de electrones

$1/2O_2 + 2e^- \rightarrow O^{2-}$

Semi-reacció para el receptor de electrones

Con la suma de estos dos:

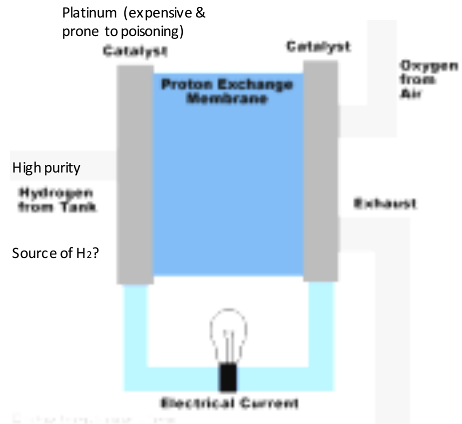
$2H^+ + O^{2-} \rightarrow H_2O$

Formación de agua

Donante Receptor

$H_2 + 1/2O_2 \rightarrow H_2O$

Reacción NETA



H_2 fuel cell

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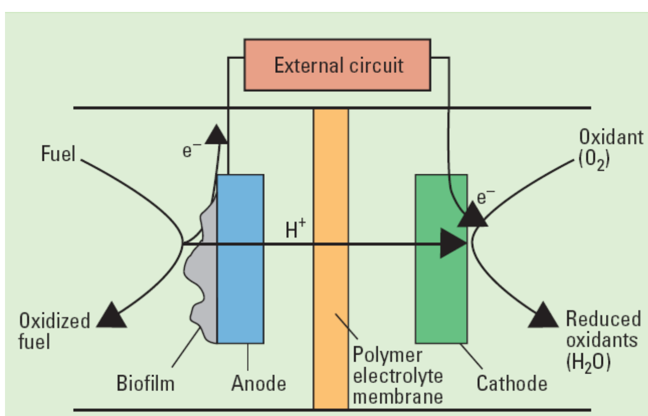
Celda de combustible microbiana Microbial fuel cell (MFC)

- Getting energy from treating wastewater
- Oxidizing organic matter (electron reservoir) and capturing electrons liberated through anode to power an external device
- Have been shown to work on wastewater directly



From Rittmann et al (ES&T)

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REDOX en sistemas ambientales

Element	Most reduced form of element	Intermediate forms			Most oxidized form of element
		OXIDATION → e^-			
C	CH_4, Alkanes	CH_3OH, many organics	CH_2O, many organics	HCOOH, CO	CO_2
H	H_2				H^+
O	H_2O				O_2
N	NH_3	N_2	N_2O	NO_2^-	NO_3^-
S	H_2S		S	SO_2	SO_4^{2-}
Fe	Fe(II)				Fe (III)
Mn	Mn(II)				Mn(IV)
Cl	Cl^-		Cl_2		ClO_4^-
Hg	Hg				Hg (II)
Cr	Cr (III)				Cr (VI)
		← REDUCTION e^-			
	Electron donors (reductants)				Electron acceptors (oxidants)

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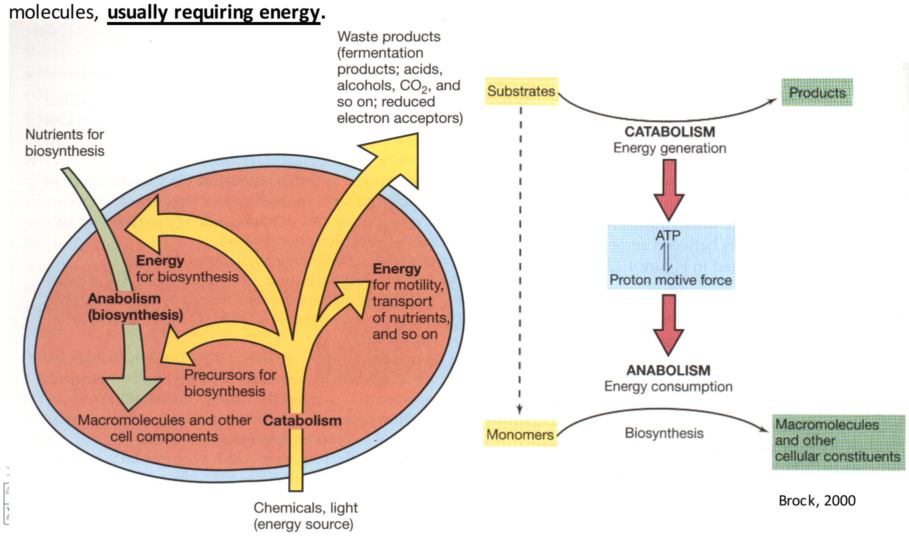
Metabolismo celular simplificado

Catabolism - The biochemical processes involved in the breakdown of organic or inorganic compounds, usually leading to the **production of energy**

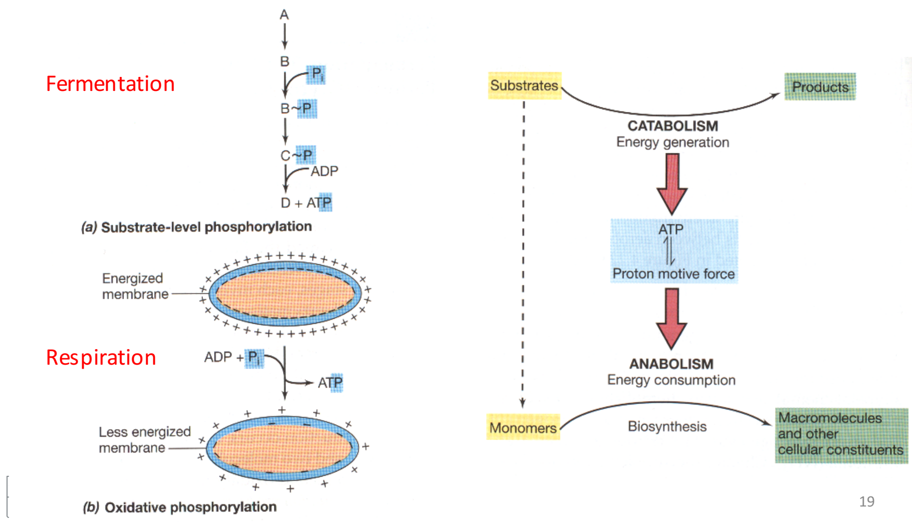
Anabolism - The biochemical processes involved in the synthesis of cell constituents from simpler molecules, **usually requiring energy**.



Brock, 2000

ATP acarrea energía!

Extra credit 1: ¿Cómo funciona el gradiente de protones (proton motive force) y que rol juega en la producción de energía en la célula?



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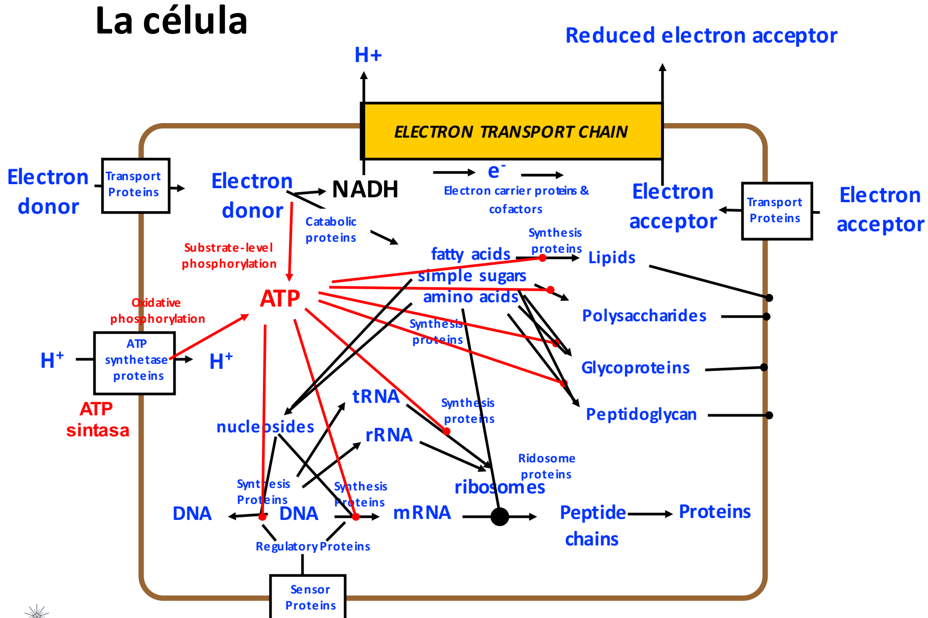
La célula



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Cl	Cl^-		Cl_2		ClO_4^-
Hg	Hg				Hg (II)
Cr	Cr (III)				Cr (VI)
	Electron donors (reductants)				Electron acceptors (oxidants)

REDUCTION ← e^-

Agrupamiento microbiano: energía y utilización de carbono

Group	Energy Source	Carbon Source
Autotrophic		
- chemolithotrophic	Inorganic redox	CO_2
- photolithotrophic	Radiant energy	CO_2
Heterotrophic (organotrophic)		
- chemoorganotrophic (chemoheterotrophic)	Organic redox	Organics
- photoheterotrophic (photoorganotrophic)	Radiant energy	organics
Mixotrophic	Inorganic redox	organics

Trophic Classification of Major Microbial Types According to Their Electron Donor, Electron Acceptor, Carbon Source, and Domain

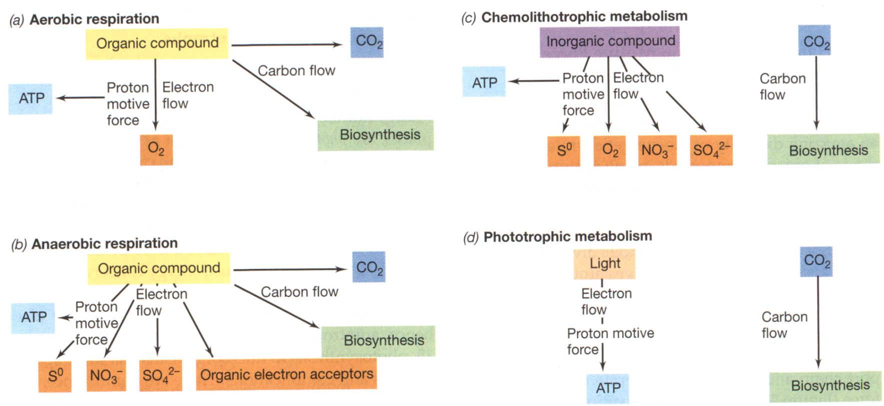
Microbial Group	Electron Donor	Electron Acceptor	Carbon Source	Domain*
Aerobic Heterotrophs	Organic	O_2	Organic	B, A, & E
Nitrifiers	NH_4^+	O_2O_2	CO_2CO_2	B & A B
Denitrifiers	Organic H_2 S°	NO_3^-, NO_2^- NO_3^-, NO_2^- NO_3^-, NO_2^-	Organic CO_2 CO_2	B B B
Methanogens	Acetate H_2	Acetate CO_2	Acetate CO_2	A A
Methanotrophs	CH_4	O_2	CH_4	B
Sulfate Reducers	Acetate H_2	SO_4^{2-} SO_4^{2-}	Acetate CO_2	B B
Sulfide Oxidizers	H_2S	O_2	CO_2	B & A
Homoacetogens	H_2	CO_2	CO_2	B
Carboxidotrophs	CO	CO_2, Fe(III), SO_4^{2-}, H^+	CO_2	B
Fermenters	Organic	Organic	Organic	B & E
Halorespirers	H_2	PCE	Acetate	B
Anode Respirers	Acetate H_2	Anode	Acetate CO_2	B B
Phototrophs	H_2O H_2S H_2	CO_2 CO_2 CO_2	CO_2 CO_2 CO_2	E & B B B

* The domains are **B**acteria, **A**rchaea, and **E**ukarya.

Agrupamiento microbiano: relación con el oxígeno

Group	**Oxygen effect**
Aerobes	
- Obligative	required
- Facultative	Not required but grow better with oxygen
- Microaerophilic	Required, but at levels lower than atmospheric
Anaerobes	
- Aerotolerant	Not required; grow no better when oxygen present
- Obligate (strict)	Harmful or lethal

Relaciones energéticas y flujo de carbono



Brock, 2000

Ciclos Biogeoquímicos

Recuerden: Requisitos de Crecimiento

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CaO	10
SO_3	15

Procesos biológicos Redox

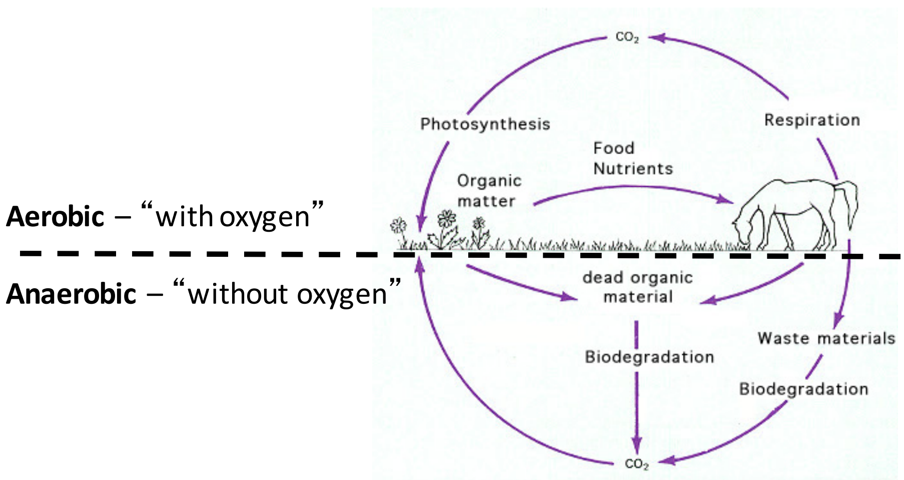
The C and O cycles are closely connected, as oxygenic photosynthesis both removes CO_2 and produces O_2 while respiratory processes both produce CO_2 and removes O_2

Schlesinger, 1991, Biogeochemistry: Analysis of Global Change.

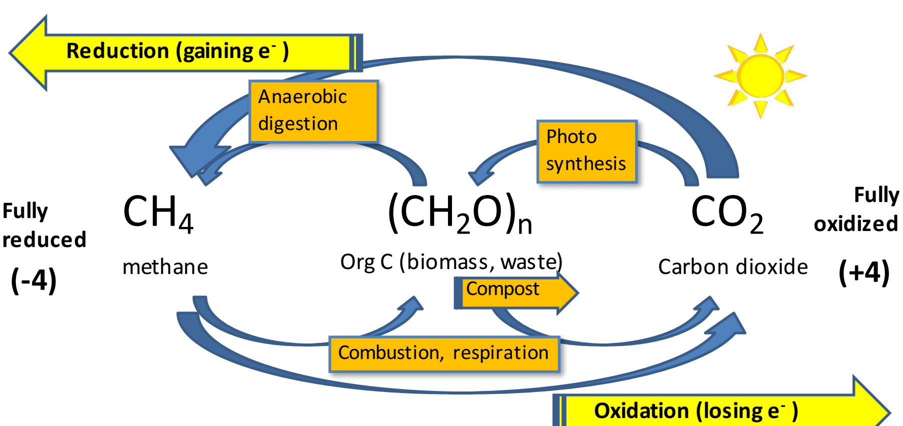
Oxidized ⟶ Reduced (columns); Reduced ⟶ Oxidized (rows)

	H_2O/O_2	C	N	S
H_2O/O_2		Photosynthesis $H_2O \rightarrow O_2$ $CO_2 \rightarrow OC$		
C	Respiration $OC \rightarrow CO_2$ $O_2 \rightarrow H_2O$		Anaerobic respiration Glucose ⟶ Acetate $NO_3 \rightarrow N_2$	$SO_4 \rightarrow H_2S$
N		Chemosynthesis nitrification $-NH_2 \rightarrow NO_3$		
S		Chemosynthesis $H_2S \rightarrow H_2SO_4$ $H_2S \rightarrow S$		

Ciclo del Carbono

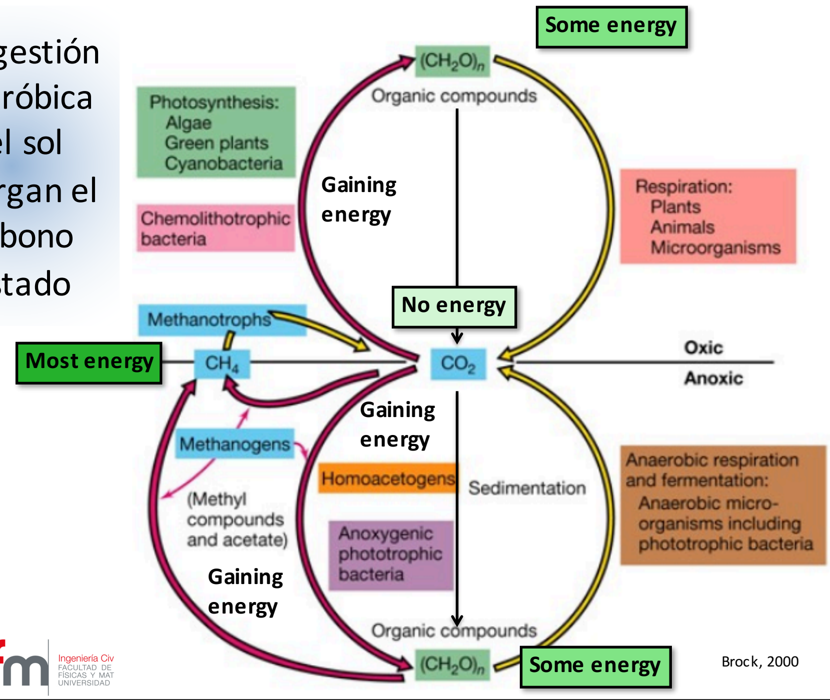


Estado energéticos del Carbono
BIORECICLAJE

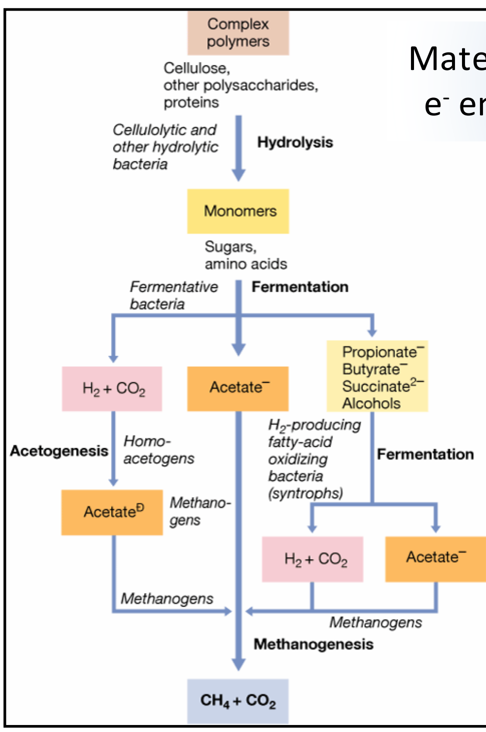


	Methane	biomass	Carbon dioxide
Energy	rich	moderate	none
Redox state	-4	In between	+4
COD (energy)	4 g OD/g (180.4 Wh /g)	Typically 1-3 g OD/g	zero

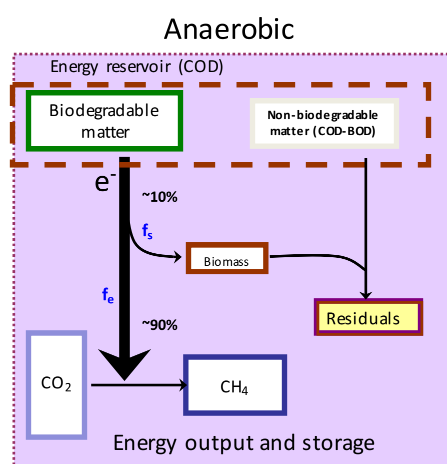
La digestión anaeróbica y el sol recargan el carbono gastado



Brock, 2000



Materia orgánica como donador de e^- en descomposición anaeróbica



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Flujo de C y e- en la digestión anaeróbica

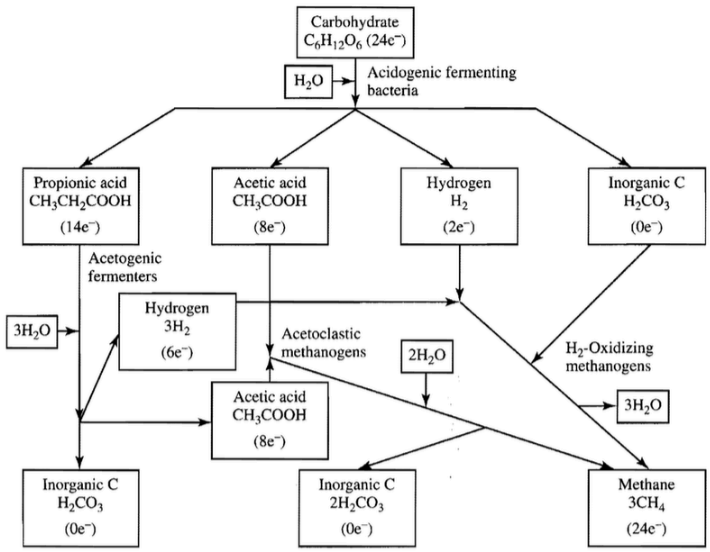
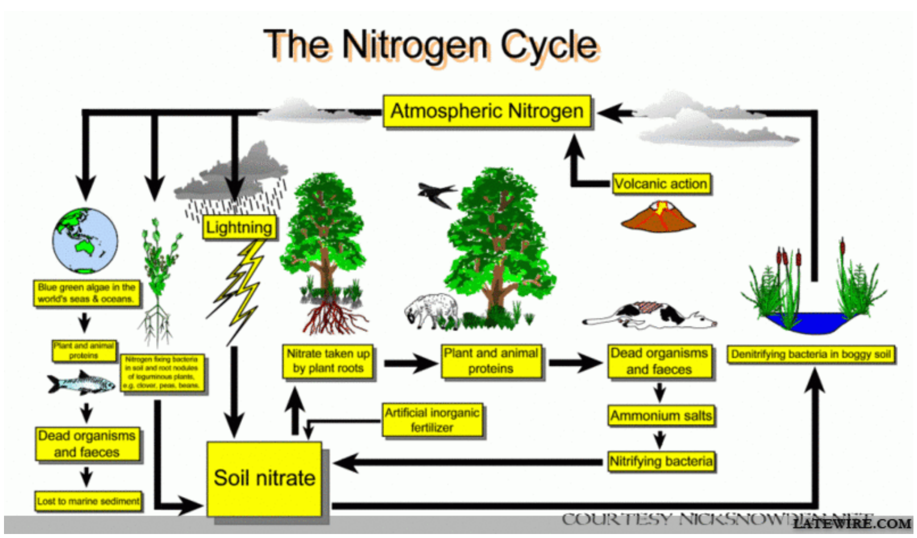


Figure 1.40 Flow of intermediate molecules in an anaerobic ecosystem that starts with carbohydrate, forms intermediate organic acids and H_2, and ultimately generates CH_4. The net reaction is $C_6H_{12}O_6 + 3\ H_2O \rightarrow 3\ CH_4 + 3\ H_2CO_3$, but four unique microbial groups are involved.

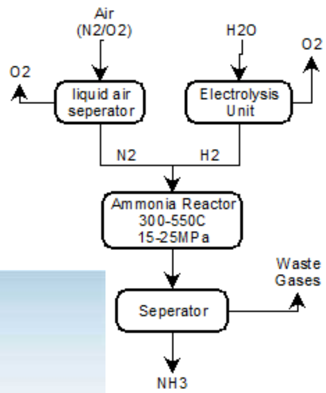


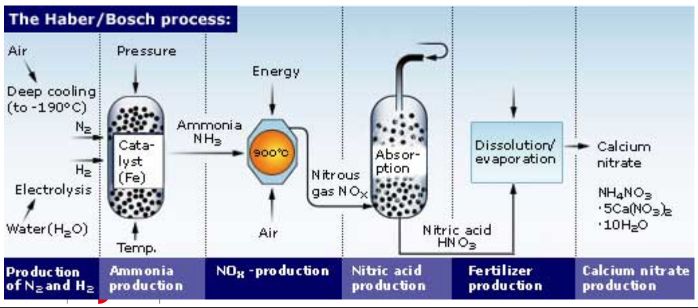
Significant amounts of energy is expended via the Haber-Bosch process to bring N from the atmosphere into the biosphere. We need to reuse N and not lose it back to the air

Proceso Haber-Bosch para producción de Ammonia a partir de N atmosférico

Enabled the agricultural revolution, but energy intensive

$$H_2 \rightleftharpoons 2H_{ad}$$
$$N_2 \rightleftharpoons N_{2,\,ad} \rightleftharpoons 2N_s$$
$$N_s + H_{ad} \rightleftharpoons NH_{ad}$$
$$NH_{ad} + H_{ad} \rightleftharpoons NH_{2,\,ad}$$
$$NH_{2,\,ad} + H_{ad} \rightleftharpoons NH_{3,\,ad}$$
$$NH_{3,\,ad} \rightleftharpoons NH_3$$

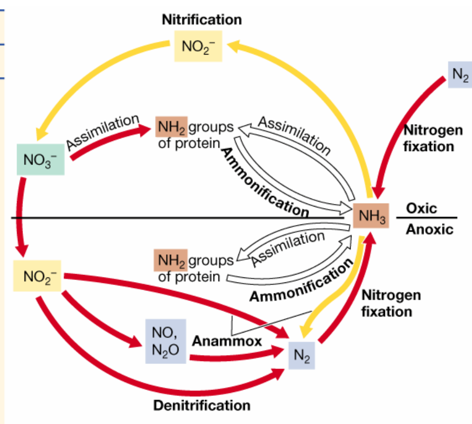




Ciclo REDOX del Nitrógeno

Key Processes and Prokaryotes in the Nitrogen Cycle

Processes	Example organisms
Nitrification ($NH_4^+ \rightarrow NO_3^-$)	
$NH_4^+ \rightarrow NO_2^-$	*Nitrosomonas*
$NO_2^- \rightarrow NO_3^-$	*Nitrobacter*
Denitrification ($NO_3^- \rightarrow N_2$)	*Bacillus, Paracoccus, Pseudomonas*
N_2 Fixation ($N_2 + 8H \rightarrow NH_3 + H_2$)	
Free-living	
Aerobic	*Azotobacter* Cyanobacteria
Anaerobic	*Clostridium*, purple and green bacteria
Symbiotic	*Rhizobium* *Bradyrhizobium* *Frankia*
Ammonification (organic-N $\rightarrow NH_4^+$)	Many organisms can do this
Anammox ($NO_2^- + NH_3 \rightarrow 2N_2$)	*Brocadia*



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Brock, 2000

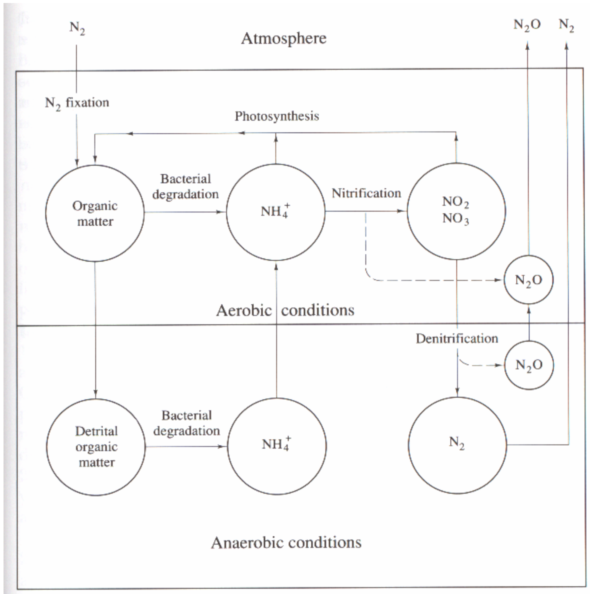


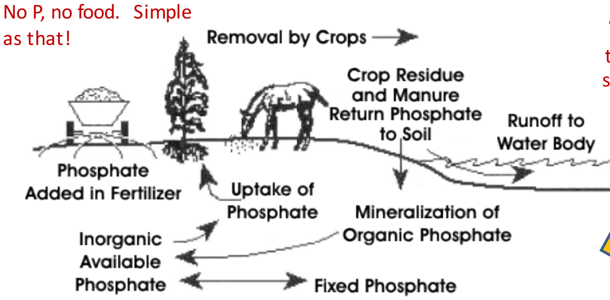
Figure 12.1 Microbial transformations of nitrogen in the global cycle. From Wollast (1981).
Schlesinger, 1991, Biogeochemistry: Analysis of Global Change.

Ciclo del Nitrógeno

Life is not possible without P.

No P, no food. Simple as that!

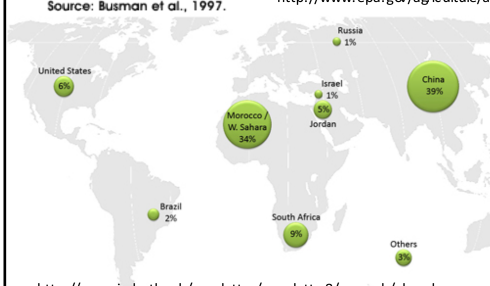
The Phosphorus Cycle



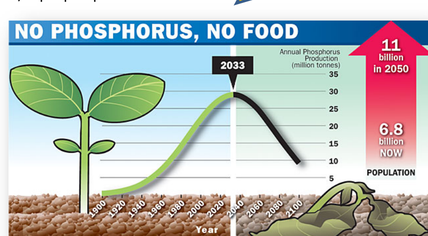
Source: Busman et al., 1997.

http://www.epa.gov/agriculture/ag101/impactphosphorus.html

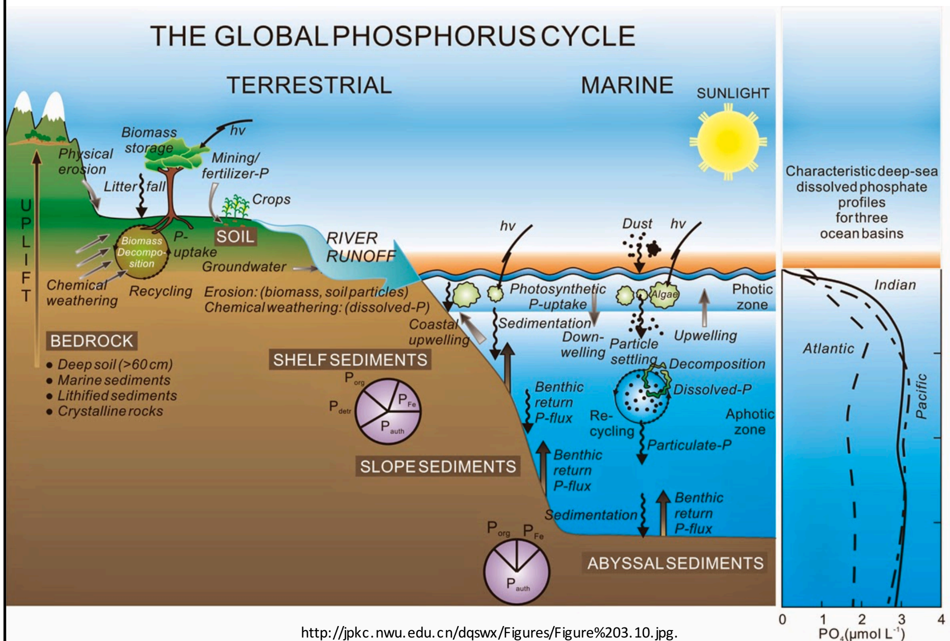
We dig up P, use it once, then lose it to the sea for good. Some say that we are approaching "**peak phosphorus.**" P will be at the heart of geopolitical conflicts since P reserves are concentrated in just a few countries



http://www.ied.ethz.ch/newsletter/newsletter8/research/phosphor



http://www.rainharvest.co.za/2010/07/peak-phosphorus-what-does-this-mean/



http://jpkc.nwu.edu.cn/dqswx/Figures/Figure%203.10.jpg.

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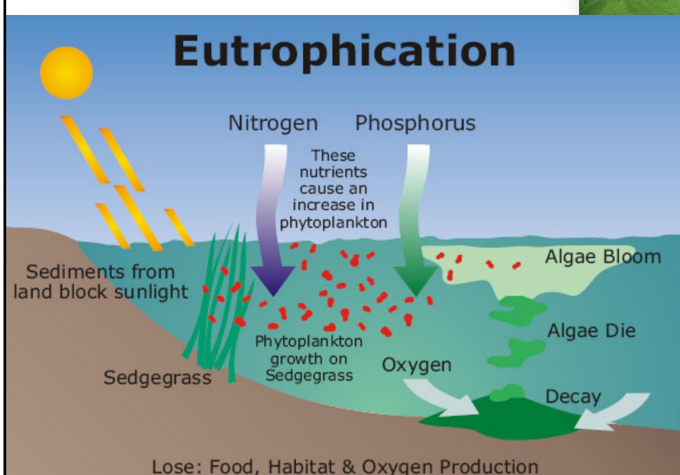
Cuando los nutrientes están mal ubicados…



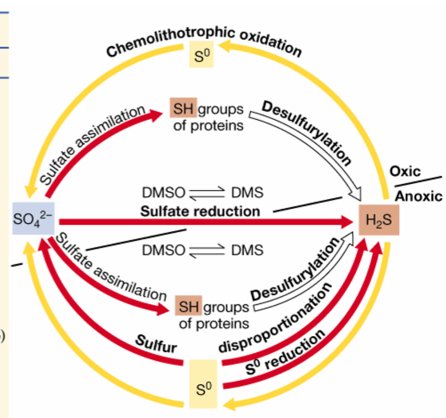
http://www.ecology.com/2012/08/27/how-food-production-impacts-water-quality/

http://05lovesgeography.blogspot.com/2011/02/eutrophication.html

Ciclo del Azufre

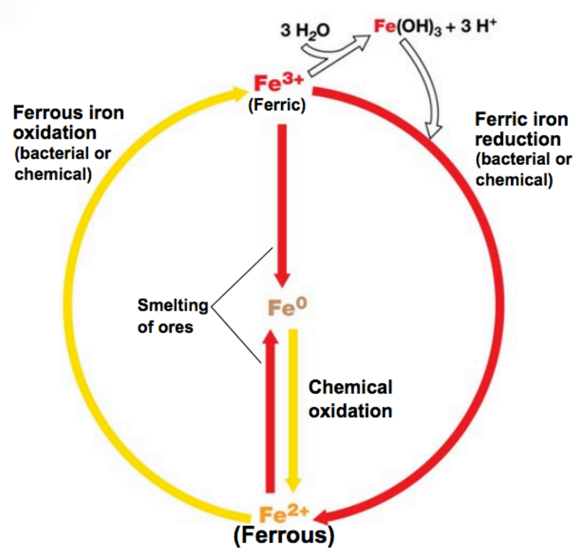
Key Processes and Prokaryotes in the Sulfur Cycle

Process	Organisms
Sulfide/sulfur oxidation ($H_2S \rightarrow S^0 \rightarrow SO_4^{2-}$)	
Aerobic	Sulfur chemolithotrophs (*Thiobacillus*, *Beggiatoa*, many others)
Anaerobic	Purple and green phototrophic bacteria, some chemolithotrophs
Sulfate reduction (anaerobic) ($SO_4^{2-} \rightarrow H_2S$)	*Desulfovibrio*, *Desulfobacter*
Sulfur reduction (anaerobic) ($S^0 \rightarrow H_2S$)	*Desulfuromonas*, many hyperthermophilic *Archaea*
Sulfur disproportionation ($S_2O_3^{2-} \rightarrow H_2S + SO_4^{2-}$)	*Desulfovibrio*, and others
Organic sulfur compound oxidation or reduction ($CH_3SH \rightarrow CO_2 + H_2S$) ($DMSO \rightarrow DMS$)	Many organisms can do this
Desulfurylation (organic–S $\rightarrow H_2S$)	Many organisms can do this

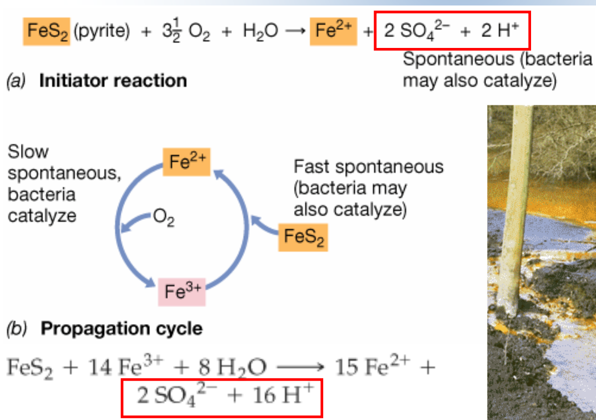


Redox cycle for sulfur. DMSO, dimethylsulfoxide; DMS, dimethylsulfide

Ciclo del Hierro

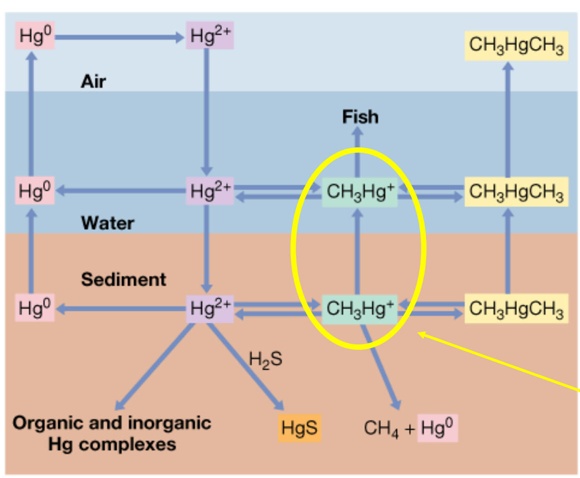


Drenaje ácido



Problem: reduced sulfure (sulfide) is oxidized to surfuric acid, making water acidic

Ciclo de Mercurio



Mercury methylation results in bioaccumulation!

Presence of sulfide affects mercury bioavailability

Brock, 2000