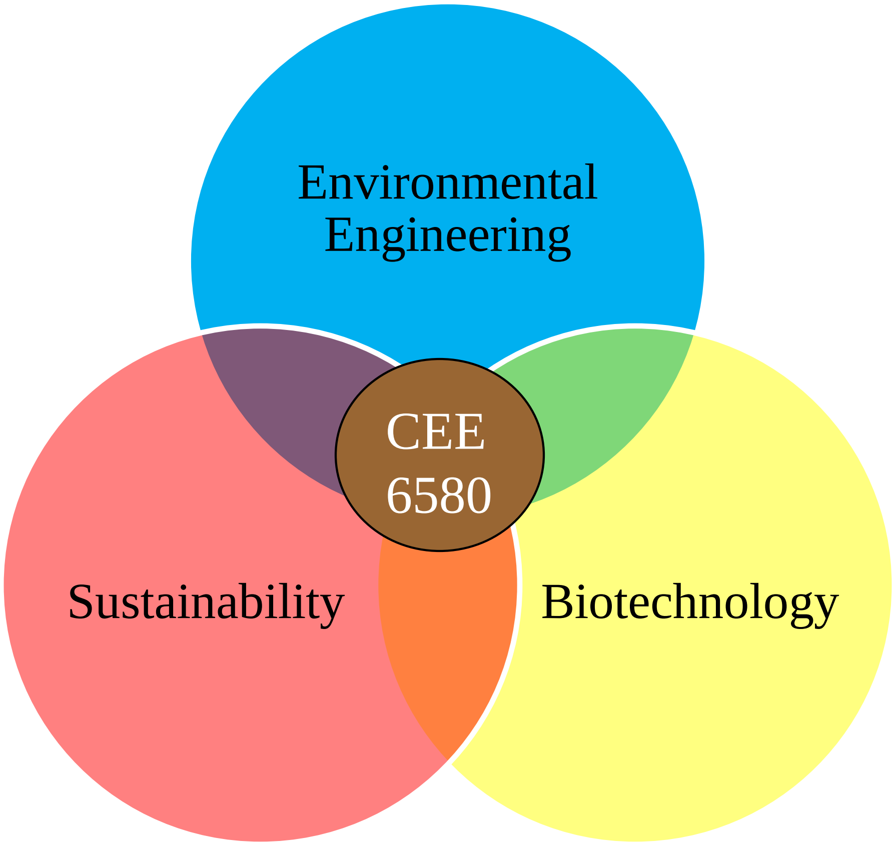
CEE6580: Microbial Biodegradation and Biocatalysis

(aka Environmental Biotechnology)

E.B. is the intersection of:



SYLLABUS:

CEE 6580 – Microbial Biodegradation and Biocatalysis

Spring, 2018, 3 credits

Tuesday & Thursday 10:10 – 11:25 am

110 Hollister (with some sessions in the ACCEL computer labs)

Instructor: Ruth E. Richardson

School of Civil and Environmental Engineering

271 Hollister Hall

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Phone: Office: 607 - 255-3233; Cell: 607-339-9894

Office Hours: (in 271 Hollister Hall):TBD after poll for best slots

TA: none

Website: Accessible through Blackboard (autoenrollment is turned on: you register, you're enrolled)

Overall Objectives to train students to:

- Think critically about major challenges to environmental sustainability and the principles of green engineering.
- Describe how biological processes can be applied to meet challenges in: bioremediation, bioenergy, green chemistry, modern wastewater treatment, biosensing/biomonitoring, and environmental toxicology.
- Recognize processes involved in biochemistry at several spatial levels (molecular, cellular, community & environmental).
- Know enzyme kinetic models and how they feed larger reactor models (biofilm and suspended growth) for predicting biodegradation and biocatalysis rates.
- Know current and emerging methodologies for tracking specific biological activities and gene expression profiles in pure and mixed communities – especially molecular biological tools (DNA, RNA and protein)
- Understand the regulatory feedback mechanisms that can be manipulated to control biological processes.
- Design and operate a microbial reactor for the treatment of waste streams <u>or</u> production of bioenergy from waste organics.
- Use online and print resources to find metabolic pathways (and corresponding enzymes/genes) for breakdown or synthesis of a specific compound and use published data to model biological processes.
- Understand the following concepts/terminology related to Enviornmental Biotechnology: bioavailability; transcriptional regulation; allosteric enzymology; operon theory, intermediary and secondary metabolism; diffusion-controlled transport in biofilms; export tags and transporter systems; horizontal gene transfer; microbial ecology; “omics”; bioenergetics & the thermodynamics of growth, biosensors
- Use online gene databases (e.g NCBI GenBank and Brenda) to find genes of interest and design primers for PCR-based methods.
- Read and understand current scientific literature in Environmental Biotechnology

- <u>Learning Objectives:</u> A list of specific learning objectives, divided into topical headings will be distributed as the topics are introduced and compiled into a signal "Learning Objectives" file to be posted on the Blackboard site. These learning objectives clearly spell out the specific skills and knowledge that students should have when they finish this course. <u>*Use them as a study guide for the exam*</u>.

- <u>Academic Integrity:</u> Each student in this course is expected to abide by the Cornell University Code of Academic Integrity. Any work submitted by a student in this course for academic credit will be the student's own work or that of the collective student group assigned to the problem set/lab report.

Grading: Final grades will be determined as follows:

Prelim Exam (1) in April:	25%
HW/Response papers:	25%
Final project	30%
Participation (in class/online disc’ns)	20%

Outline

Week #	Week of	Topic
1	1/23	Course Overview Microbial metabolism at several scales Microbial diversity (metabolic and genetic)
2	1/30	Bioenergetics: predicting yields Enzyme-catalyzed reactions: Predicting and finding biochemical pathways (Use of online resources)
3	2/6	Kinetics Begin Biroemediation focus Finding enzyme parameters and inhibitors using online resources
4	2/13	Bioremediation and Biodegradation topics
5	2/20	(Note: no class Tuesday 2/20 - minibreak) Genetics & Genomics Organization and regulation of metabolic pathways
6	2/27	Molecular biology techniques
7	3/6	Diagnostic E.B. Applications
8	3/13	Advanced Wastewater treatment bioreactors
9	3/20	Advanced Wastewater treatment (cont'd) Biofuels and Waste-to-Energy
10	3/27	Biofuels and Waste-to-Energy (continued)
	Spring break	Spring break no classes
11	4/10	The "Omics" Era Systems Biology and Synthetic Biology
12	4/17	**Exam;** Biosynthesis & Green Chemistry Biorefineries
13	4/24	Case Studies and Final Presentations
14	5/1	Final Presentations

Introductions

- Name
- Major & year
- Research of key topic of interest
- Something fun you like to do

Three general application areas in this class

1. Waste treatment
2. Biosynthesis/Bioproducts
3. Diagnostic biotechnology

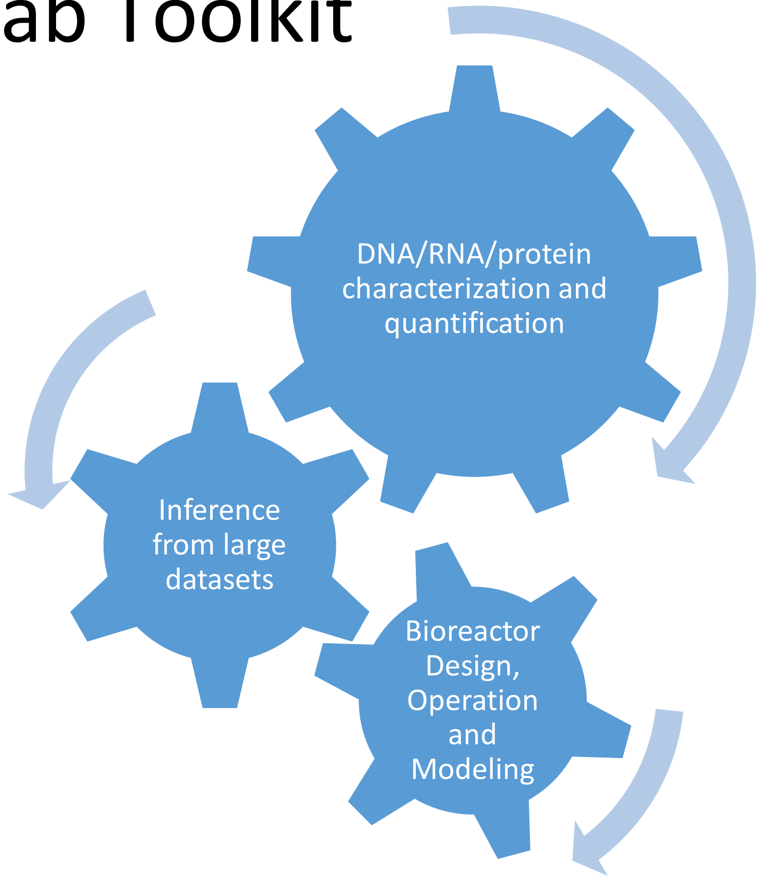
In groups of 2-3 people come up with lists of examples from 1,2, & 3 above

(have one person write these down and hand in)

Richardson Lab Research Areas

- Bioremediation
- Bioenergy
- Sustainable WW treatment & Sanitation
- Carbon and Nitrogen cycling
- Pathogen detection

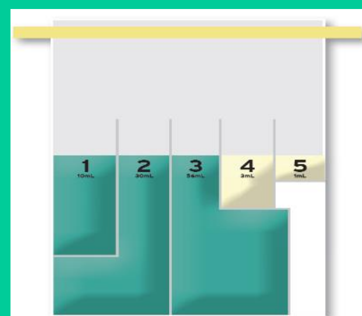
Richardson Lab Toolkit



Some research examples

Diagnostics: Rapid Fecal Indicator Bacteria monitoring in remote/low resource settings

- Desirable features
  - Good quantitative range
  - Rapid
  - Low/no electricity requirement
  - Affordable

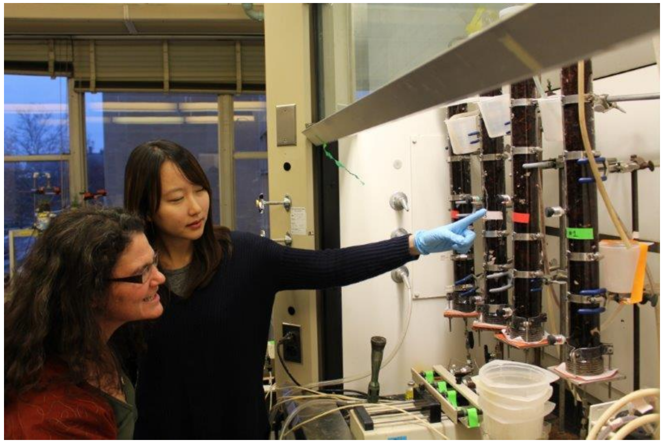


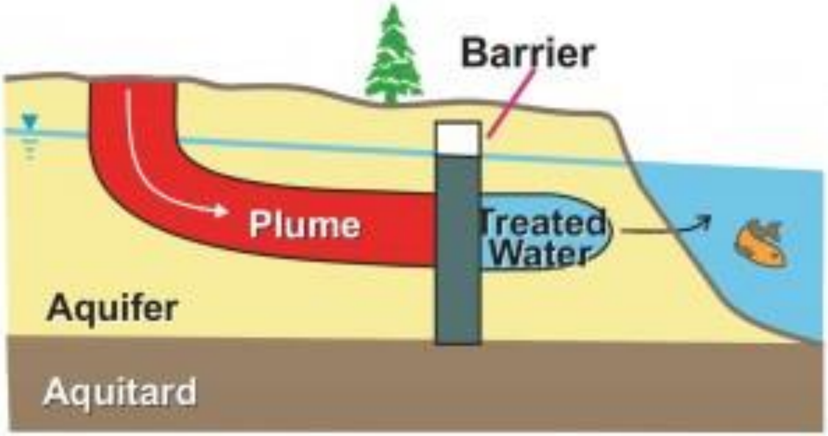
Compartment Bag Test
-24-48 hr assay
-ambient incubation (25-40°C)
- Quant range: 1-100 CFU/100mL
-~$8 per test

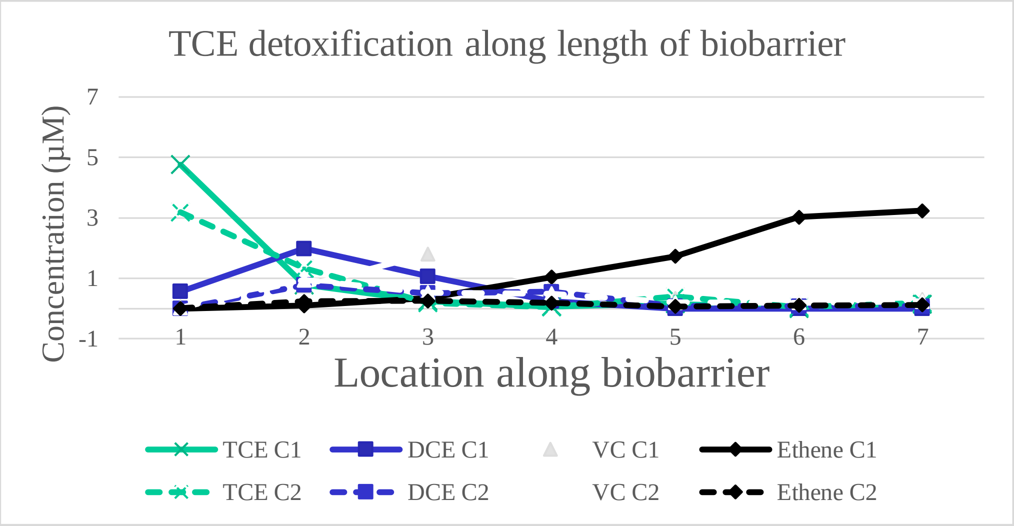
Biomeme two3 handheld qPCR device
-<1 hr assay
-cell phone controlled
-~$2 per test (device ~$4k)

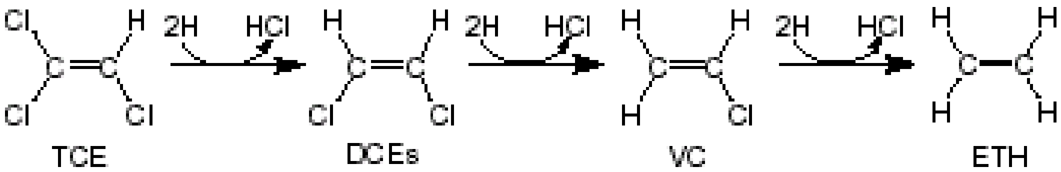
Waste treatment

- Biobarriers for bioremediation of groundwater
- We study it for Trichloroethene

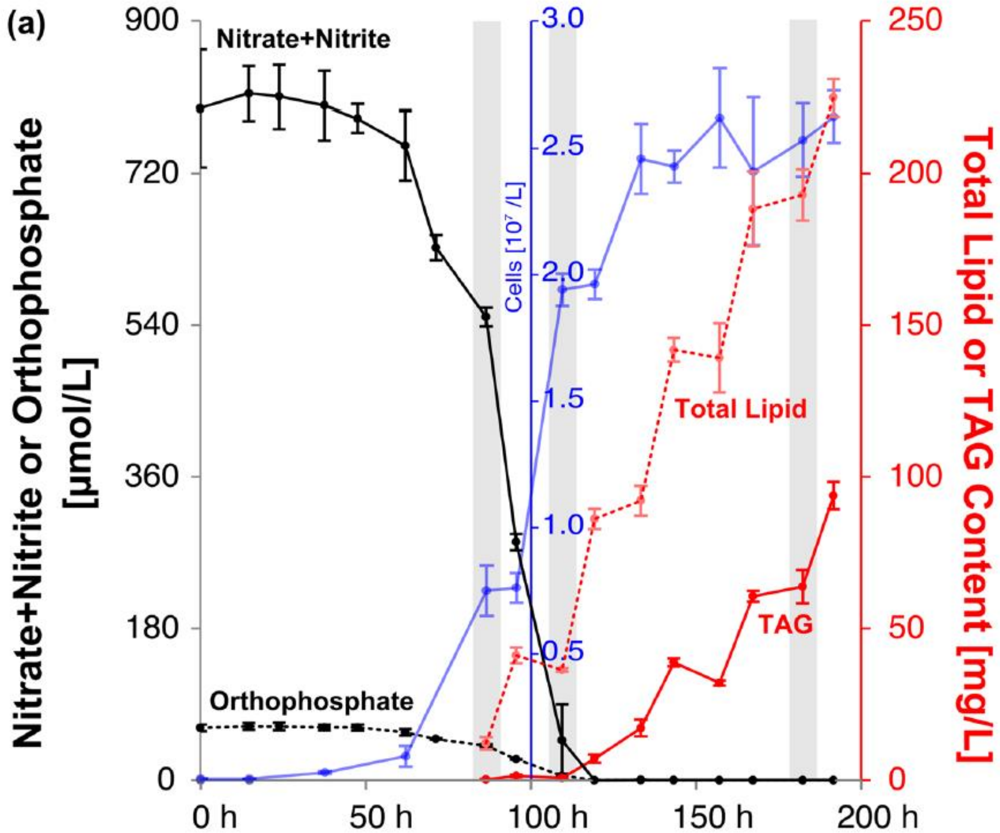


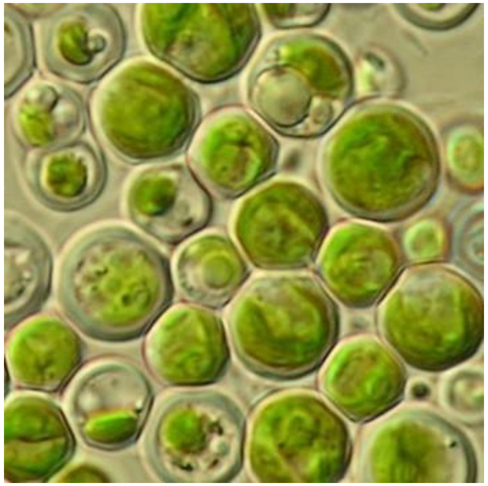






Bioproducts: Biodiesel feedstock from marine *Chlorella* algae



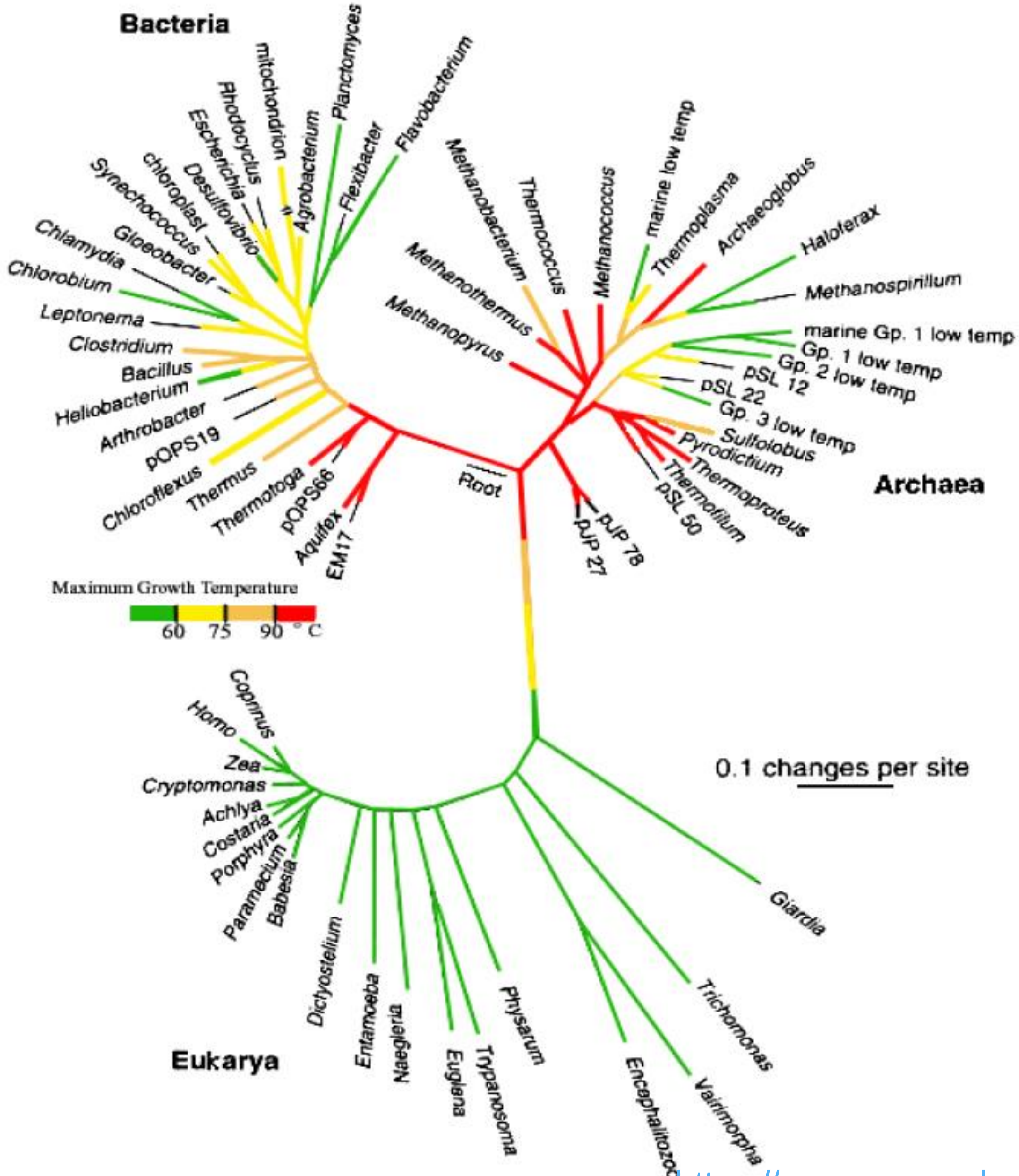


TAG = oil (precursor for Biodiesel)

Cellular lifeforms share some features

- Evolve from common ancestor (based on ribosomal RNA phylogenetic analysis)
- All cells are comprised mostly of water and macromolecules (protein, carbs, lipids, nucleic acids)
- All cells have genetic material that is inherited by offspring (one or more chromosomes)
- Conservation of core metabolic pathways ("Intermediary metabolism")

The Big Tree of Life



https://www.researchgate.net/publication/1931610

CEE6580
second lecture overview

- Brief review/powwow on the readings.
- Metabolism - Theoretical bioenergetics of cell growth
  - Predicting yields based on overall metabolism (example of an aerobic heterotroph)
- Normalizing all sorts of compounds using the unit of electron-equivalents (eeqs) which is the same as a mole of electrons.
- Other common measures of numbers of electrons are "oxygen demand" (as in COD, BOD) and Coulombs (96400 C per mole of electrons).
- Free energy predictions and corrections based on non-standard conditions

Readings review

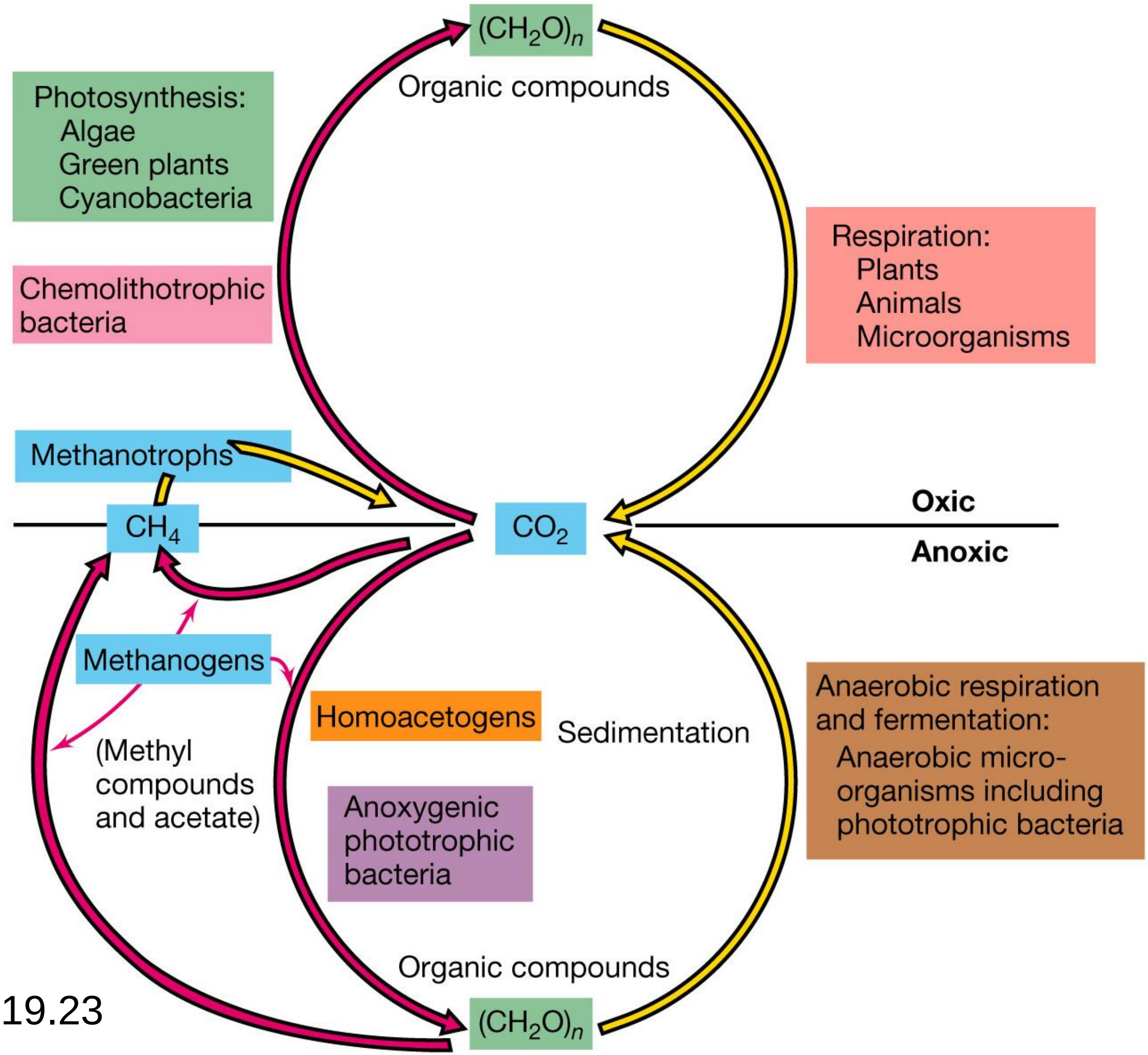
- Wackett Ch 1: intro to subject
- Wackett Ch 2: brief history of research in microbial transformations
  - What was Winogradsky noted for?
  - What was von Leeuenhoek noted for?
- McDonough & Braungart:
  - 12 principles of green engineering
  - 3 tenets: waste equals food; Use current solar income; celebrate diversity

TOPIC: Microbial Metabolism

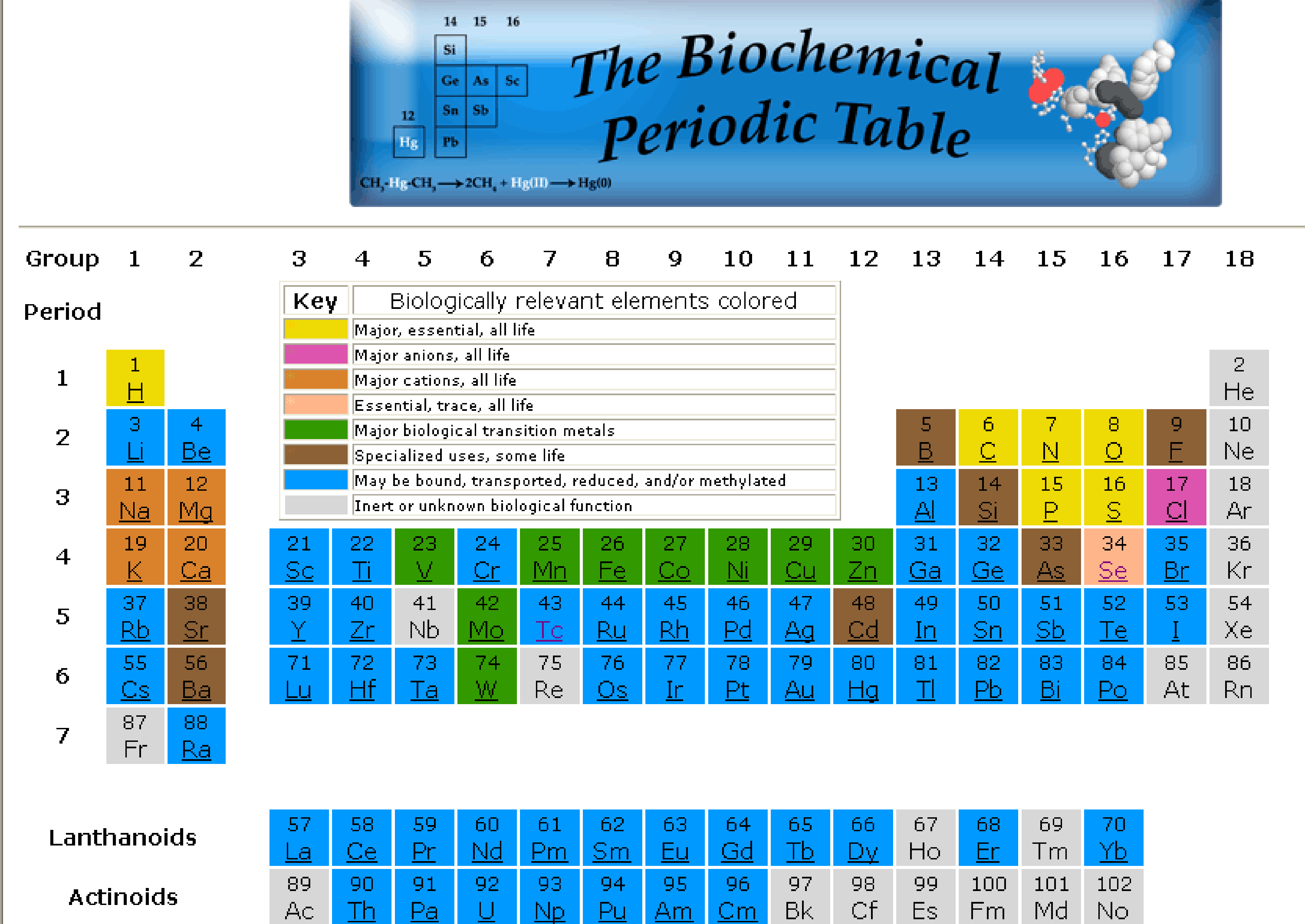
Learning Objectives

- Define the nuanced differences between biodegradation, bioremediation, biocatalysis, biotechnology and biogeochemistry.
- Be able to draw black boxes around metabolism at multiple scales.
- Explain the difference between mechanisms and summary reactions.
- Trace the flow of carbon and electrons in common microbial metabolisms (chemolithoautotrophs, photoautotrophs, chemoorganoheterotrophs ("heterotrophs").
- Recommend strategies for enrichment culturing of specific catabolic capabilities.
- Explain why it is more difficult to enrich for organisms that PRODUCE compounds of interest than those that biodegrade them (note exceptions - e.g. antibiotic production).
- Recognize the differences between central (intermediate) metabolism and secondary metabolism - and explain how secondary metabolism relates to biodegradation & biocatalysis
- Be able to balance coupled redox reactions AND predict yield of cell growth given metabolic summary reactions
- Describe eeq, COD and coulombs. Convert easily amongst them.

The Global Carbon cycle is affected by microbes

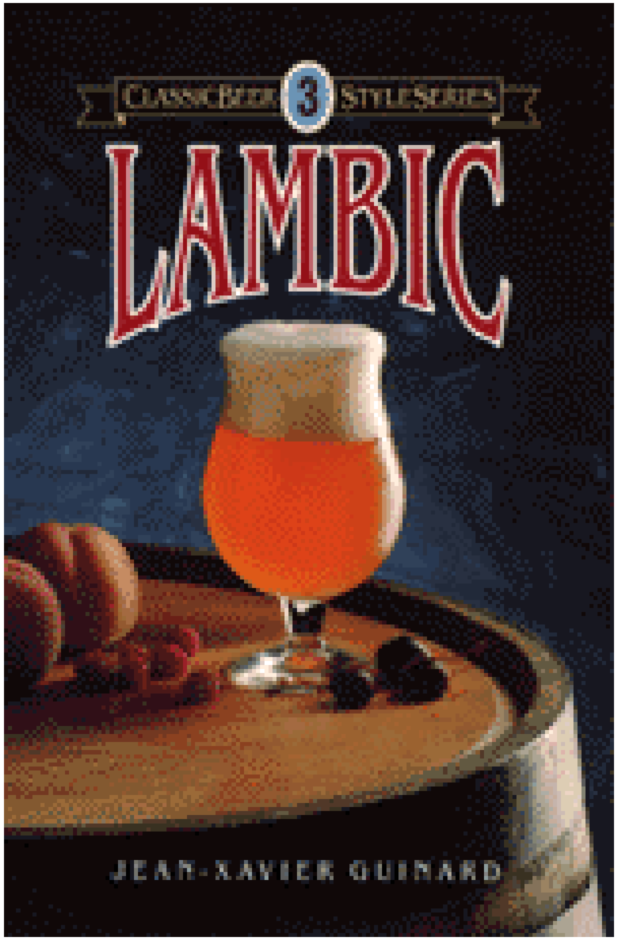


Brock Fig 19.23

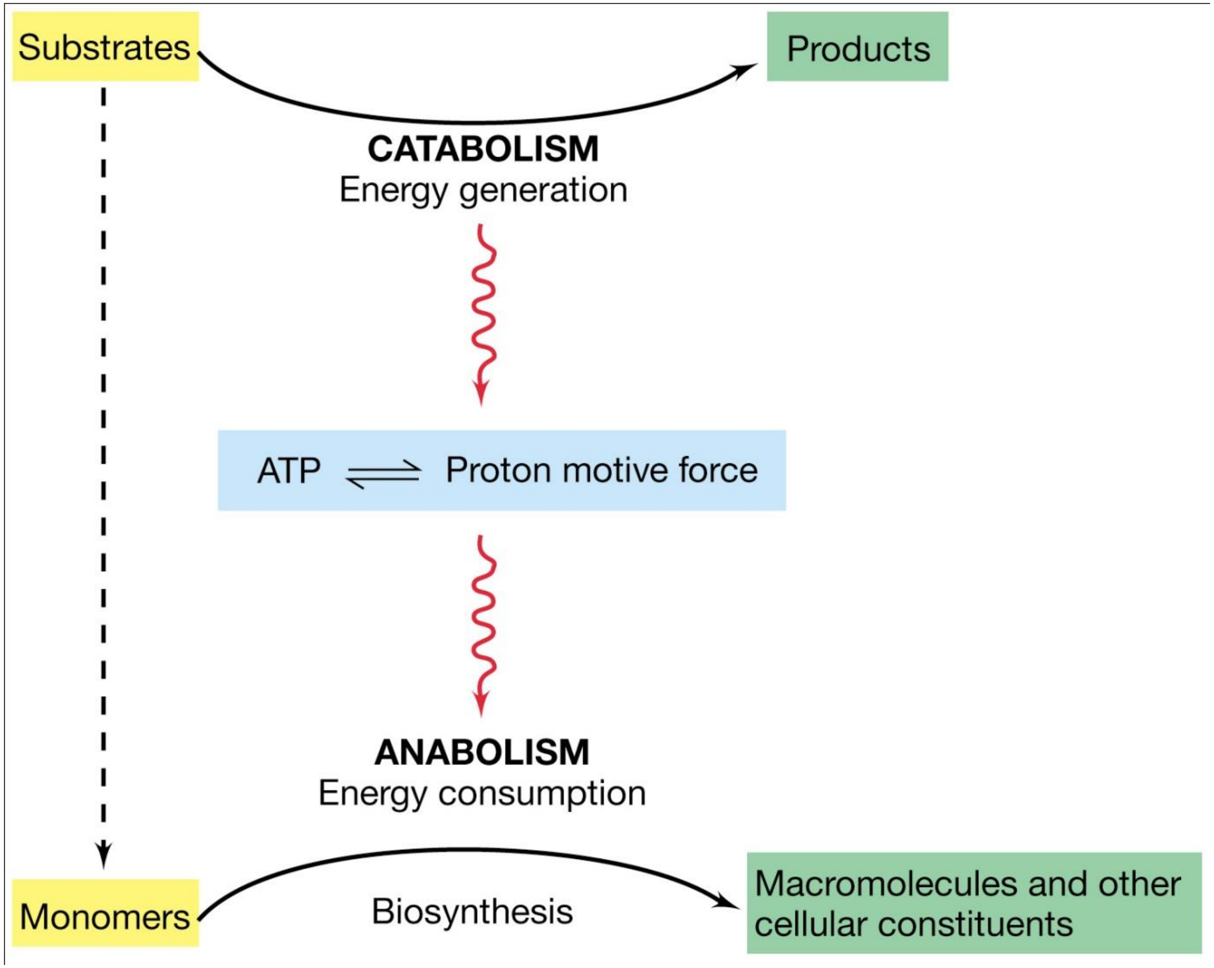


Biochemical Periodic Table: http://umbbd.ahc.umn.edu/periodic/periodic.html

“Reactor scale” metabolism

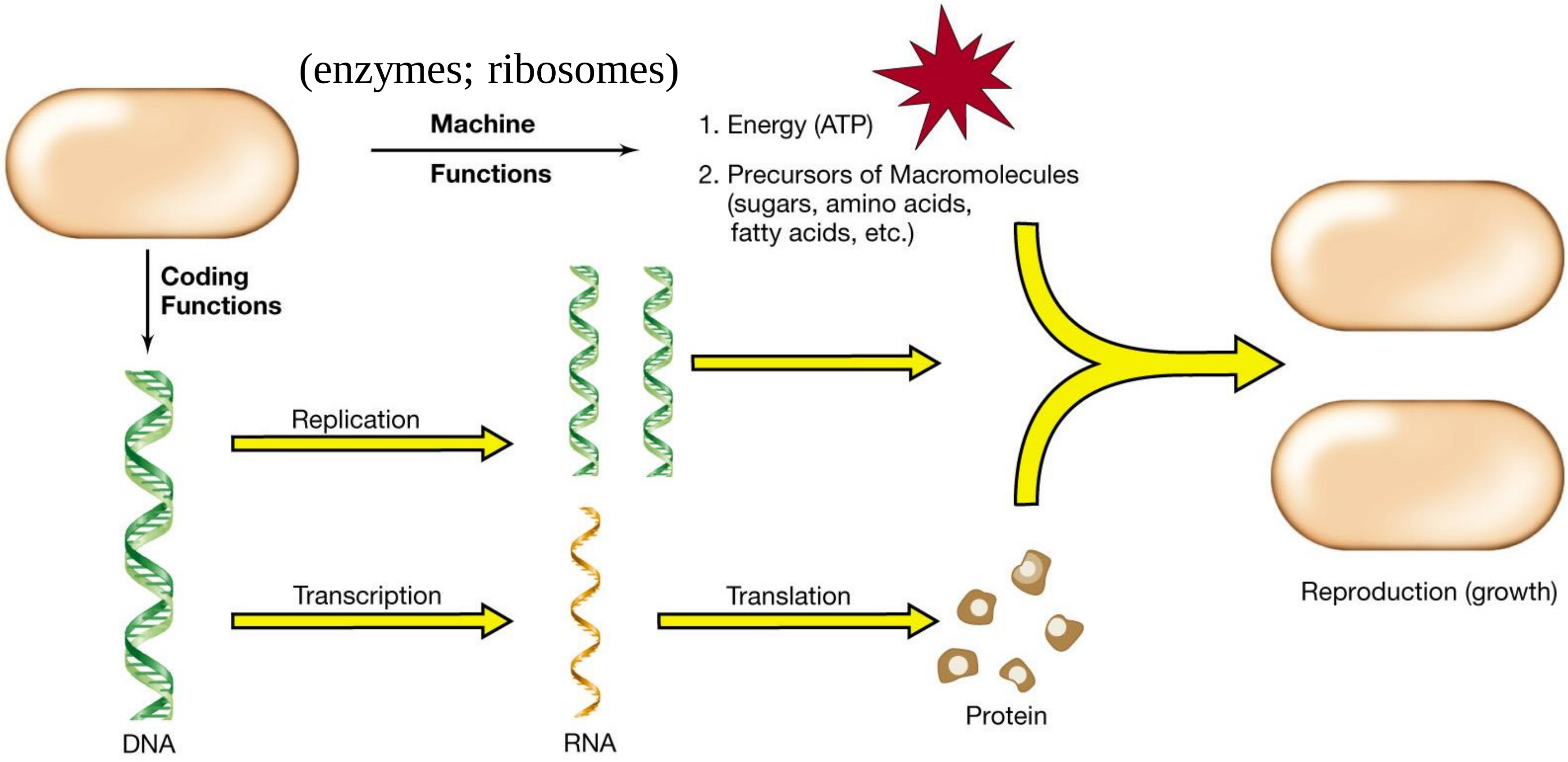


Cellular Metabolism: the art of Energy Coupling



Brock Fig 5.24

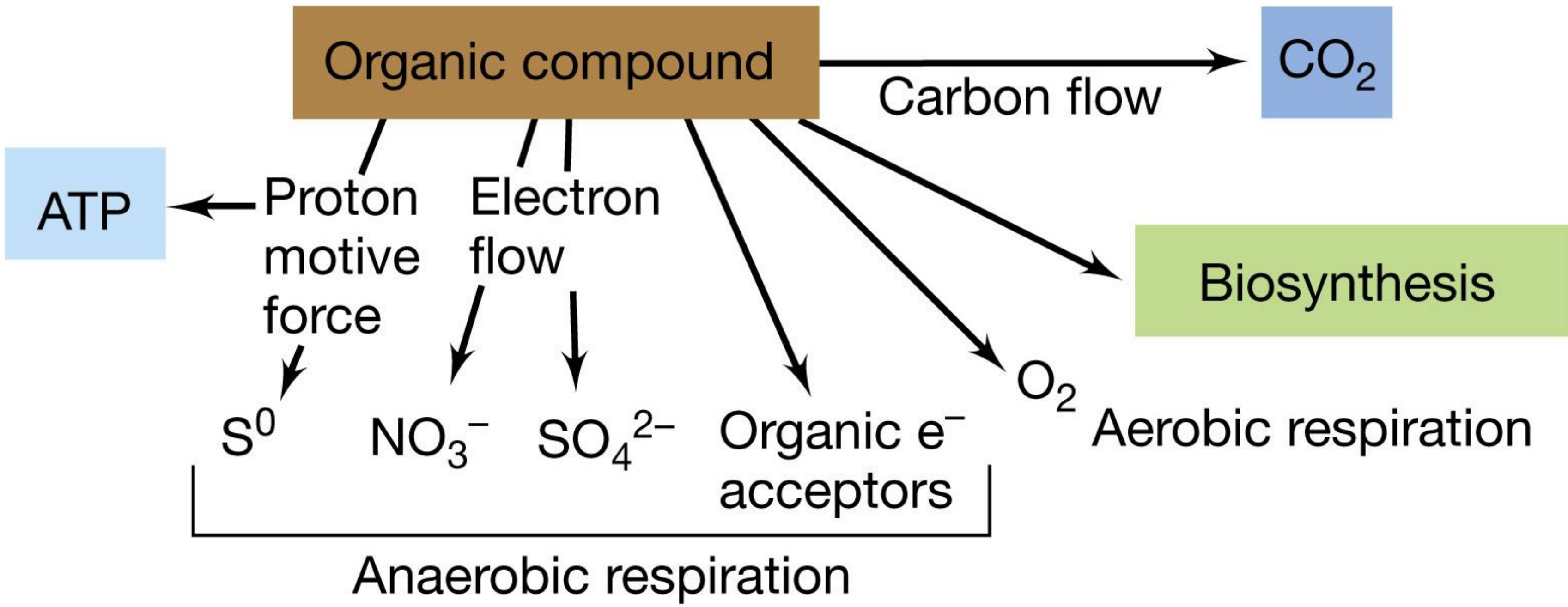
Cells have both Coding and Machine functions



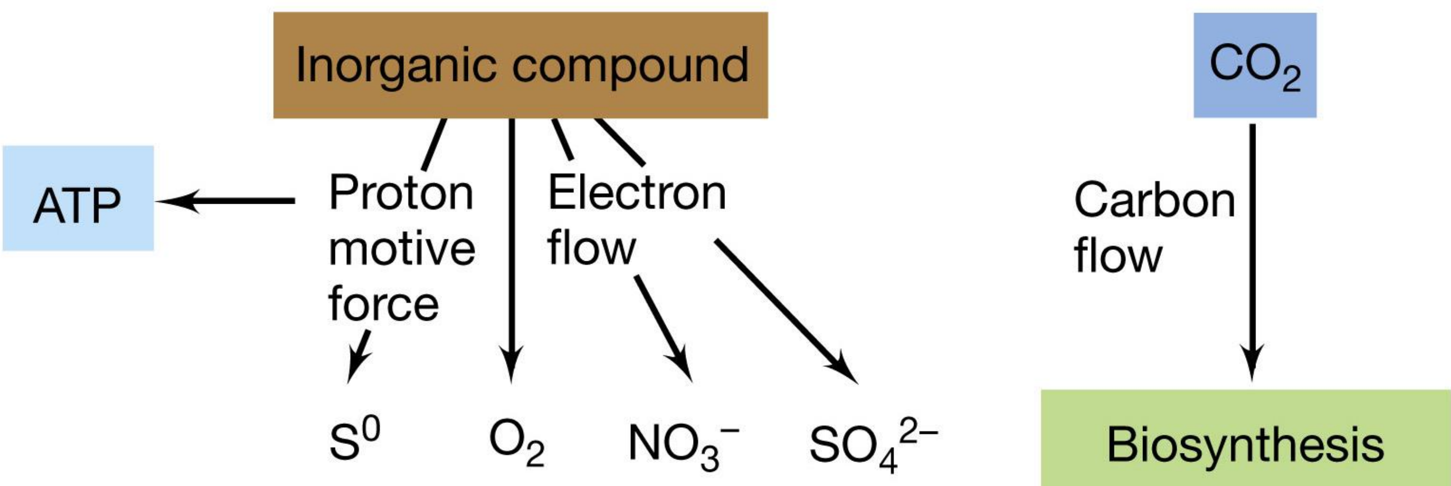
Brock Fig 1.4

Metabolism

- Catabolism (reactions/pathways that generate energy) and anabolism (rxns/pthys that synthesize key biomass compounds) are intricately linked
- Anabolic rxns require energy (& raw materials)
- Catabolic rxns generate that energy



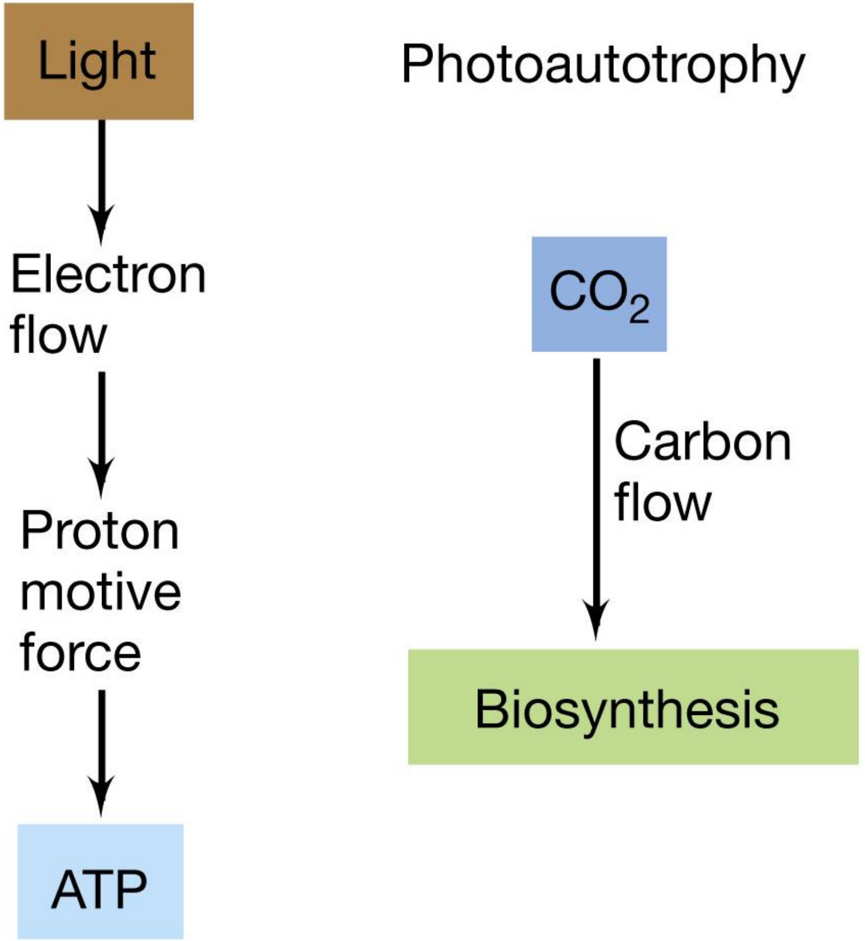
(a) **Chemoorganotrophic metabolism** e.g. *E. coli*, humans, Denitrifiers,



e.g. nitrifiers (*Nitrosomonas*, *Nitrospira*)

(b) **Chemolithotrophic metabolism**

Brock Fig 5.23 a & b



Photoautotrophy: light for energy, CO2 for biosynthesis

Brock Fig 5.23c

Coupled redox reactions

$$H_2 \rightarrow 2\,e^- + \boxed{2\,H^+}$$

Electron-donating half reaction

$$\tfrac{1}{2}O_2 + 2\,e^- \rightarrow \boxed{O^{2-}}$$

Electron-accepting half reaction

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

Formation of water

$$\underset{\text{Electron donor}}{H_2} + \underset{\text{Electron acceptor}}{\tfrac{1}{2}O_2} \rightarrow H_2O$$

Net reaction

Fig 5.8

The redox tower

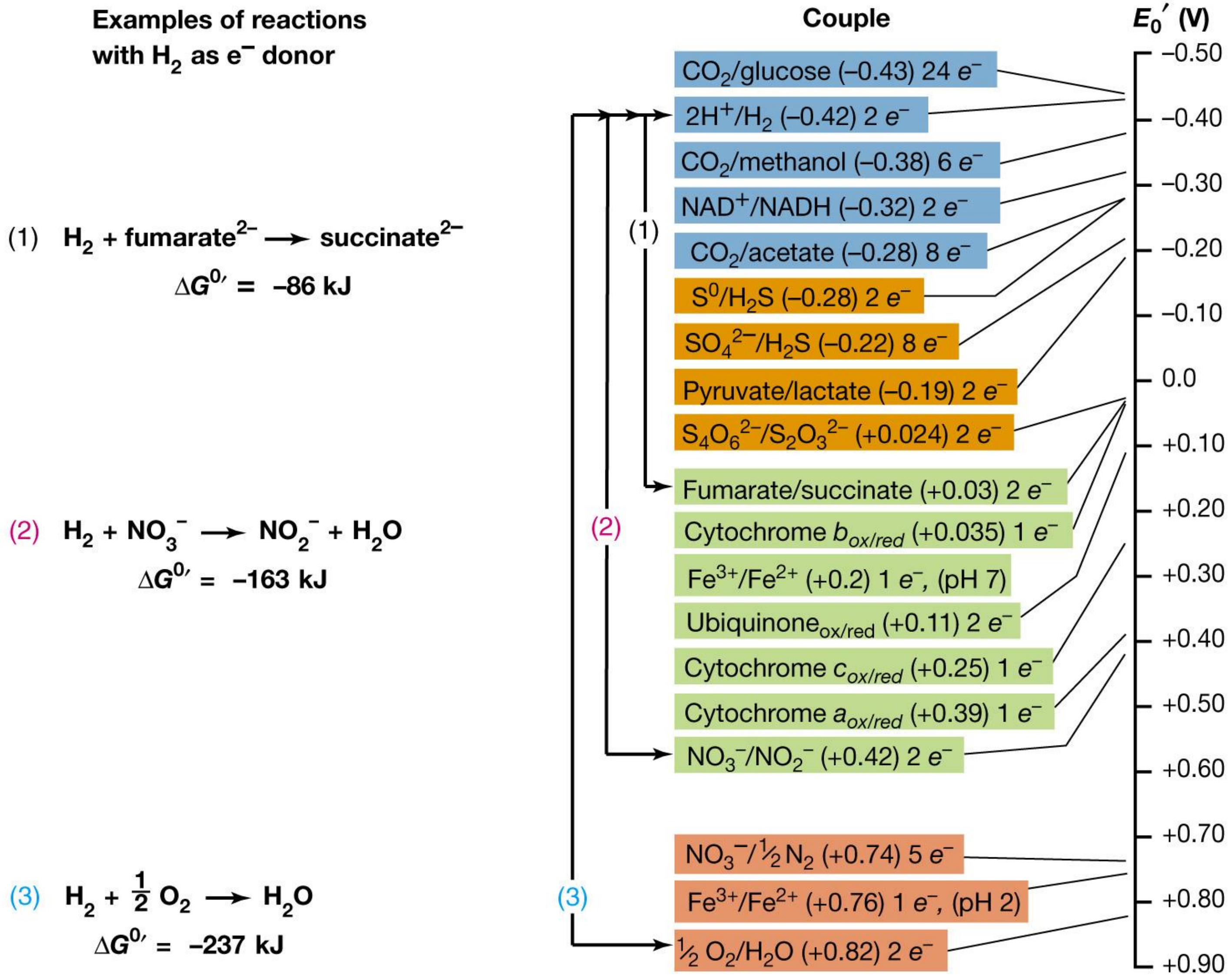
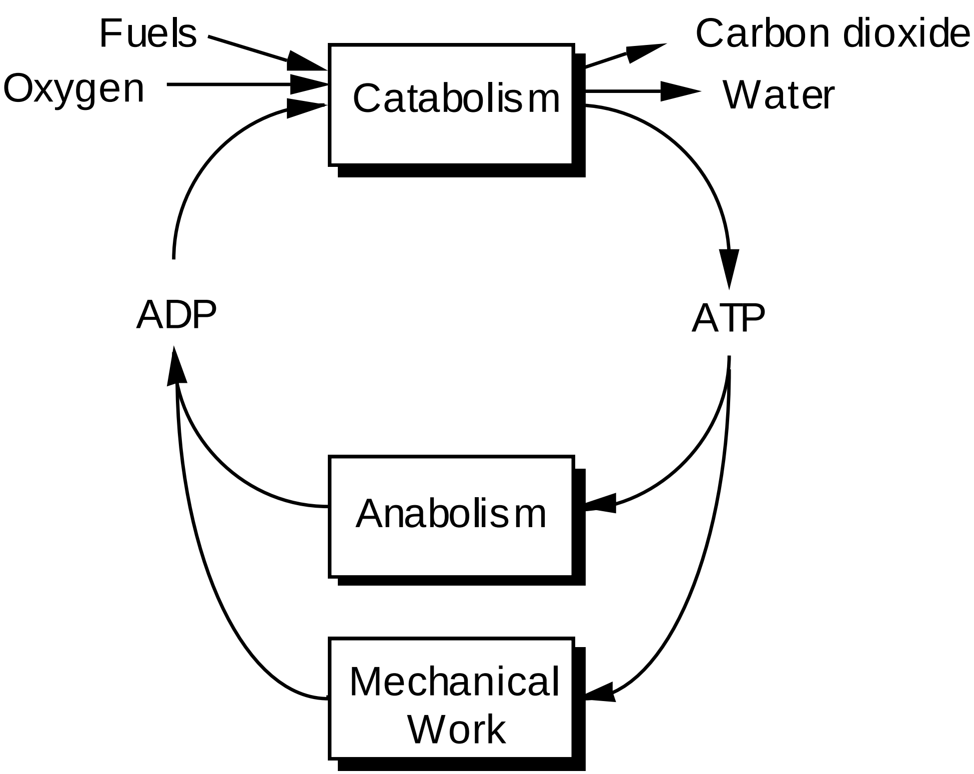


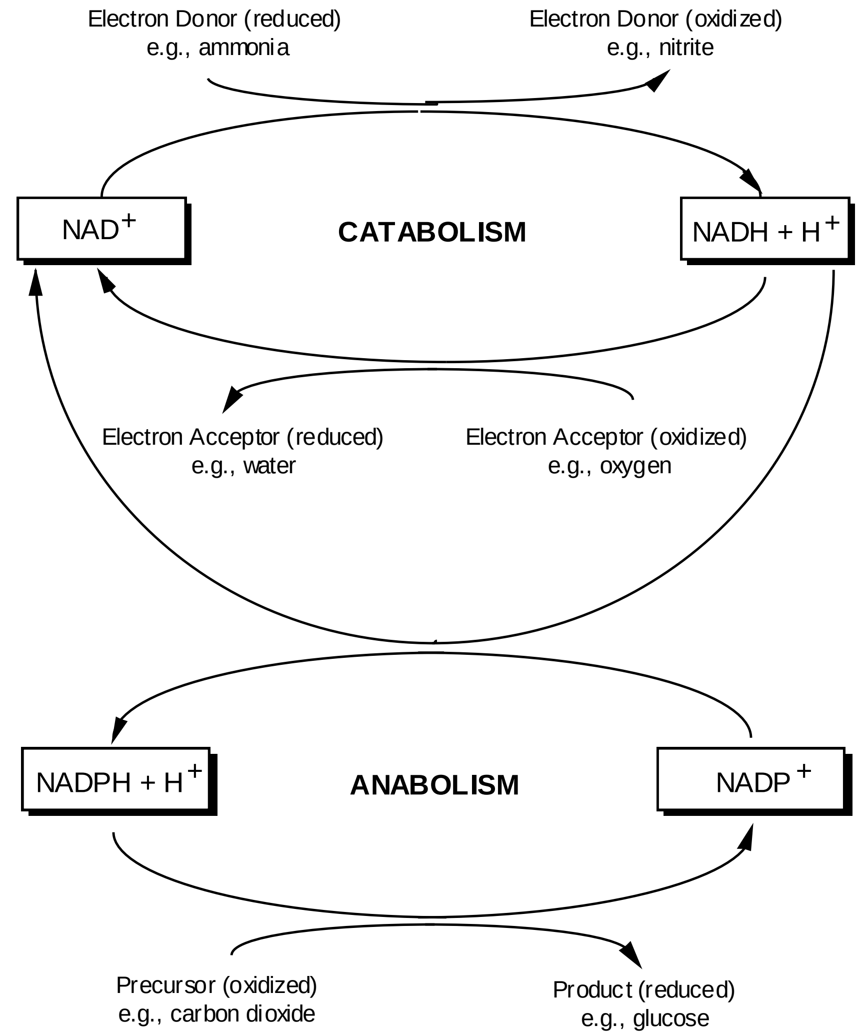
Fig 5.9

ATP & NADH as common currencies of energy and electrons, respectively

ENERGY FLOW IN METABOLISM

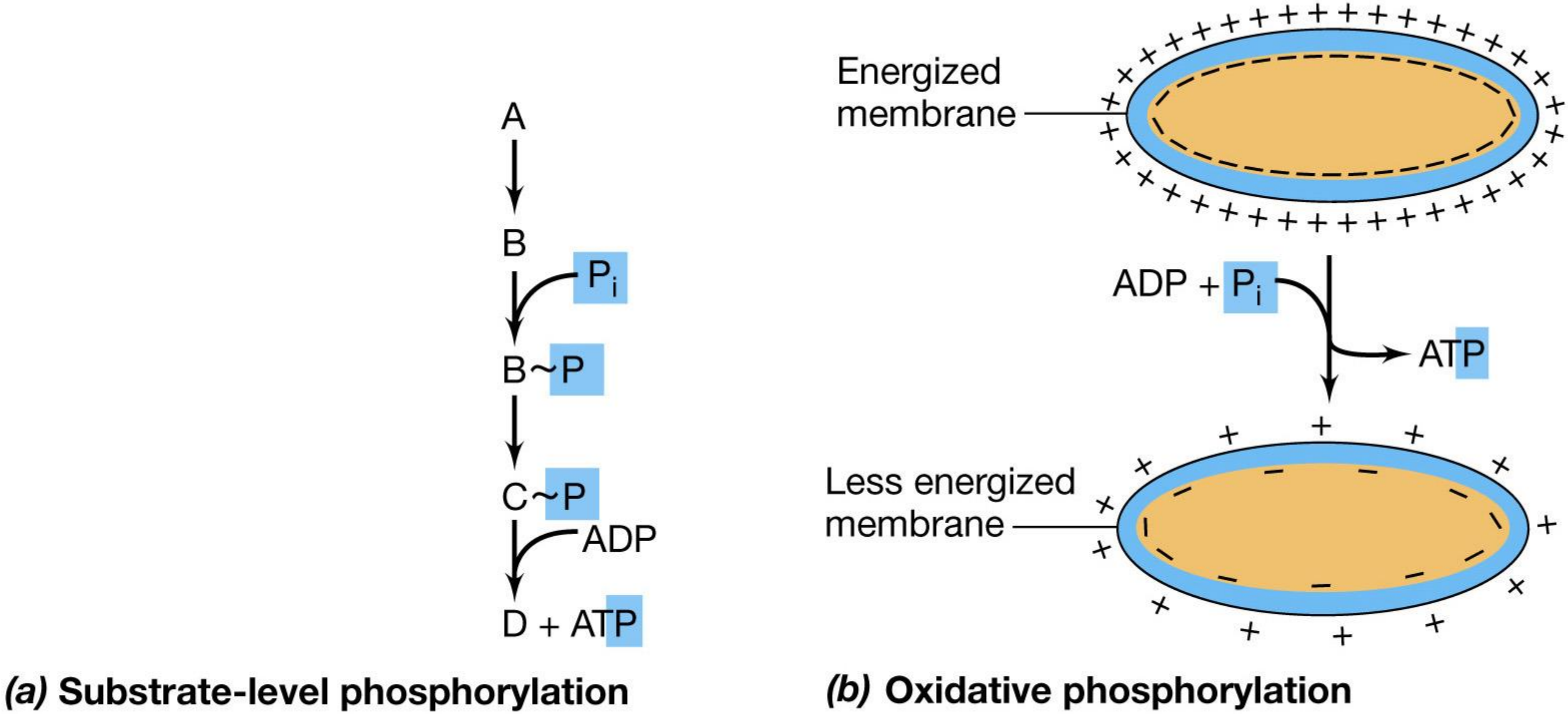


ELECTRON FLOW IN METABOLISM



From CEE451 reader Chapter 17

Two methods of ATP production



Brock Figure: 05-13a-b

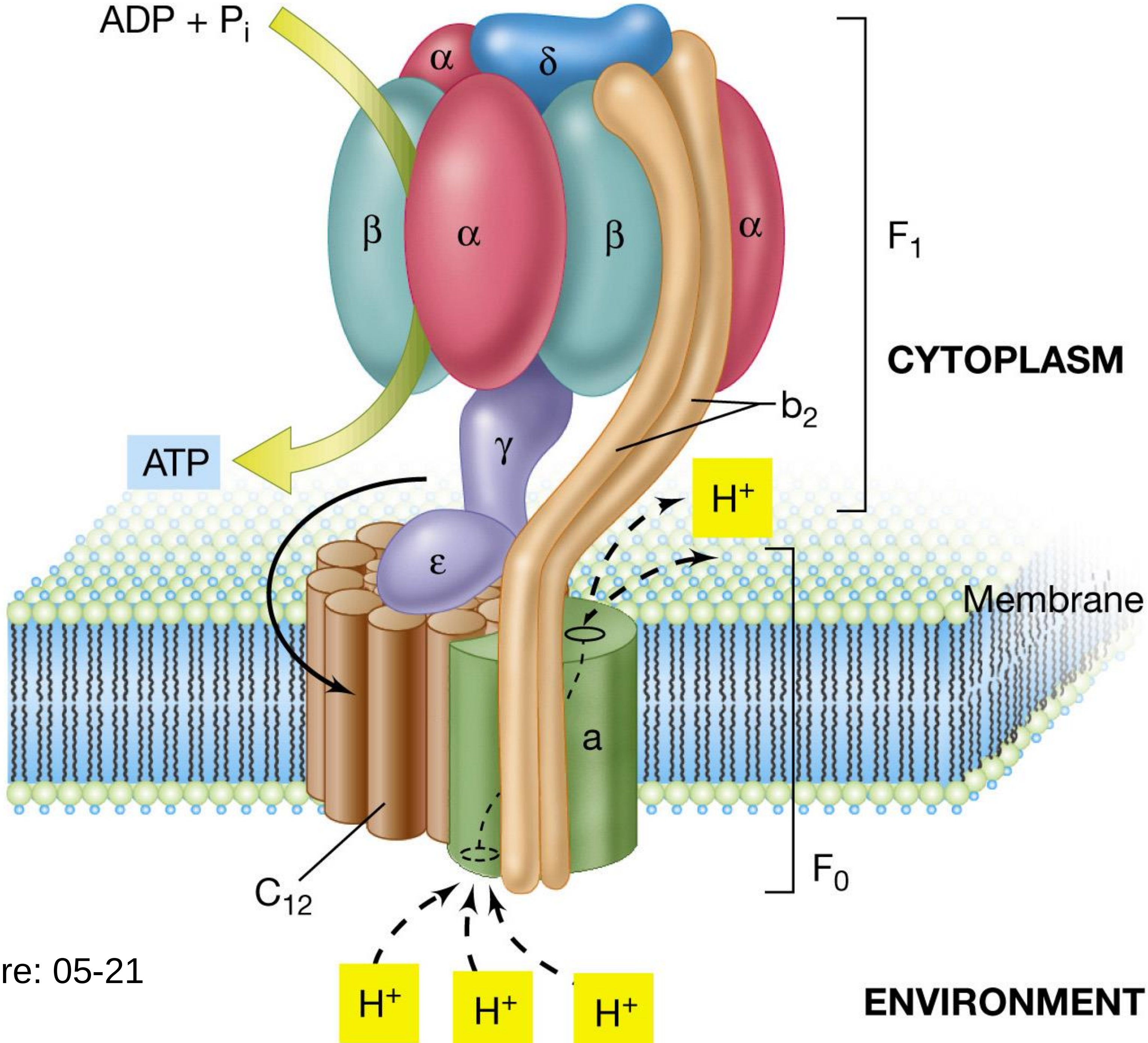

ADP + P_i
α
δ
β
α
β
α
F_1
CYTOPLASM
b_2
ATP
γ
H^+
ε
Membrane
a
C_{12}
F_0
H^+
H^+
H^+
ENVIRONMENT

Figure: 05-21

Cell membrane-bound Electron Transport Chain in typical aerobes:

- -shuttles e-s to O2 (forming water)
- -pumps protons (H+) out of the cell

Note: both e-s and H+ are brought by NADH + H+

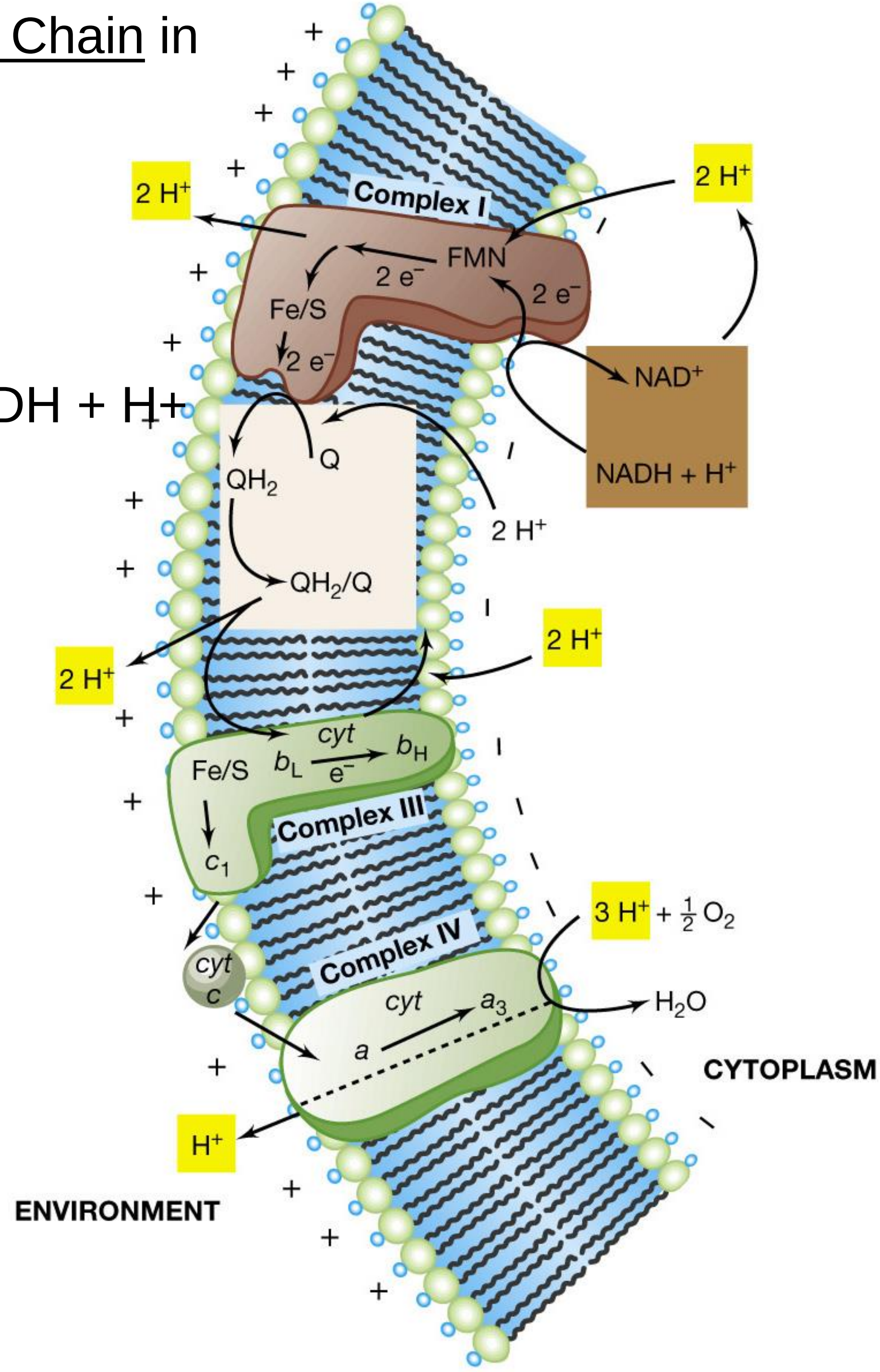


Figure: 05-20

Redox tower for common ETC components

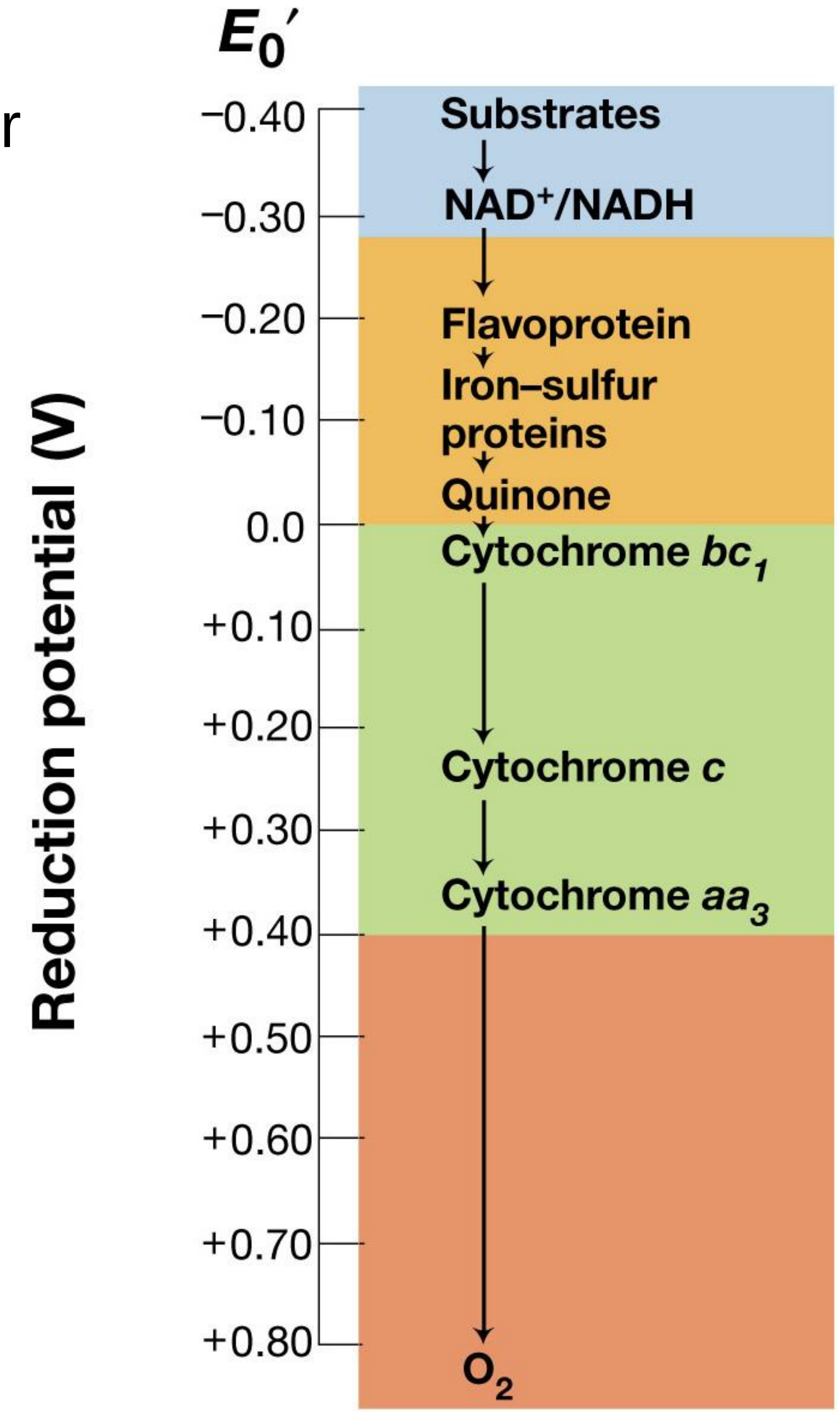


Figure: 05-19

Free energies of reactions

- The free energy associated with any catabolic reaction (any reaction, really) can be predicted
  - Using free energies of formation
  - Or (for redox rxns) coupling of standard half reactions
- For catabolic redox reactions (i.e. respirations) eeq are
  - donated by electron donors
  - accepted by electron acceptors
  - Electron balance
- Sum of half reactions involved equals the standard free energy per eeq transferred from donor to

HALF REACTIONS

kcal/eeq

Reactions for Bacterial Cell Synthesis (R_c)

	Ammonia as Nitrogen Source:		
1.	$^1/_{20}\ C_5H_7O_2N + {}^9/_{20}\ H_2O$	$= {}^1/_5\ CO_2 + {}^1/_{20}\ HCO_3^- + {}^1/_{20}\ NH_4^+ + H^+ + e^-$	
	Nitrate as Nitrogen Source:		
2.	$^1/_{28}\ C_5H_7O_2N + {}^{11}/_{28}\ H_2O$	$= {}^1/_{28}\ NO_3^- + {}^5/_{28}\ CO_2 + {}^{29}/_{28}\ H^+ + e^-$	

Reactions for Electron Acceptors (R_a)

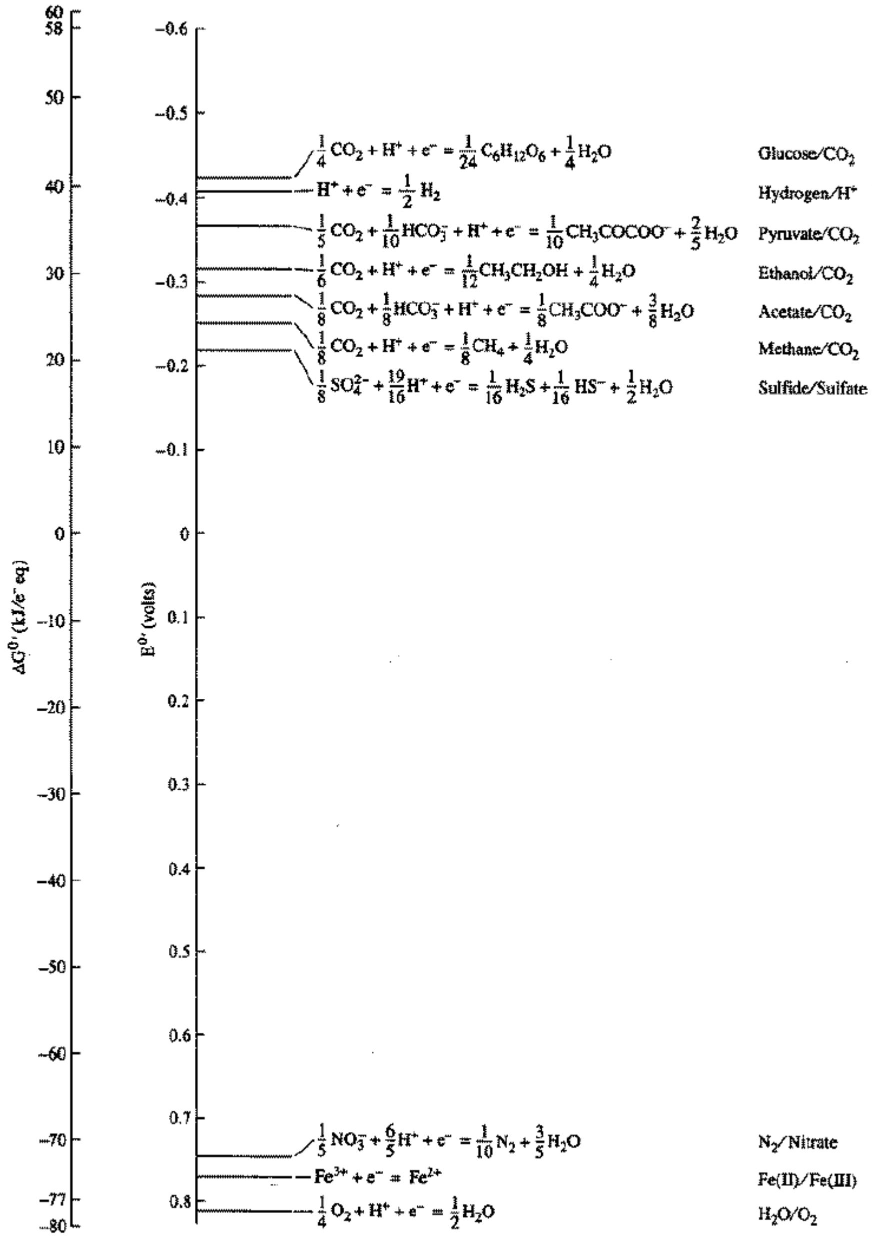
	Oxygen:		
3.	$^1/_2\ H_2O$	$= {}^1/_4\ O_2 + H^+ + e^-$	18.675
	Nitrate:		
4.	$^1/_{10}\ N_2 + {}^3/_5\ H_2O$	$= {}^1/_5\ NO_3^- + {}^6/_5\ H^+ + e^-$	17.128
	Sulfate:		
5.	$^1/_{16}\ H_2S + {}^1/_{16}\ HS^- + {}^1/_2\ H_2O$	$= {}^1/_8\ SO_4^= + {}^{19}/_{16}\ H^+ + e^-$	–5.085
	Carbon Dioxide:		
6.	$^1/_8\ CH_4 + {}^1/_4\ H_2O$	$= {}^1/_8\ CO_2 + H^+ + e^-$	–5.763

Reactions for Electron Donors (R_d)

Organic Donors

	Domestic Wastewater:		
7.	$^1/_{50}\ C_{10}H_{19}O_3N + {}^9/_{25}\ H_2O$	$= {}^9/_{50}\ CO_2 + {}^1/_{50}\ NH_4^+ + {}^1/_{50}\ HCO_3^- + H^+ + e^-$	–7.6
	Protein:		
8.	$^1/_{66}\ C_{16}H_{24}O_5N_4 + {}^{27}/_{66}\ H_2O$	$= {}^8/_{33}\ CO_2 + {}^2/_{33}\ NH_4^+ + {}^{31}/_{33}\ H^+ + e^-$	–7.7
	Carbohydrate (Cellulose, Starch, Sugars):		
9.	$^1/_{24}\ C_6H_{12}O_6 + {}^1/_4\ H_2O$	$= {}^1/_4\ CO_2 + H^+ + e^-$	–10.0
	Grease, Fat, and Oil:		
10.	$^1/_{46}\ C_8H_{16}O + {}^{15}/_{46}\ H_2O$	$= {}^4/_{23}\ CO_2 + H^+ + e^-$	–6.6

• From Rittman & McCarty Env'l Biotech 2001



Balanced catabolic summary reaction for aerobic glucose respiration

		$\Delta G^o(w)$* (kcal/eeq)
Rxn (9)	$^1/_{24}\ C_6H_{12}O_6 + {}^1/_4\ H_2O = {}^1/_4\ CO_2 + H^+ + e^-$	–10.0
–Rxn (3)	$^1/_4\ O_2 + H^+ + e^- = {}^1/_2\ H_2O$	–18.675
	$^1/_{24}\ C_6H_{12}O_6 + {}^1/_4\ O_2 = {}^1/_4\ CO_2 + {}^1/_4\ H_2O$	–28.675

Note: 1 eeq reduces ¼ mole O2 to water (1/4 mole O2 = 32/4 = 8 g O2)

Therefore 1 eeq of an electron donor imposes 8 g of Oxygen Demand (OD)

Other examples of chemoorganotrophy

- Fermentation of sugar to lactate

Lactate:

15. $\frac{1}{12} CH_3CHOHCOO^- + \frac{1}{3} H_2O = \frac{1}{6} CO_2 + \frac{1}{12} HCO_3^- + H^+ + e^-$ −7.873 x (-1)

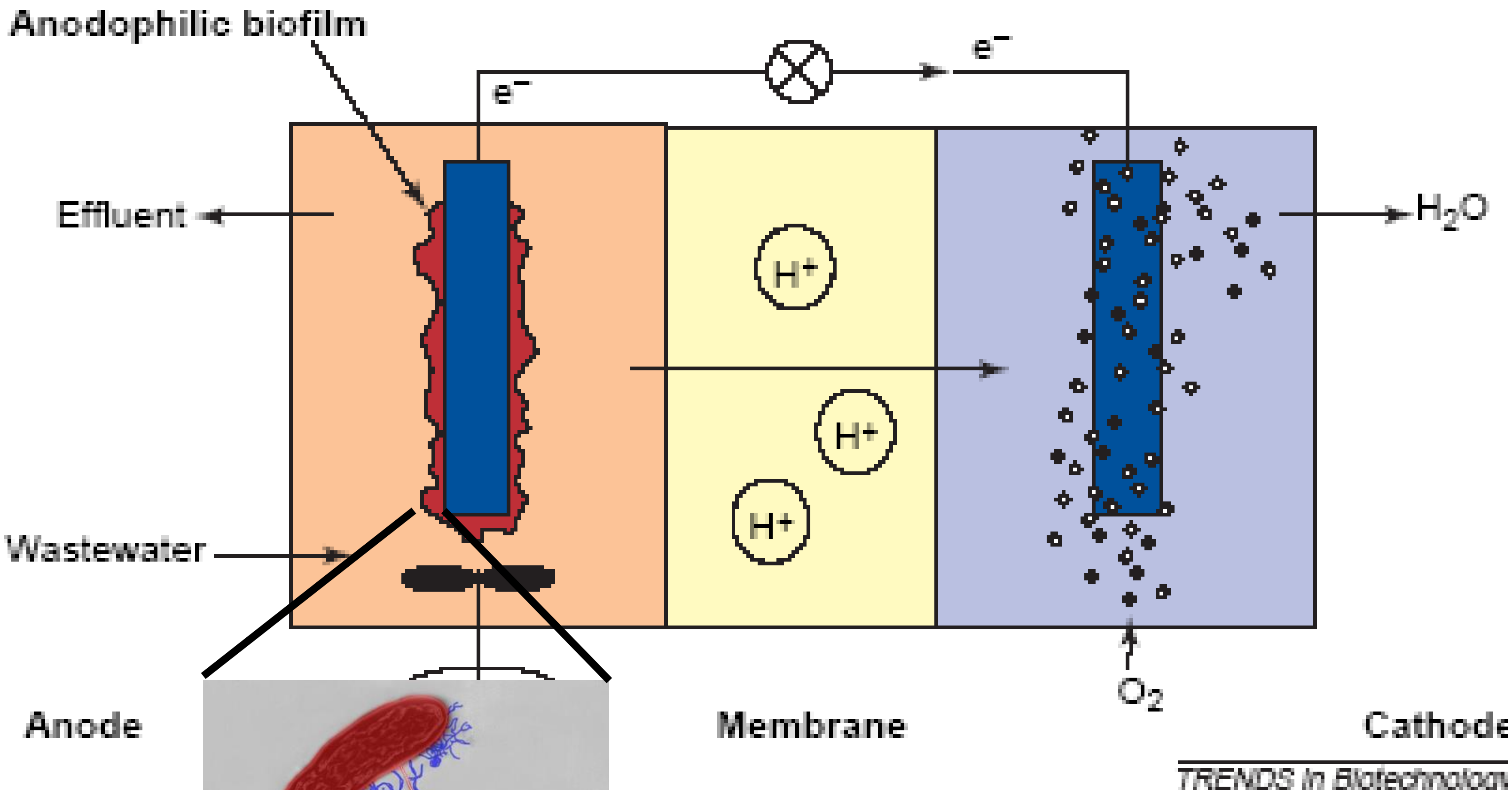
Carbohydrate (Cellulose, Starch, Sugars):

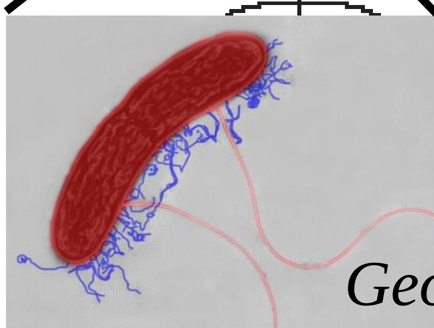
9. $\frac{1}{24} C_6H_{12}O_6 + \frac{1}{4} H_2O = \frac{1}{4} CO_2 + H^+ + e^-$ −10.0

Sum: $$\frac{1}{24} C_6H_{12}O_6 + \frac{1}{12} HCO_3^- = \frac{1}{12} H_2O + \frac{1}{12} CH_3CHOHCOO^- + \frac{1}{12} CO_2$$

$\Delta G^{o(w)}$= -2.127 kcal/ee

Microbial Fuel Cells tap into electron flow directly



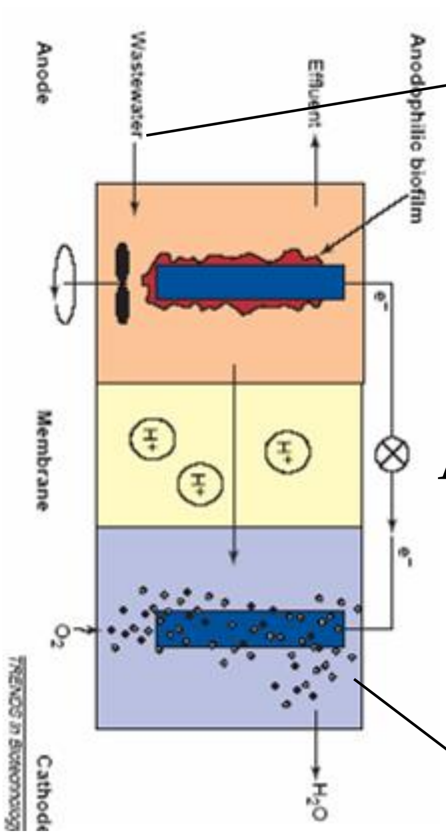


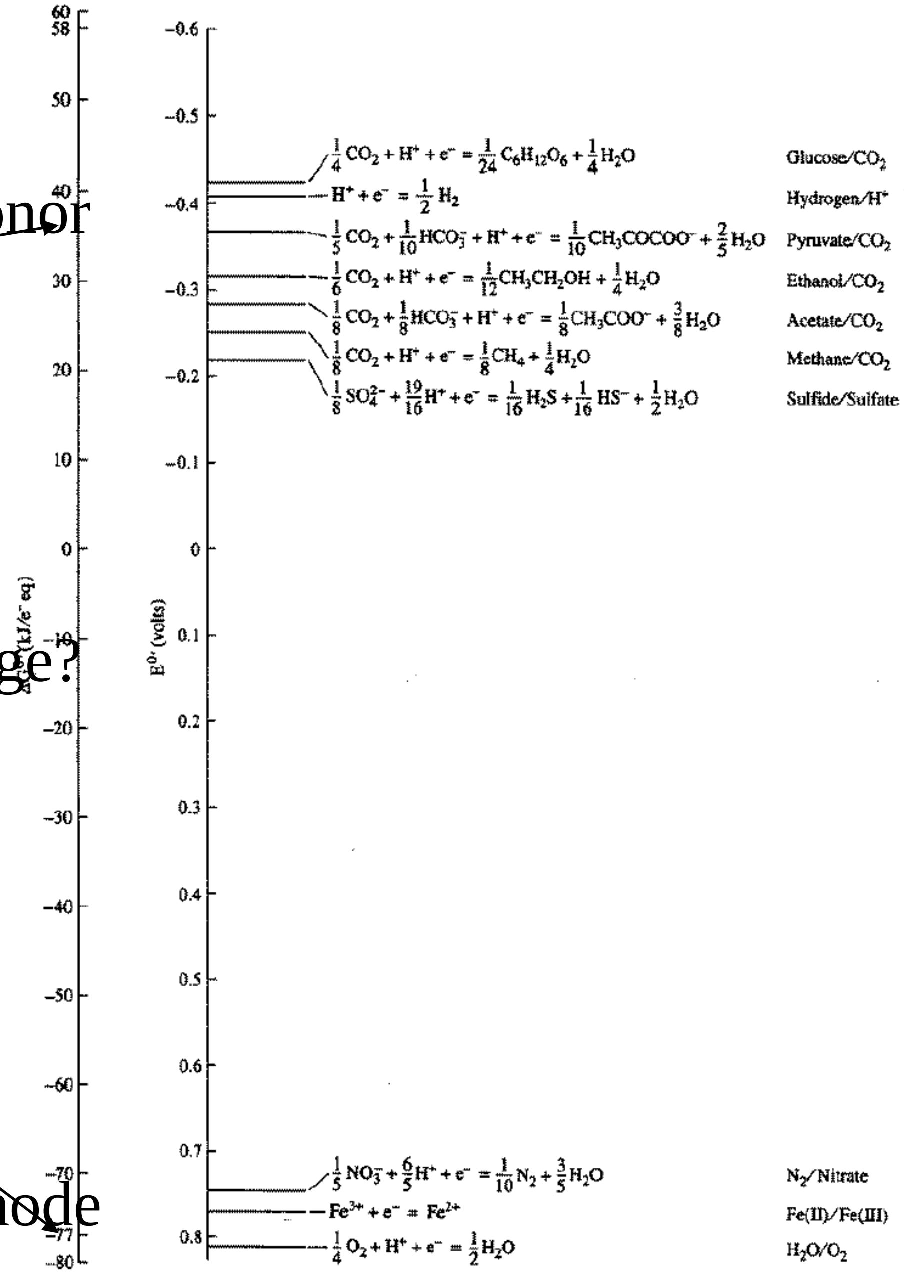
Geobacter with naonowires (blue) and flagella

E donor

Anode voltage?

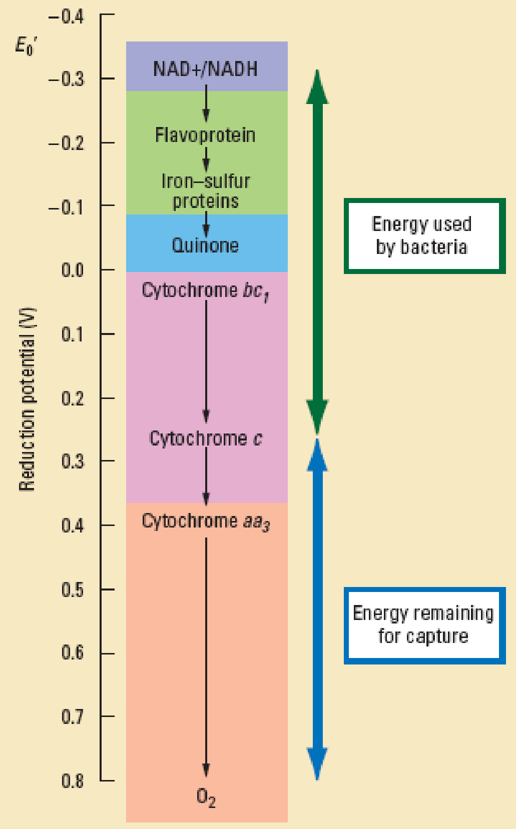
E acceptor in cathode





Respiratory chain shows how the voltage that could be recovered in a microbial fuel cell (MFC) is dependent on where electrons exit the chain of respiratory enzymes

In the case shown here, bacteria could derive energy from the potential between NADH (the reduced form of nicotinamide adenine dinucleotide) and cytochrome *c* (green arrow), whereas the MFC could be used to recover energy from the potential between cytochrome *c* and oxygen (blue arrow). Actual potentials depend on concentrations and potentials of specific enzymes and electron acceptors. (Respiratory-chain and standard potentials shown here are adapted with permission from a figure in Ref. 51 for *Paracoccus denitrificans*.)



From logan et al 2006, ES&T

eeq conversions

- 1 eeq (1 mole e’s)= 8 g OD (oxygen demand) = 96,400 Coulombs
- In electrical engineering:
  - 1 Coulomb/s = 1 Ampere (a unit of current w symbol I)
- Power in an electrical system = Volts*Current = Watts