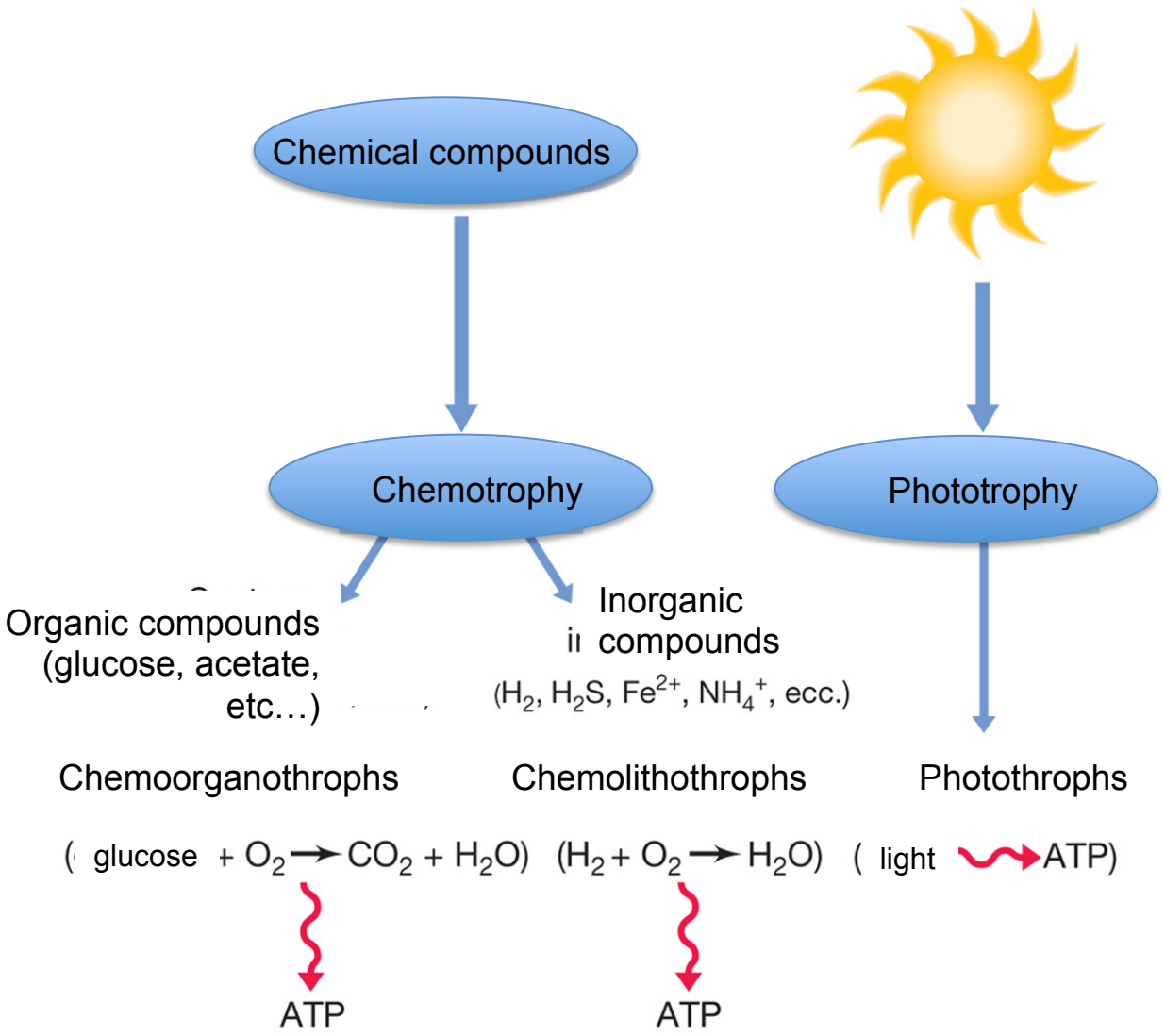
12. APPLIED BIOCHEMISTRY

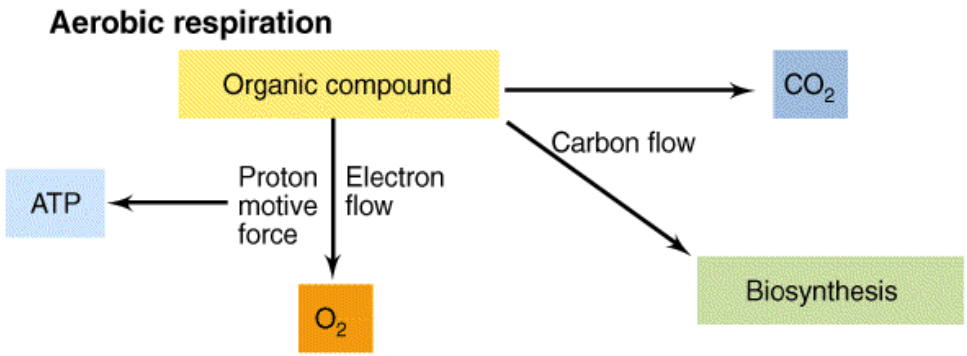
Energy source

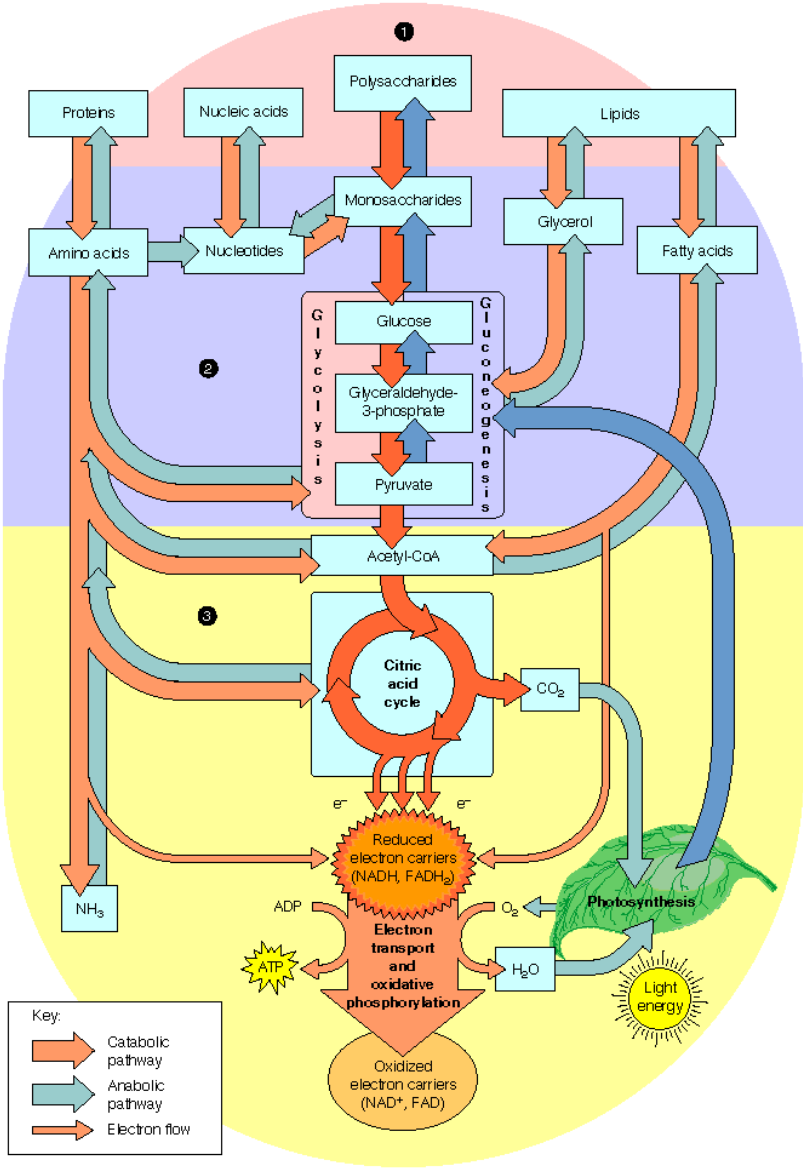


Energy and carbon source

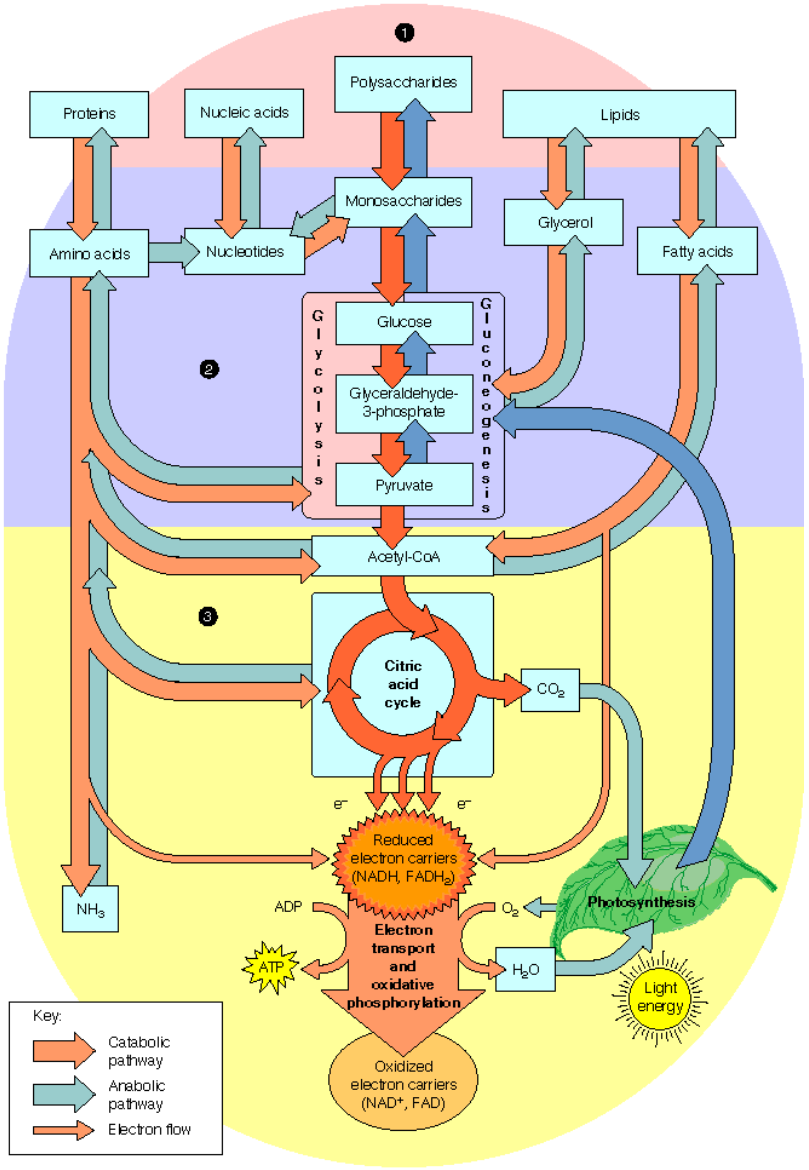
Carbon Source	Energy Source: Light (*photo-*)	Energy Source: Chemical compounds (*chemo-*)
Carbon dioxide (*auto-*)	***Photoautotrophs*** • Plants, algae, and cyanobacteria use H_2O to reduce CO_2, producing O_2 as a byproduct • Photosynthetic green sulfur and purple sulfur bacteria do not use H_2O nor produce O_2	***Chemoautotrophs*** • Hydrogen, sulfur, and nitrifying bacteria
Organic compounds (*hetero-*)	***Photoheterotrophs*** • Green nonsulfur and purple nonsulfur bacteria	***Chemoheterotrophs*** • Aerobic respiration: most animals, fungi, and protozoa, and many bacteria • Anaerobic respiration: some animals and bacteria • Fermentation: some bacteria and yeasts

Alternative energy generating patterns

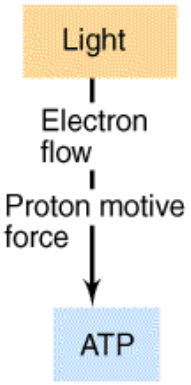


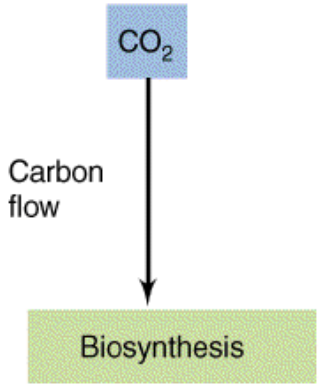


Alternative energy generating patterns

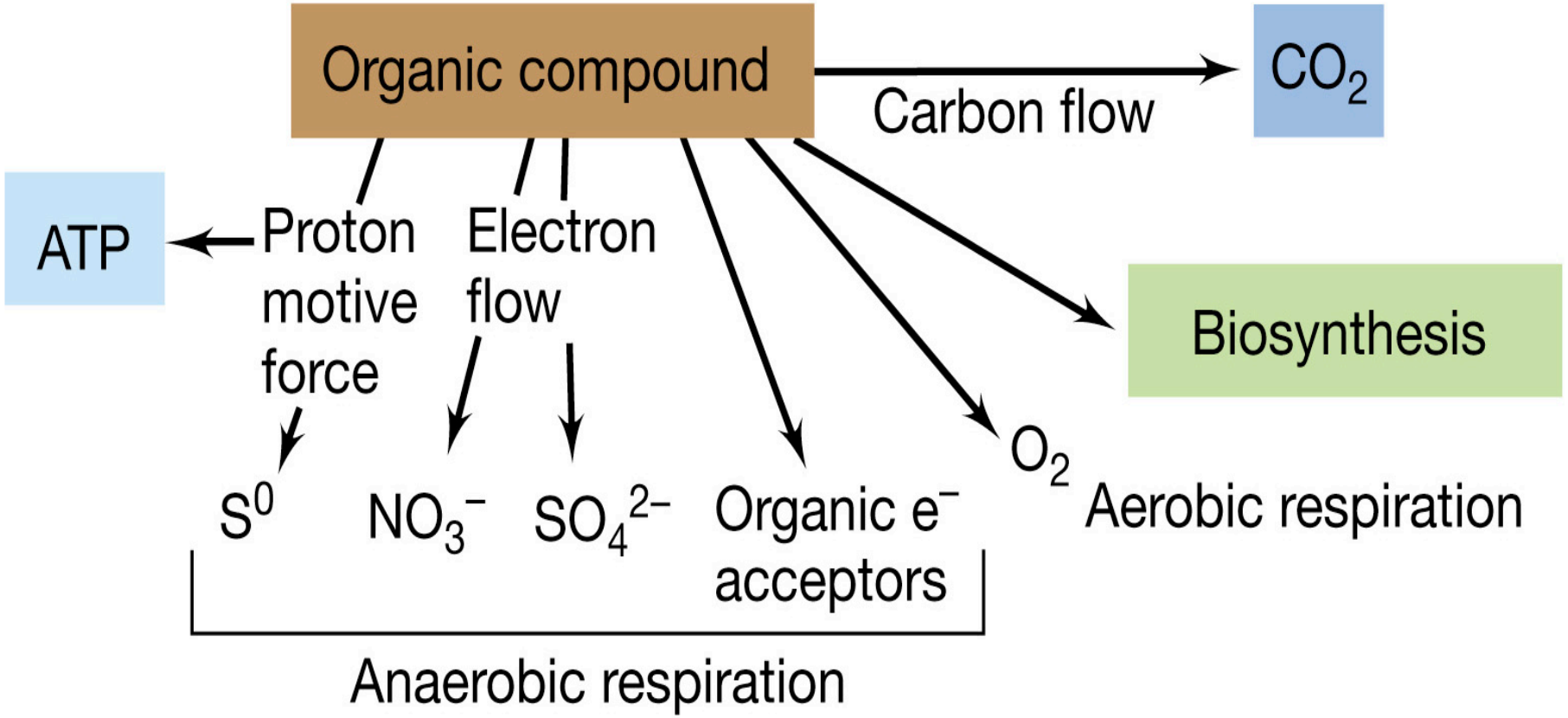


Phototrophic metabolism





Chemoorganotrophic metabolism

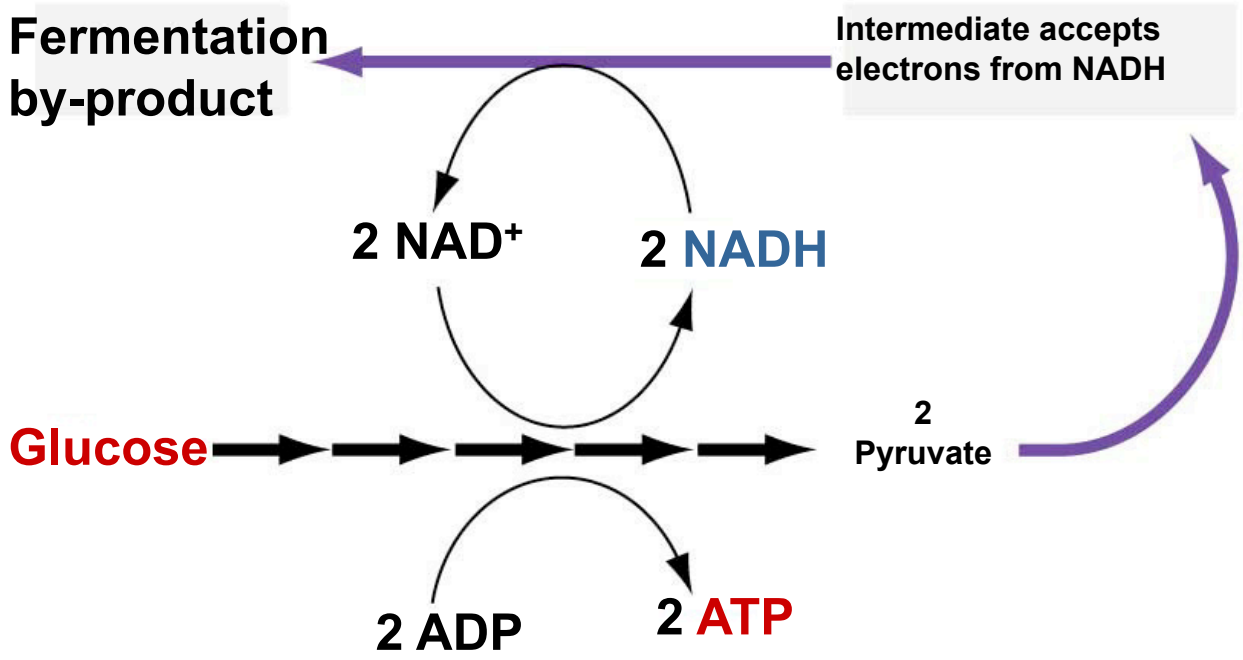


Chemoorganotrophic metabolism

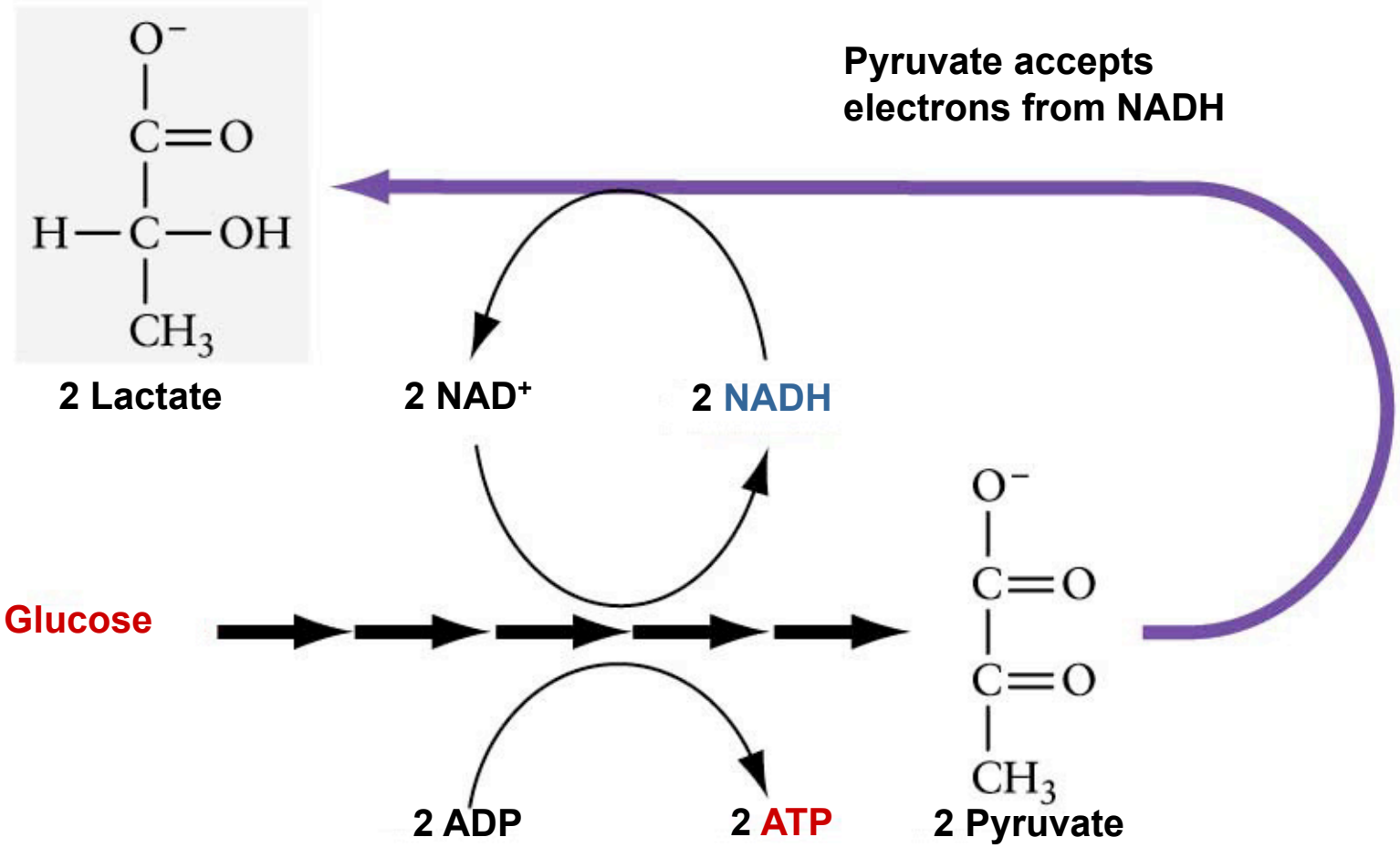
Electron acceptor	**Final product**	**Name of the process**
O_2	H_2O	Aerobic respiration
NO_3	NO_2, NH_3 or N_2	Anaerobic respiration: denitrification *Bacillus, Pseudomonas*
SO_4	S or H_2S	Anaerobic respiration: sulphates reduction *Desulfovibrio*
fumarate	succinate	Anaerobic respiration: Organic e^- acceptor *E.coli*
CO_2	CH_4	Metanogenesis (Archea)

Chemoorganotrophic metabolism

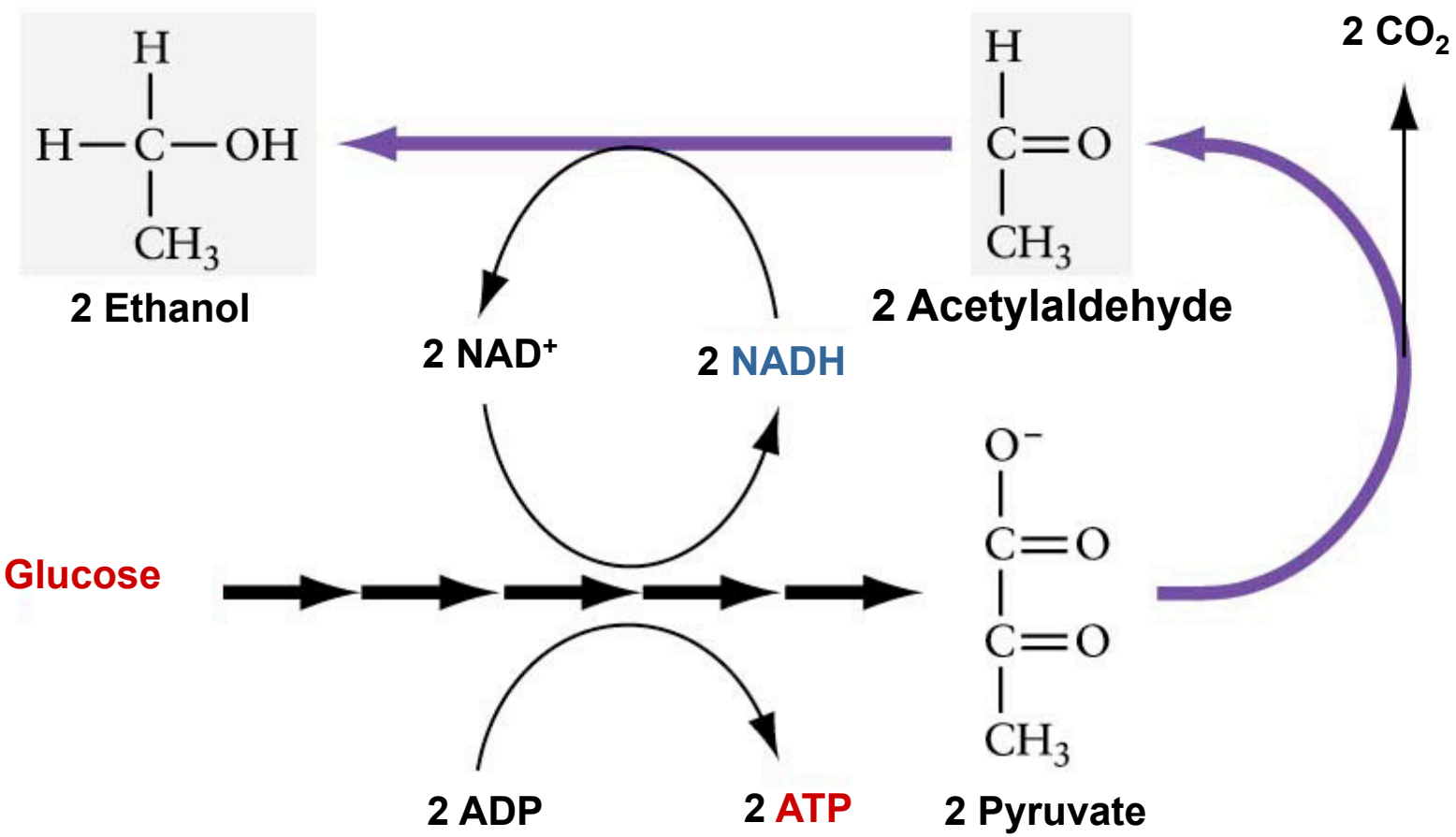
Fermentation pathways allows cells to regenerate NAD^+ for glycolsis



Chemoorganotrophic metabolism

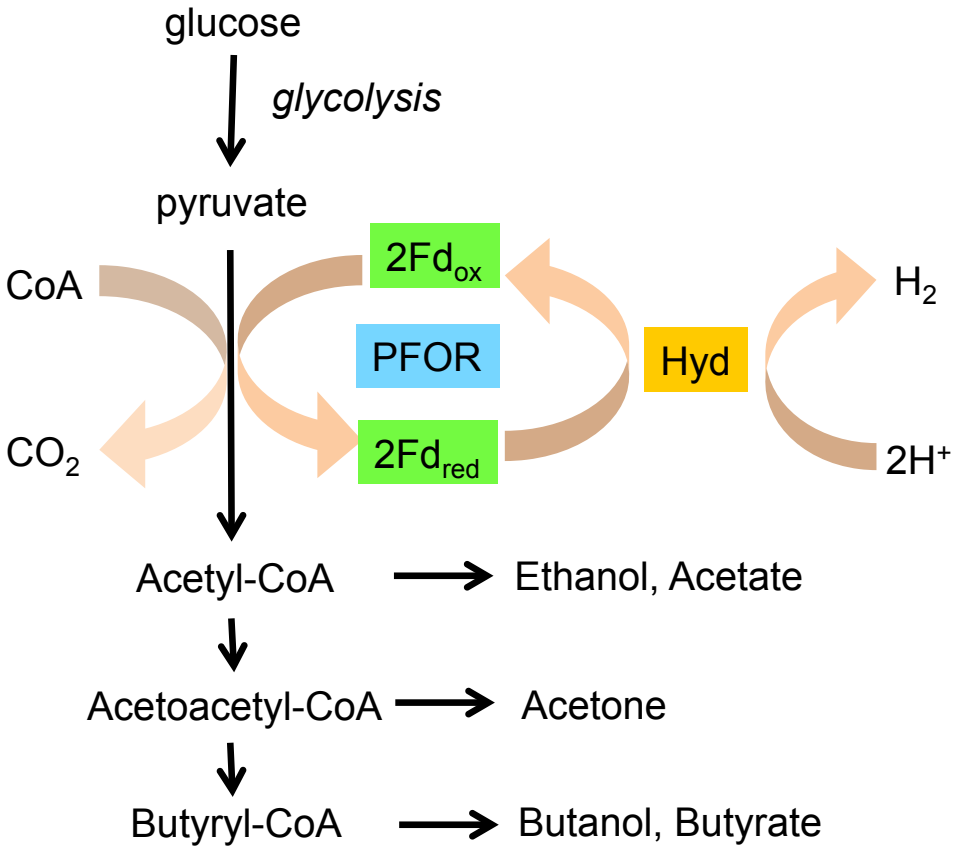


Chemoorganotrophic metabolism



Anaerobic Chemoorganotrophs: -Fermentors

- Genus *Clostridium*
  - Gram-positive rods found in soil
  - Endospores
- Ferment wide variety of compounds
- Representitives:
  - *C. tetani,*
  - *C. perfringens,*
  - *C. botulinum*

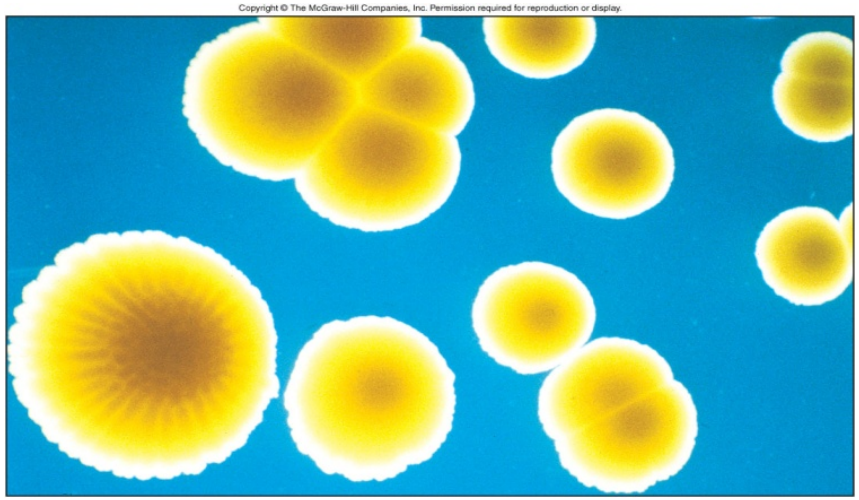


Anaerobic Chemotrophs

- *Propionibacterium* species are Gram-positive rods
- Organisms produce propionic acid as end product of fermentation
  - Found in anaerobic micro environments
  - Essential in the production of Swiss cheese
  - Also ferment lactic acid

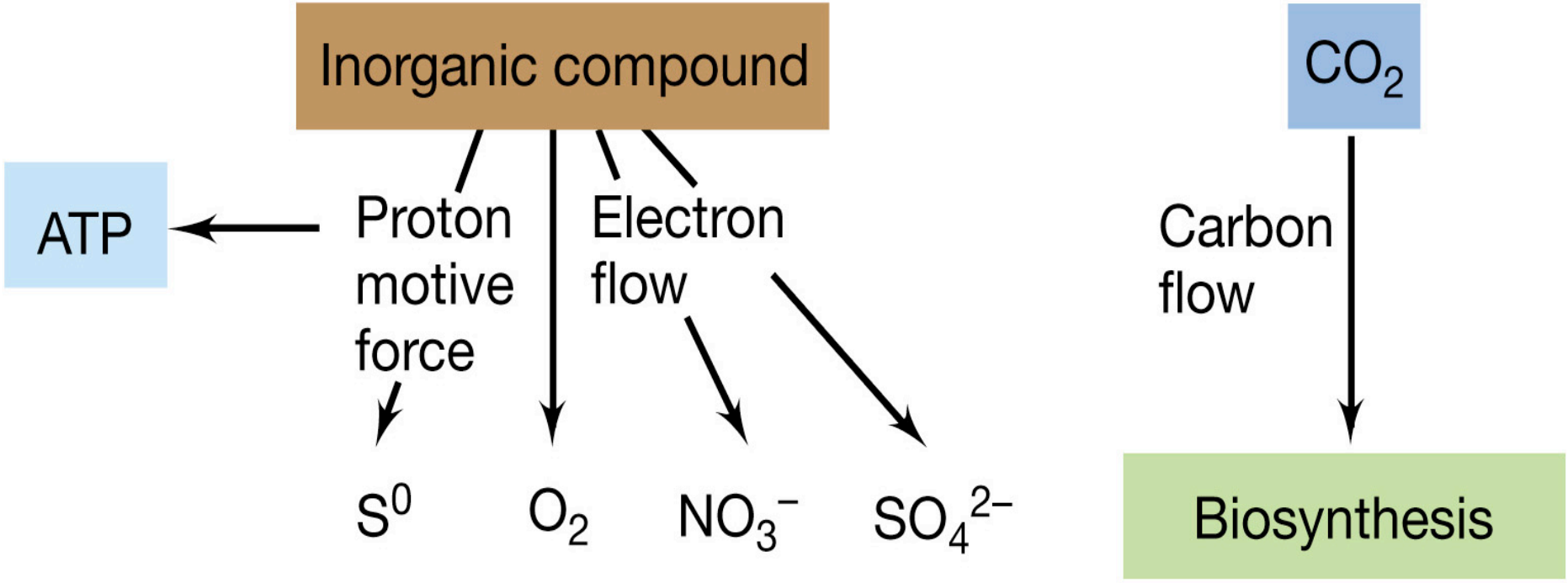
Aerobic Chemoorganotrophs: Obligate Aerobes

- Obligate aerobes obtain energy using aerobic respiration exclusively
- Characteristic genera include
  - *Micrococcus*
    - Gram-positive cocci found in soil and dust
    - Produce yellow pigmented colonies



- *Mycobacterium*
  - Gram-positive bacterium
  - Live on dead and decaying matter
- *Pseudomonas*
  - Gram-negative rods
  - Motile and often pigmented
  - Common opportunistic pathogen
- *Thermus* and *Deinococcus*
  - Both have scientific and commercial uses
    - *Thermus* produces *Taq polymerase*
    - *Deinococcus* used to clean up radioactive contamination

Chemolitotrophic metabolism



Chemolithotrophs

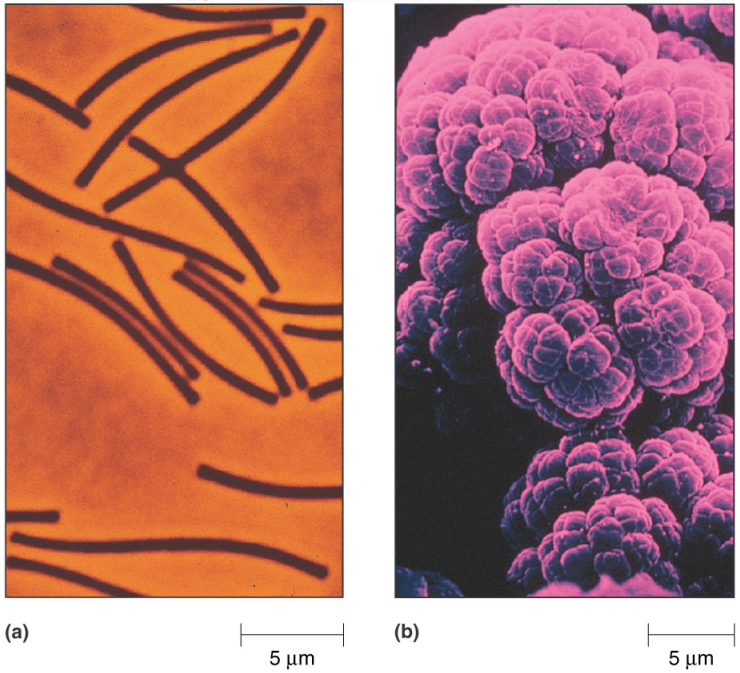
- Chemolithotrophs oxidize reduced inorganic chemicals (e.g. H_2) to produce energy
  - Rare organisms
  - Not O_2 tolerant
  - Terminal electron acceptor usually carbon dioxide or sulfur
  - Members of the domain *Archaea*

Group	Energy source	Final products	Microrganism
Hydrogen bacteria	H_2	H_2O	*Alcaligenes*, *Pseudomonas*
Metanogenes	H_2	H_2O	*Methanobacterium*
Carboxydobacteria	CO	CO_2	*Rhodospirillum*, *Azotobacter*
Nitrifying bacteria	NH_3	NO_2	*Nitrosomonas*
Nitrifying bacteria	NO_2	NO_3	*Nitrobacter*
S-oxidizing bacteria	H_2S or S	SO_4	*Thiobacillus*, *Sulfolobus*
Fe-bacteria	Fe^{++}	Fe^{+++}	*Gallionella*, *Thiobacillus*

Chemolithotroph: Methanogens

- Members of Domain *Archaea*
- Found in sewage, swamps, marine sediments and digestive tract of mammals
- Highly sensitive to oxygen
- Produce energy (ATP) the reaction:

$4H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$



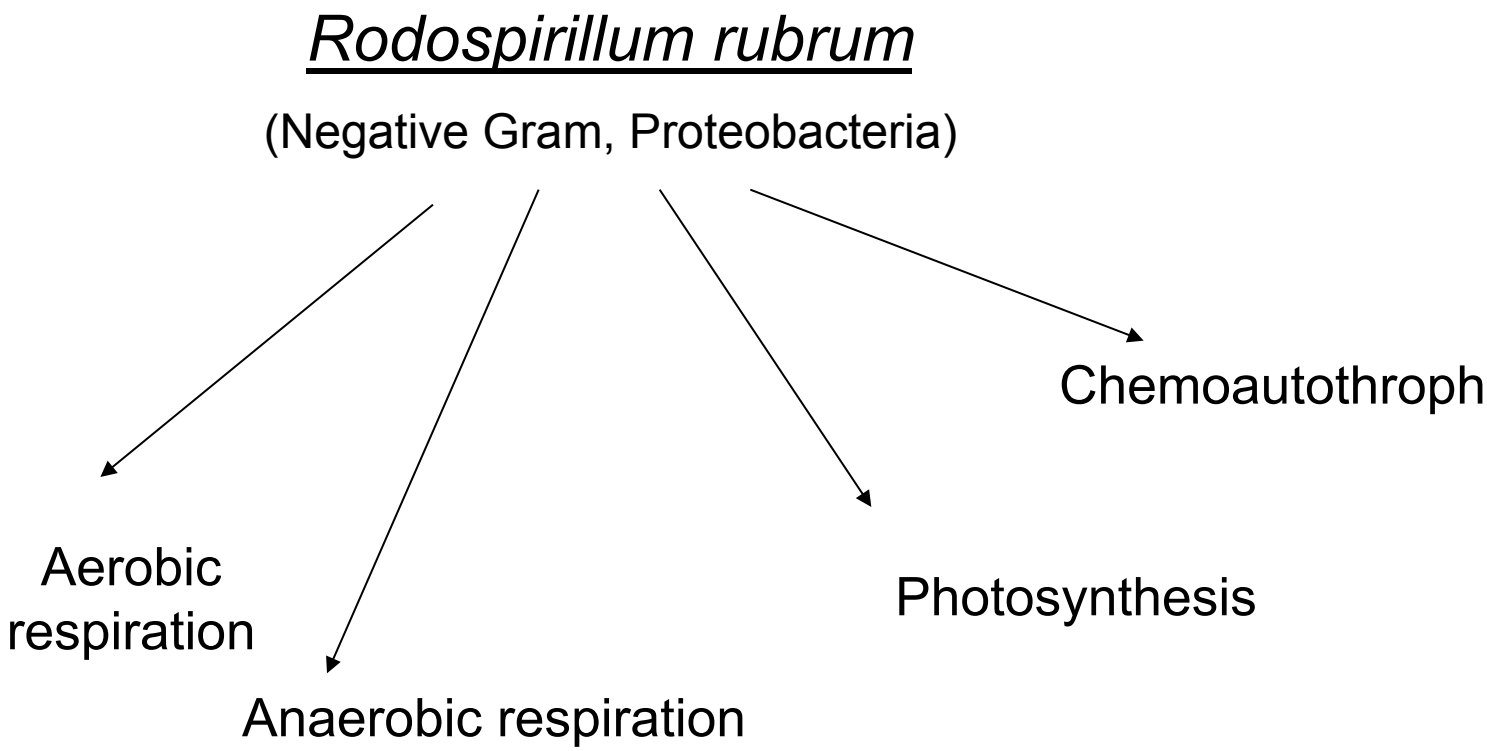
Aerobic Chemolithotrophs: Hydrogen-Oxidizing Bacteria

- Gram-negative bacteria
- Tend to be thermophilic
  - Found in hot springs, up to 95°C

$$H_2 + \tfrac{1}{2}O_2 \longrightarrow H_2O$$

(energy source) (terminal electron acceptor)

Metabolic versatility



BIOREMEDIATION

Bioremediation relies largely on the enzymatic activities of living organisms, usually microbes, to catalyze the destruction of pollutants or their transformation to less harmful forms.

Why are microorganisms so important in this process?
They have extraordinary metabolic diversity!

A complex process depending on many factors including:

- ambient environmental conditions (pH, temperature, lack of nutrients & molecular oxygen
- composition of the microbial community
- nature and amount of pollution present

BIOREMEDIATION

"use of living organisms (e.g., bacteria) to clean up oil spills or remove other pollutants from soil, water, and wastewater.“

Source: United States Environmental Protection Agency, Office of Compliance and Assurance

“clean-up of pollution from soil, groundwater, surface water and air, using biological, usually microbiological processes”

Source: Philp et al., 2001

BIOREMEDIATION

Types of pollutants

Organic pollutants → catabolized

- Naturally occurring
- Xenobiotics - substances foreign to an entire biological system, i.e. artificial substances, which did not exist in nature before their synthesis by humans
- Metals from ore extraction and manufacturing

CONTAMINANTS FOR BIODEGRADATION

Readily degradable	Somewhat degradable	Difficult to degrade	Generally recalcitrant
fuel oils, gasoline	creosote, coal tars	chlorinated solvents (TCE)	dioxins
ketones and alcohols	pentachloro-phenol (PCP)	some pesticides and herbicides	polychlorinated biphenyls (PCB)
monocyclic aromatics			
bicyclic aromatics (naphthalene)			

How Microbes Use the Contaminant

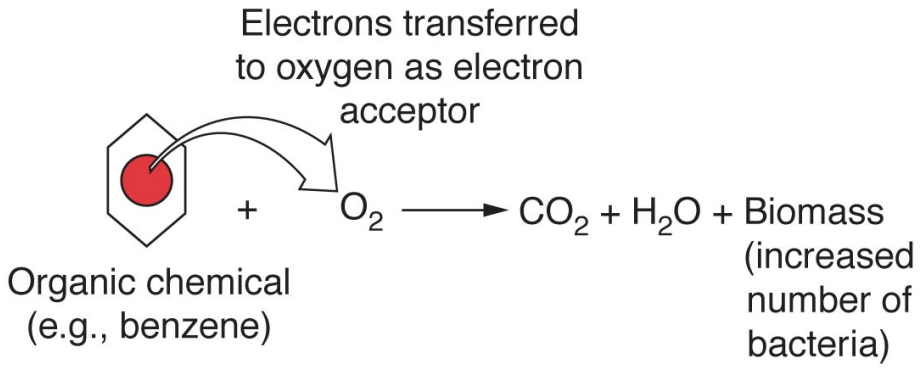
- Contaminants may serve as:
  - Primary substrate
    - enough available to be the sole energy source
  - Secondary substrate
    - provides energy, not available in high enough concentration
  - Cometabolic substrate
    - fortuitous transformation of a compound by a microbe relying on some other primary substrate

USE OF CONTAMINANTS AS PRIMARY SUBSTRATE

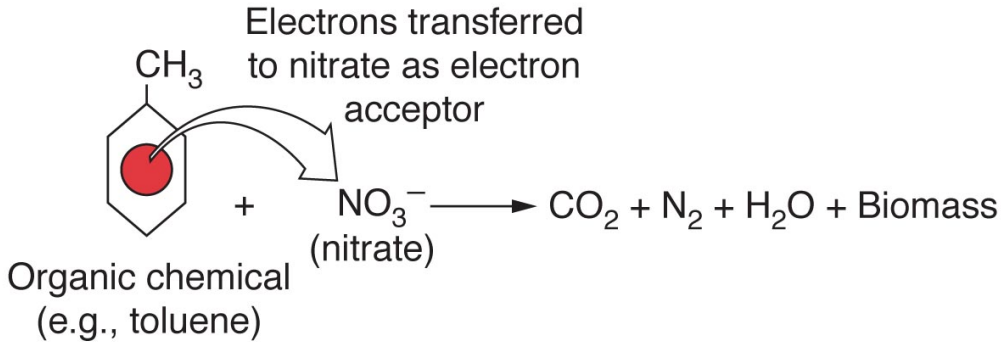
- Aerobic metabolism
  - Microbes use O_2 in their metabolism to degrade contaminants

- Anaerobic metabolism
  - Microbes substitute another chemical for O_2 to degrade contaminants
    - Nitrate, iron, sulfate, carbon dioxide, uranium, technicium, perchlorate

Aerobic biodegradation

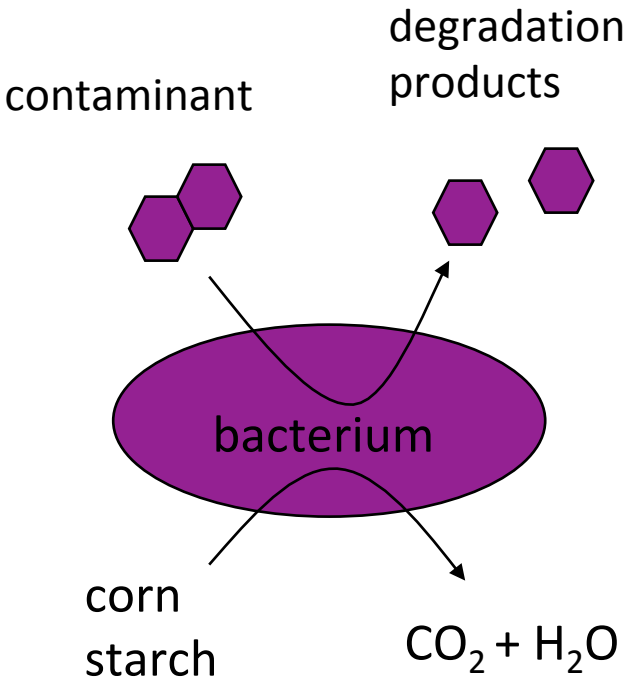


Anaerobic biodegradation

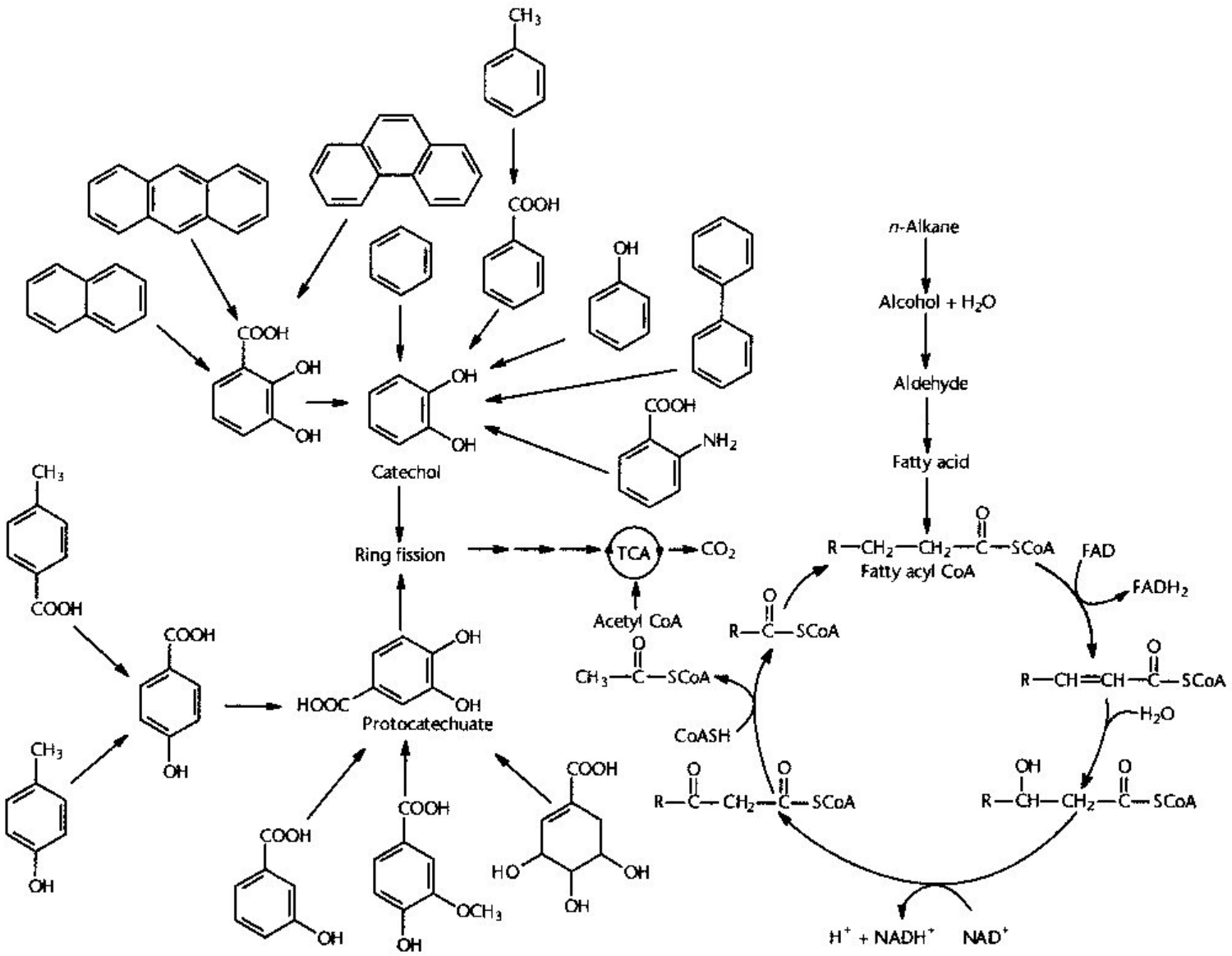


Cometabolism

Bacterium uses some other carbon and energy source to partially degrade contaminant (organic aromatic ring compound)



Secondary metabolism



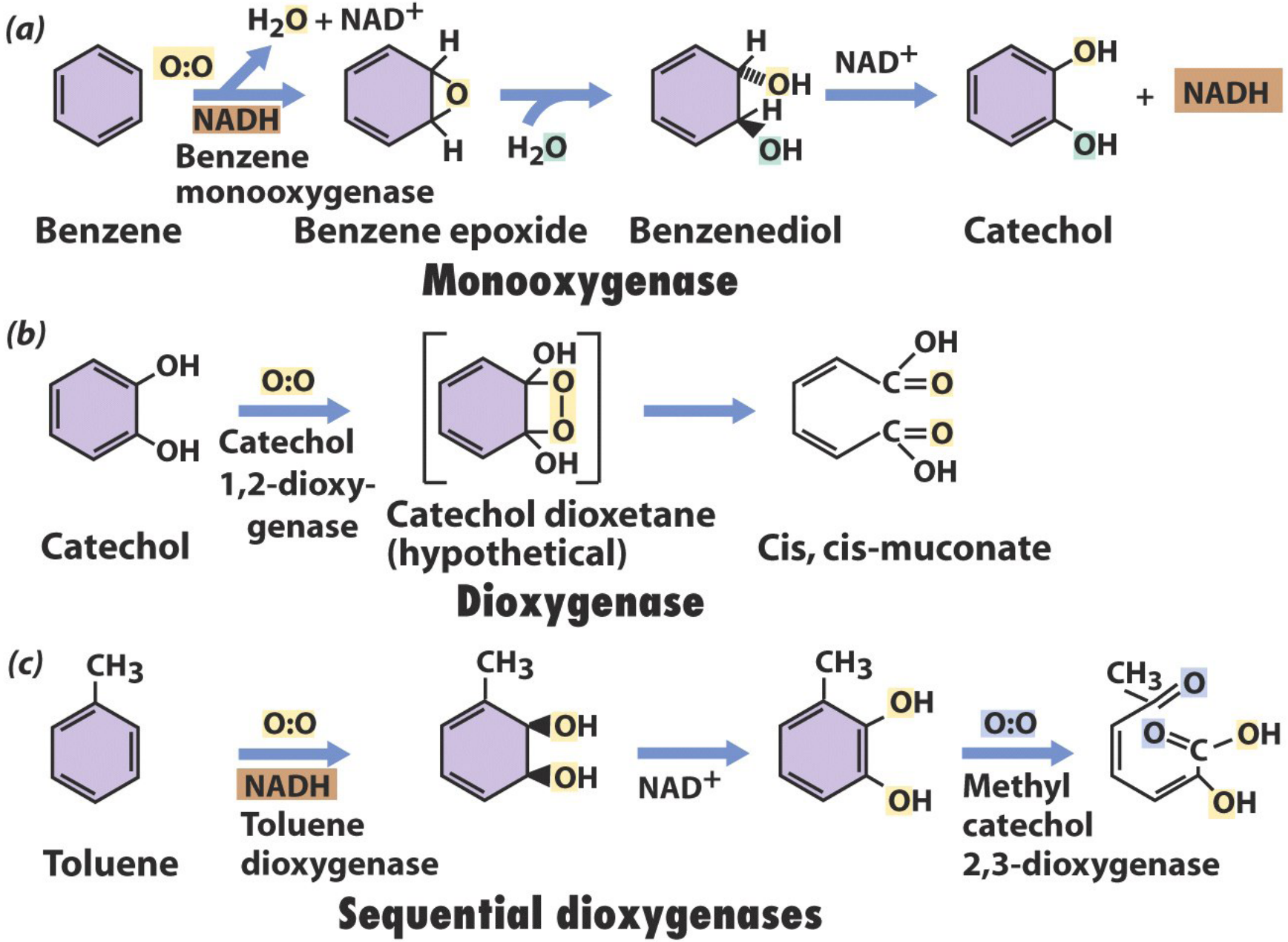


Figure 17-56 Brock Biology of Microorganisms 11/e

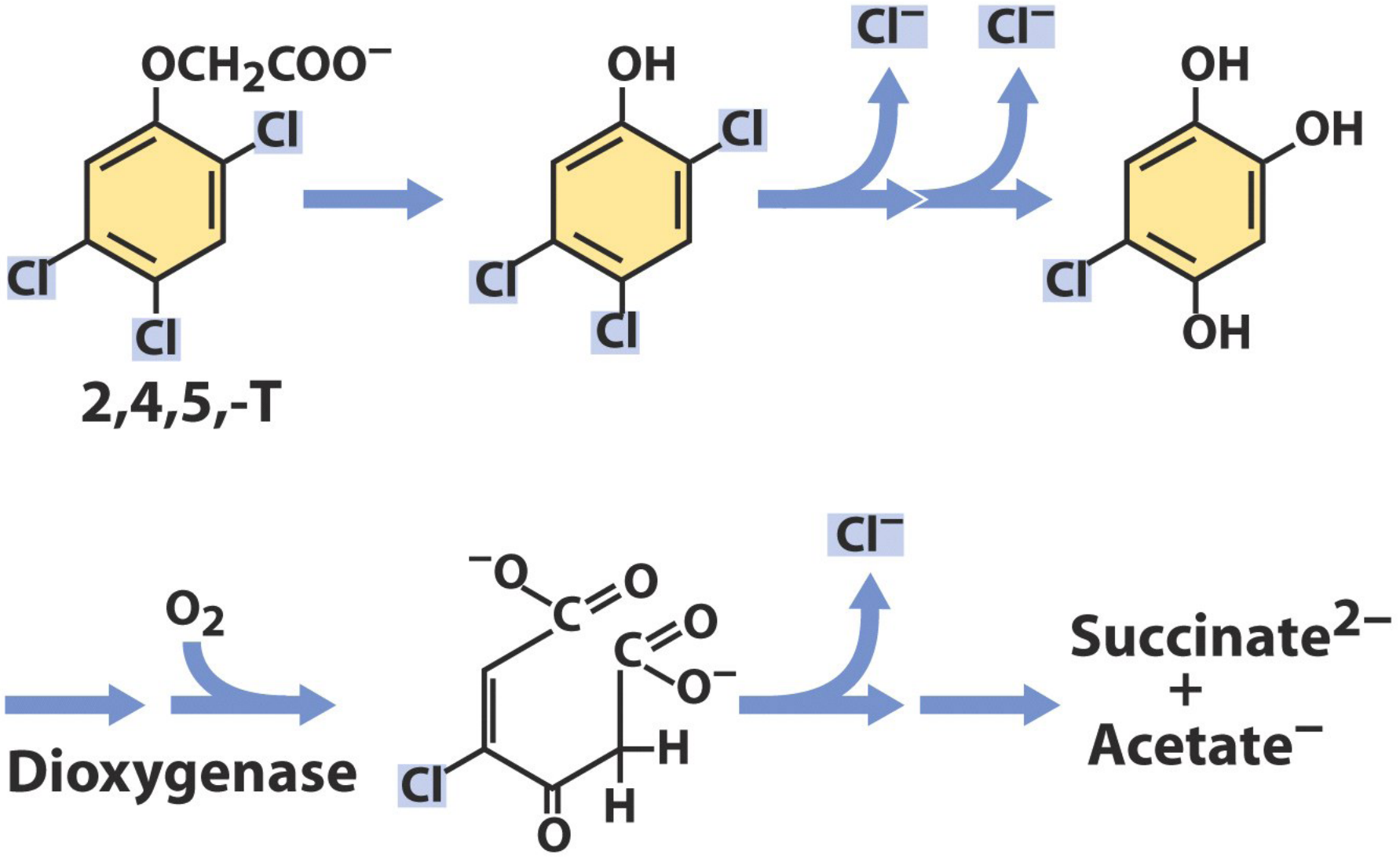


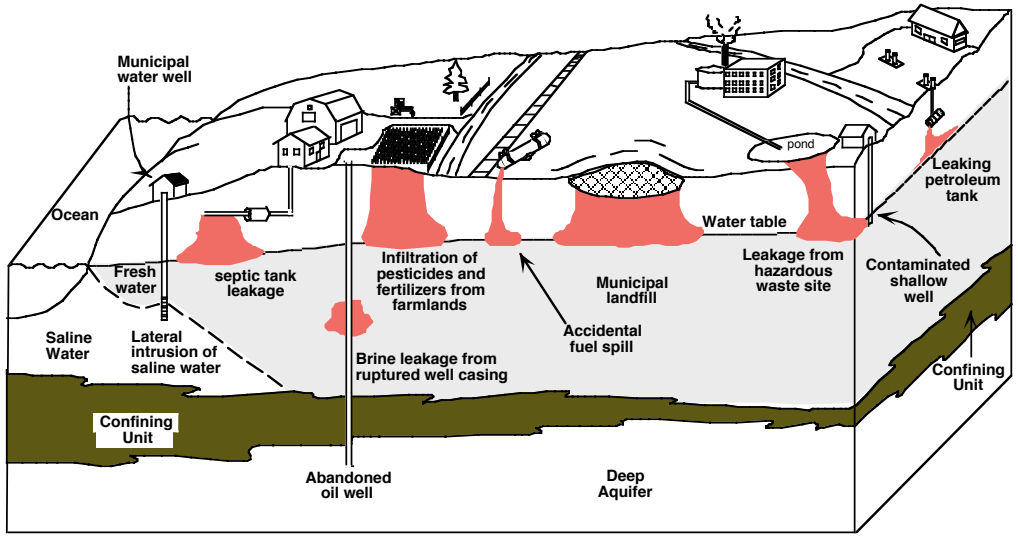
Figure 19-47b Brock Biology of Microorganisms 11/e

Soil and Subsurface Contaminants

- Benzene and related fuel components (BTEX)
- Pyrene and other polynuclear aromatics
- Chlorinated aromatics and solvents
- Herbicides and pesticides
- Nitroaromatic explosives and plasticizers

Sources of Contamination

- Industrial spills and leaks
- Surface impoundments
- Storage tanks and pipes
- Landfills
- Burial areas and dumps
- Injection wells



Wastewater Treatment

Treatment depends on three factors:

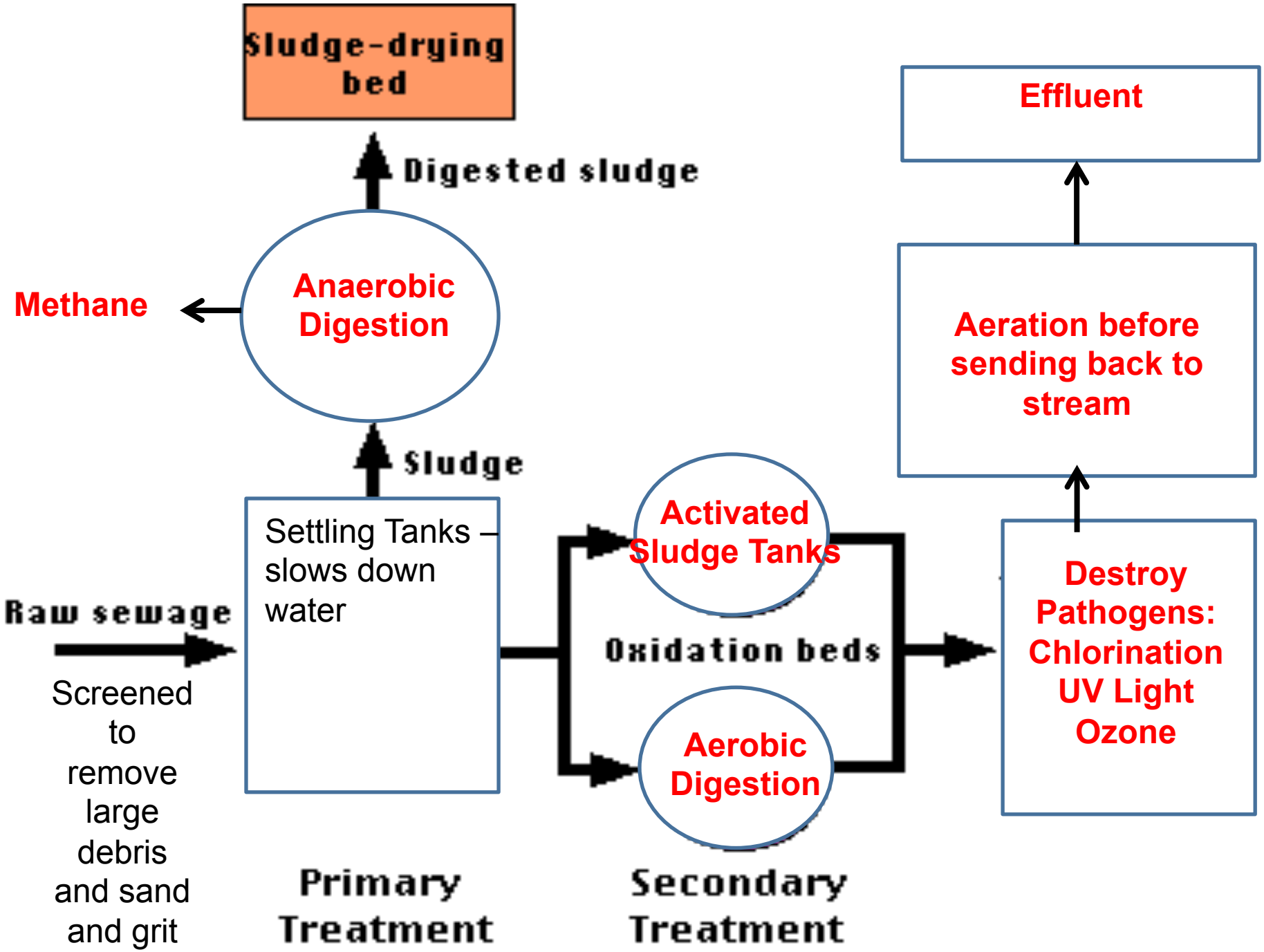
1) Slow water down - removes larger particles

2) Aerobic decomposition of organic material: natural bacteria decompose organic material IF enough dissolved oxygen is present in the water

3) Destroy pathogens (disease causing bacteria)

Biotic Transformations

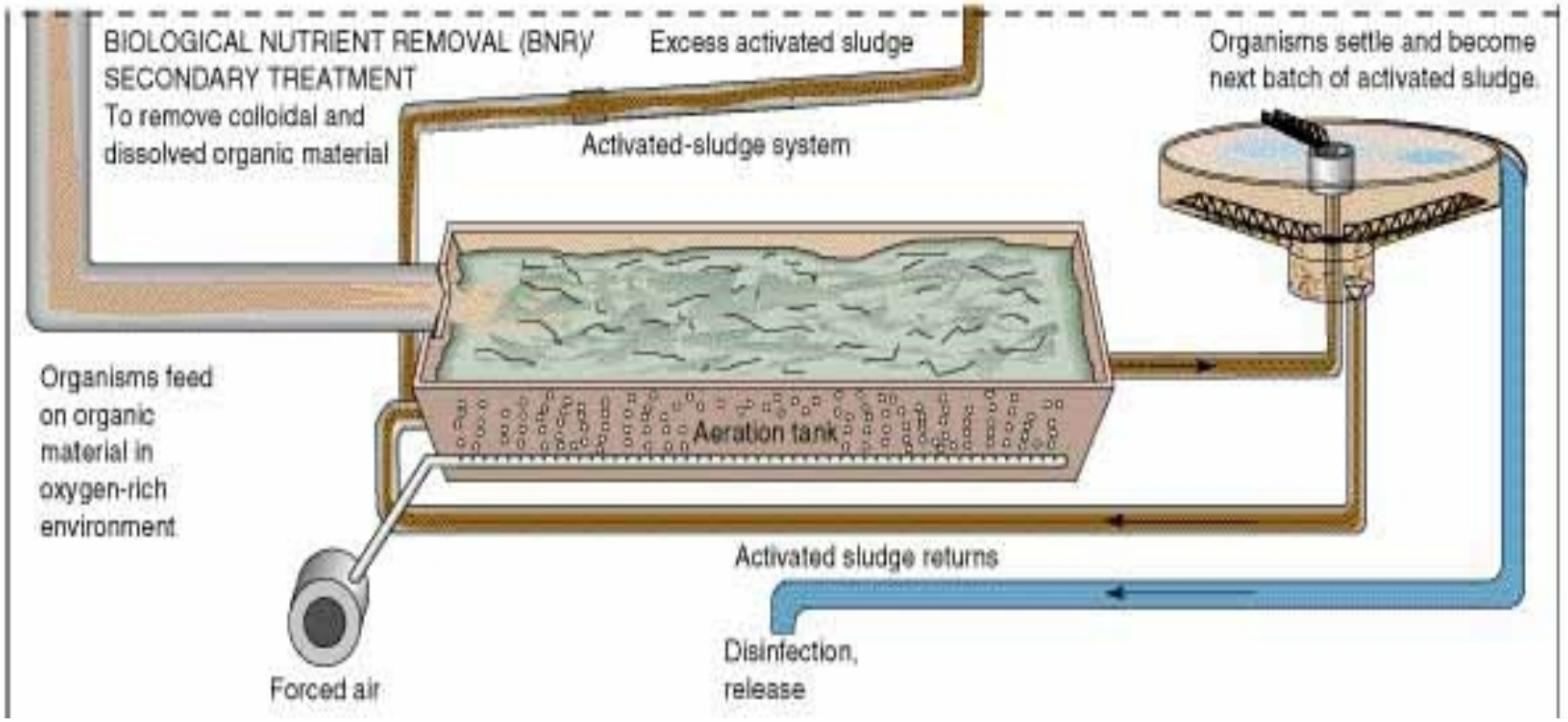
- Result of metabolic activity of microbes
- Aerobic and anaerobic biodegradation
- Reduces aqueous concentrations of contaminant
- Reduction of contaminant mass
- Most significant process resulting in reduction of contaminant mass in a system


Sludge-drying bed
Digested sludge
Effluent
Anaerobic Digestion
Methane
Aeration before sending back to stream
Sludge
Activated Sludge Tanks
Settling Tanks – slows down water
Raw sewage
Oxidation beds
Destroy Pathogens: Chlorination UV Light Ozone
Aerobic Digestion
Screened to remove large debris and sand and grit
Primary Treatment
Secondary Treatment

Wastewater Treatment

Secondary Treatment

- Secondary treatment is a biological process
- Utilizes bacteria and algae to metabolize organic matter in the wastewater



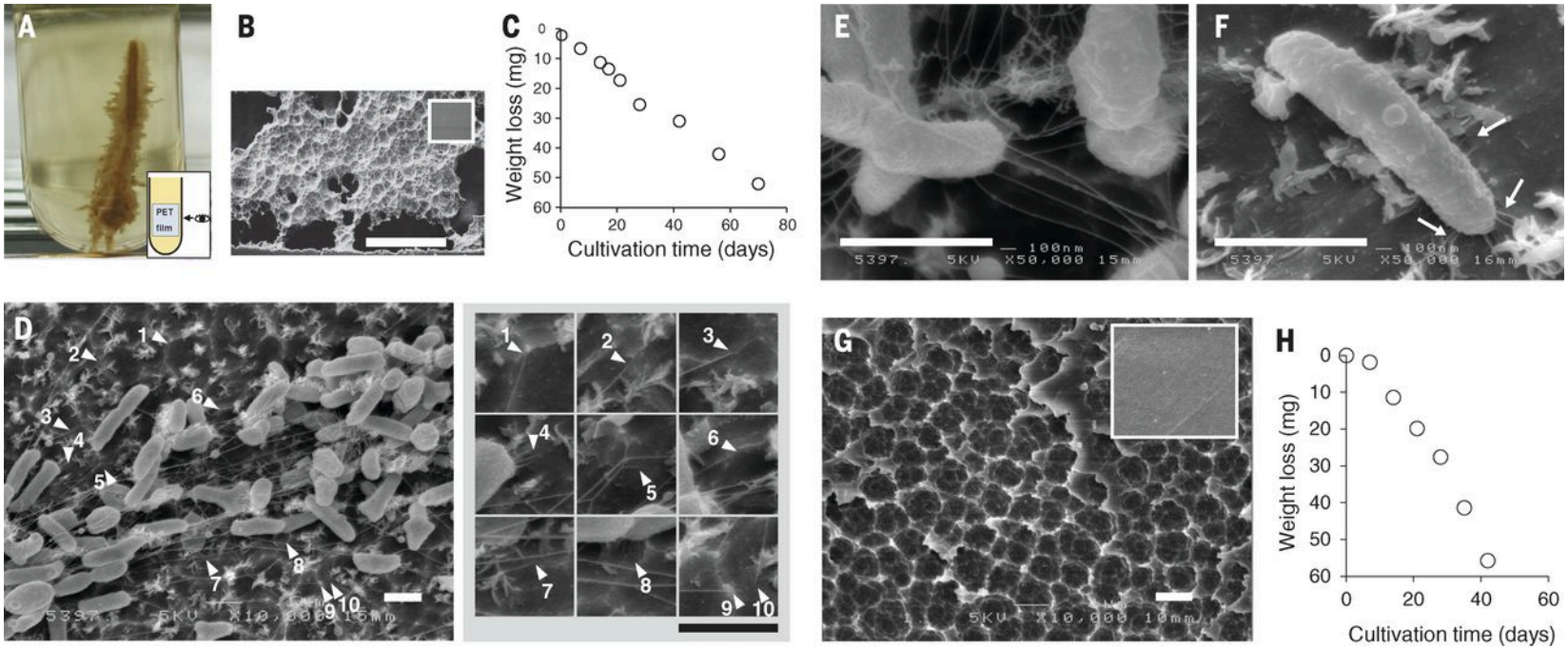
SHARE

REPORT

A bacterium that degrades and assimilates poly(ethylene terephthalate)

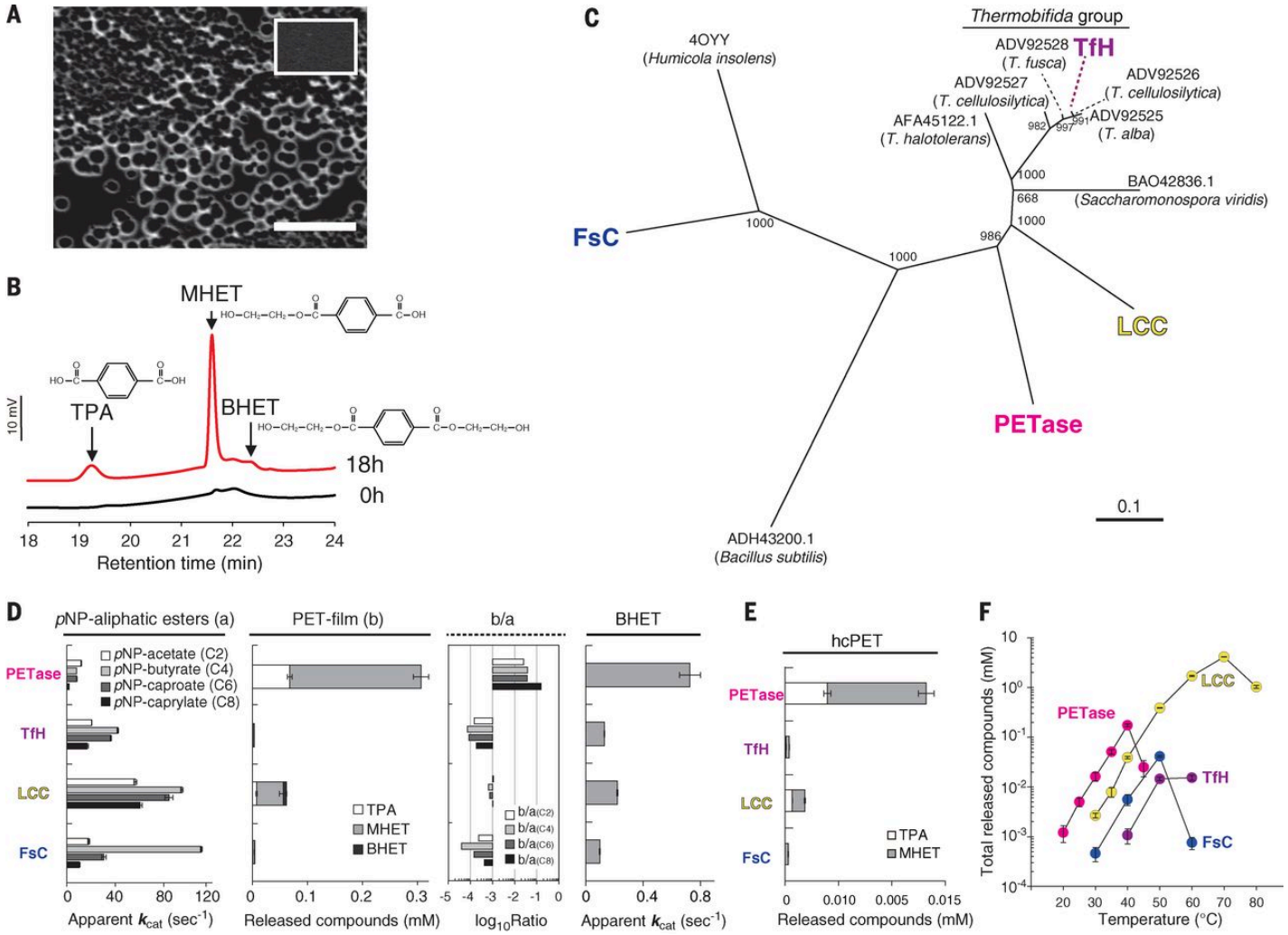
Shosuke Yoshida[1,2,*], Kazumi Hiraga[1], Toshihiko Takehana[3], Ikuo Taniguchi[4], Hironao Yamaji[1], Yasuhito Maeda[5], Kiyotsuna Toyohara[5], Kenji Miyamoto[2,†], Yoshiharu Kimura[4], Kohei Oda[1,†]

Fig. 1 Microbial growth on PET. Microbial growth on PET. The degradation of PET film (60 mg, 20 × 15 × 0.2 mm) by microbial consortium no. 46 at 30°C is shown in (A) to (C). The MLE (modified lettuce and egg) medium (10 mL) was changed biweekly. (**A**) Growth of no. 46 on PET film after 20 days. (**B**) SEM image of degraded PET film after 70 days. The inset shows intact PET film. Scale bar, 0.5 mm. (**C**) Time course of PET film degradation by no. 46. PET film degradation by *Ideonella sakaiensis* 201-F6 at 30°C is shown in (D) to (H). The YSV (yeast extract–sodium carbonate–vitamins) medium was changed weekly. (**D** to **F**) SEM images of *I. sakaiensis* cells grown on PET film for 60 hours. Scale bars, 1 μm. Arrow heads in the left panel of (D) indicate contact points of cell appendages and the PET film surface. Magnifications are shown in the right panel. Arrows in (F) indicate appendages between the cell and the PET film surface. (**G**) SEM image of a degraded PET film surface after washing out adherent cells. The inset shows intact PET film. Scale bar, 1 μm. (**H**) Time course of PET film degradation by *I. sakaiensis*



Shosuke Yoshida et al. Science 2016;351:1196-1199

Fig. 2 ISF6_4831 protein is a PETase. ISF6_4831 protein is a PETase. Effects of PETase on PET film are shown in (A) and (B). (**A**) SEM image of the treated PET film surface. The inset shows intact PET film. Scale bar, 5 μm. (**B**) High-performance liquid chromatography spectrum of the products released from the PET film. (**C**) Unrooted phylogenetic tree of known PET hydrolytic enzymes. (**D**) Substrate specificity of four phylogenetically distinct PET hydrolytic enzymes (b/a indicates the ratio of the values in the middle-left panel to those in the leftmost panel). (**E**) Activity of the PET hydrolytic enzymes for highly crystallized PET (hcPET). (**F**) Effect of temperature on enzymatic PET film hydrolysis.



Shosuke Yoshida et al. Science 2016;351:1196-1199

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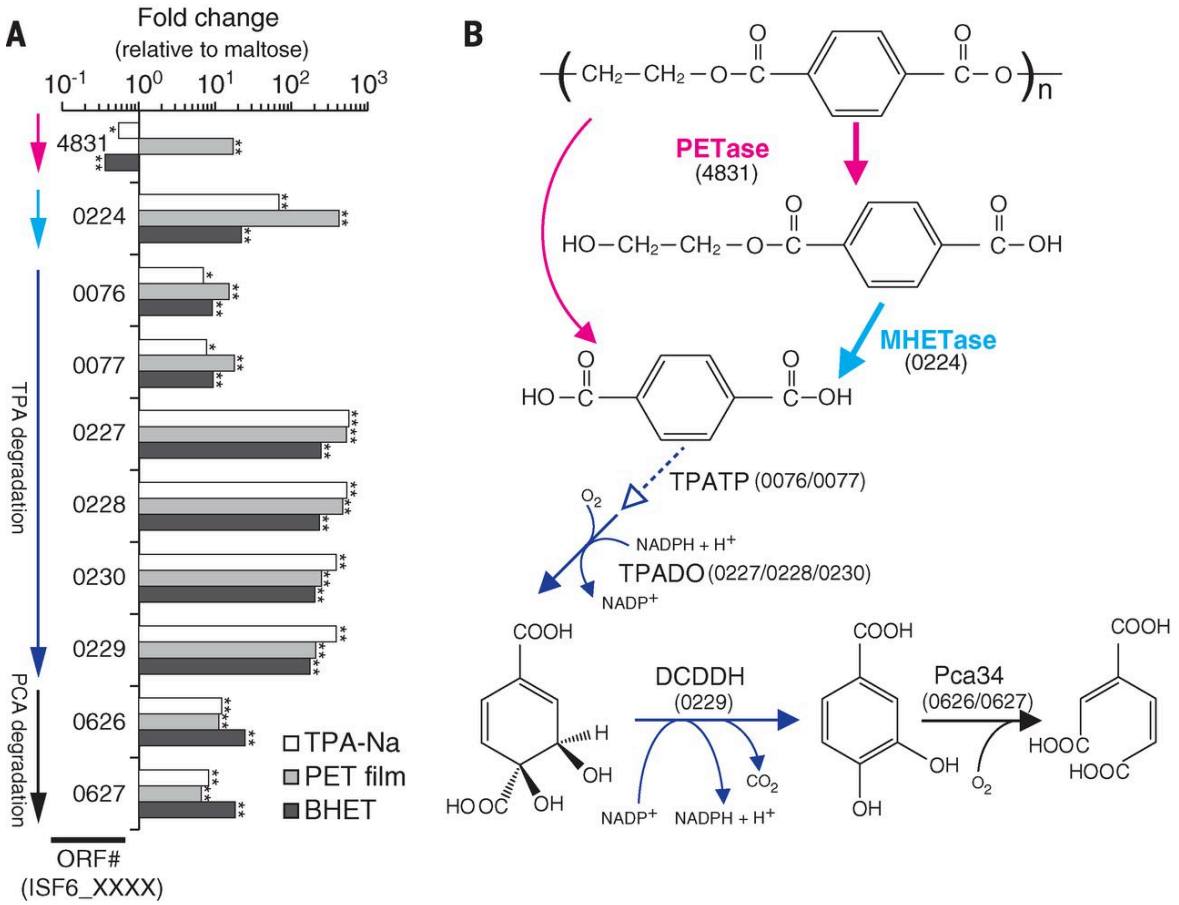
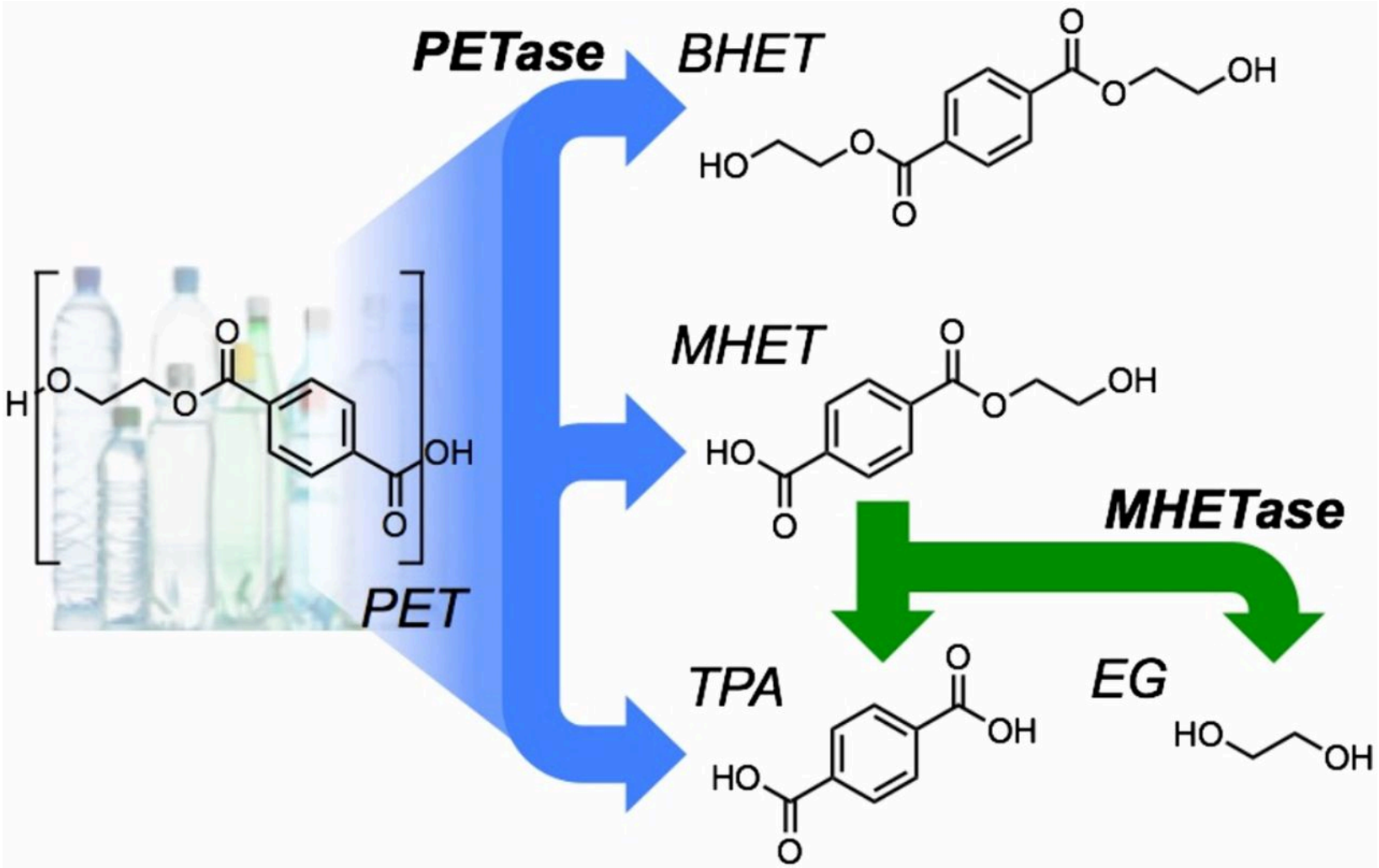
Fig. 3 PET metabolism by I. sakaiensis. (**A**) Transcript levels of selected genes when grown on TPA-Na, PET film, or BHET, relative to those when grown on maltose (**B**) Predicted *I. sakaiensis* PET degradation pathway. The cellular localization of PETase and MHETase was predicted first (supplementary text, section S1). Extracellular PETase hydrolyzes PET to produce MHET (the major product) and TPA. MHETase, a predicted lipoprotein, hydrolyzes MHET to TPA and EG. TPA is incorporated through the TPA transporter (TPATP) (*17*) and catabolized by TPA 1,2-dioxygenase (TPADO), followed by 1,2-dihydroxy-3,5-cyclohexadiene-1,4-dicarboxylate dehydrogenase (DCDDH). The resultant PCA is ring-cleaved by PCA 3,4-dioxygenase (Pca34). The predicted TPA degradation pathway is further described in the supplementary text (section S2).



- PETase (PET-digesting enzyme) converts PET to mono(2-hydroxyethyl) terephthalic acid (MHET), with trace amounts of terephthalic acid (TPA) and bis(2-hydroxyethyl)-TPA as secondary products.
- A second enzyme, MHETase (MHET-digesting enzyme), further converts MHET into the two monomers, TPA and ethylene glycol (EG).

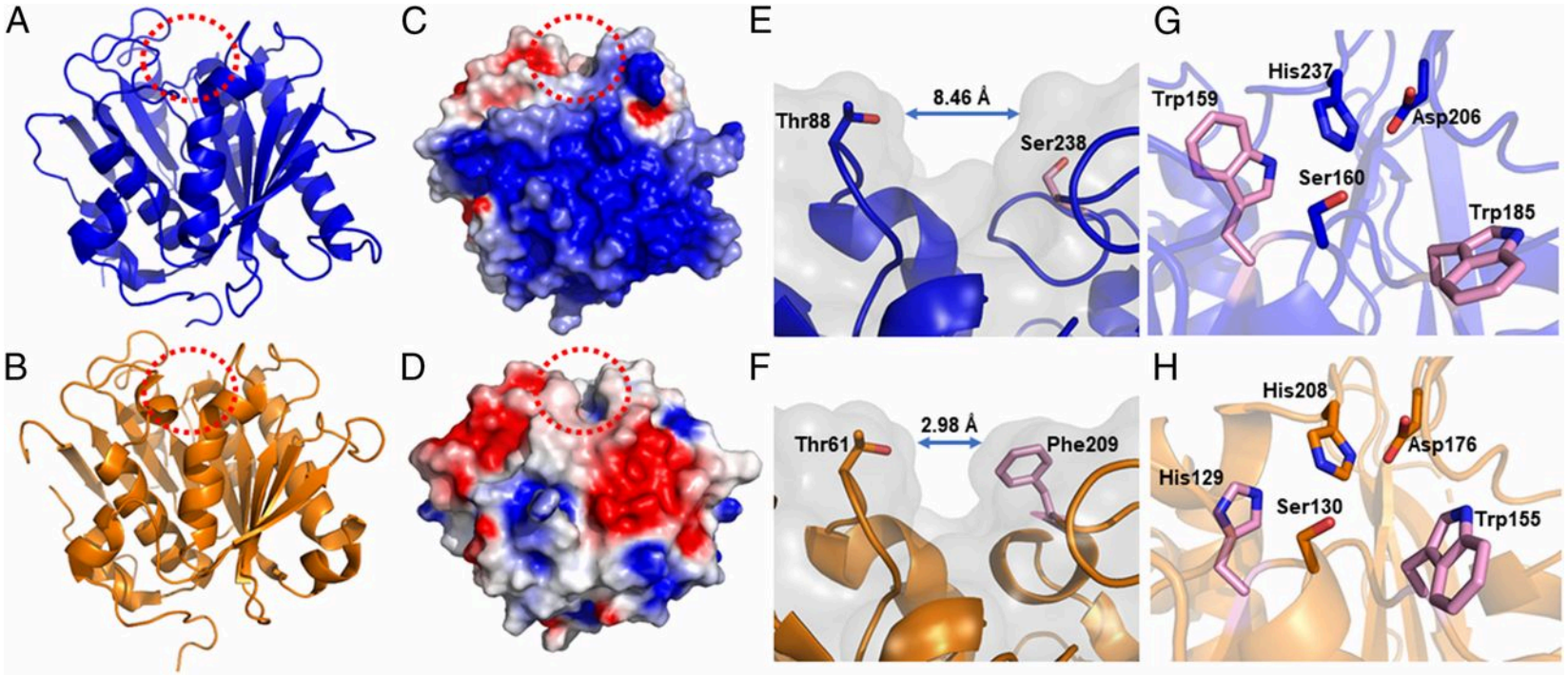
Shosuke Yoshida et al. Science 2016;351:1196-1199

PETase catalyzes the depolymerization of PET to bis(2-hydroxyethyl)-TPA (BHET), MHET, and TPA. MHETase converts MHET to TPA and EG.



Harry P. Austin et al. PNAS 2018;115:19:E4350-E4357

Structure of PETase.



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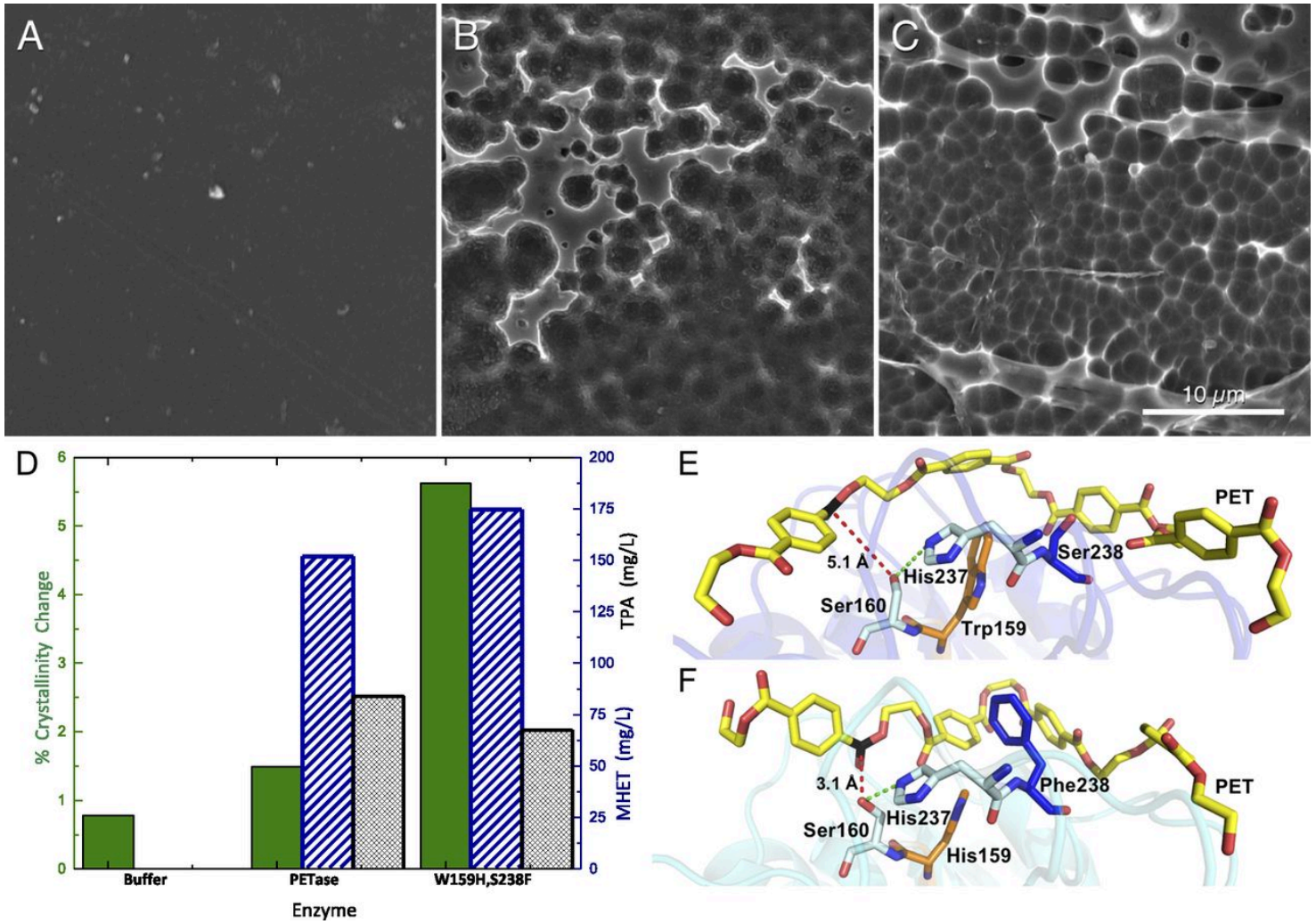
Comparison of PETase and the engineered enzyme S238F/W159H with PET. (A) Buffer-only control of PET coupon.



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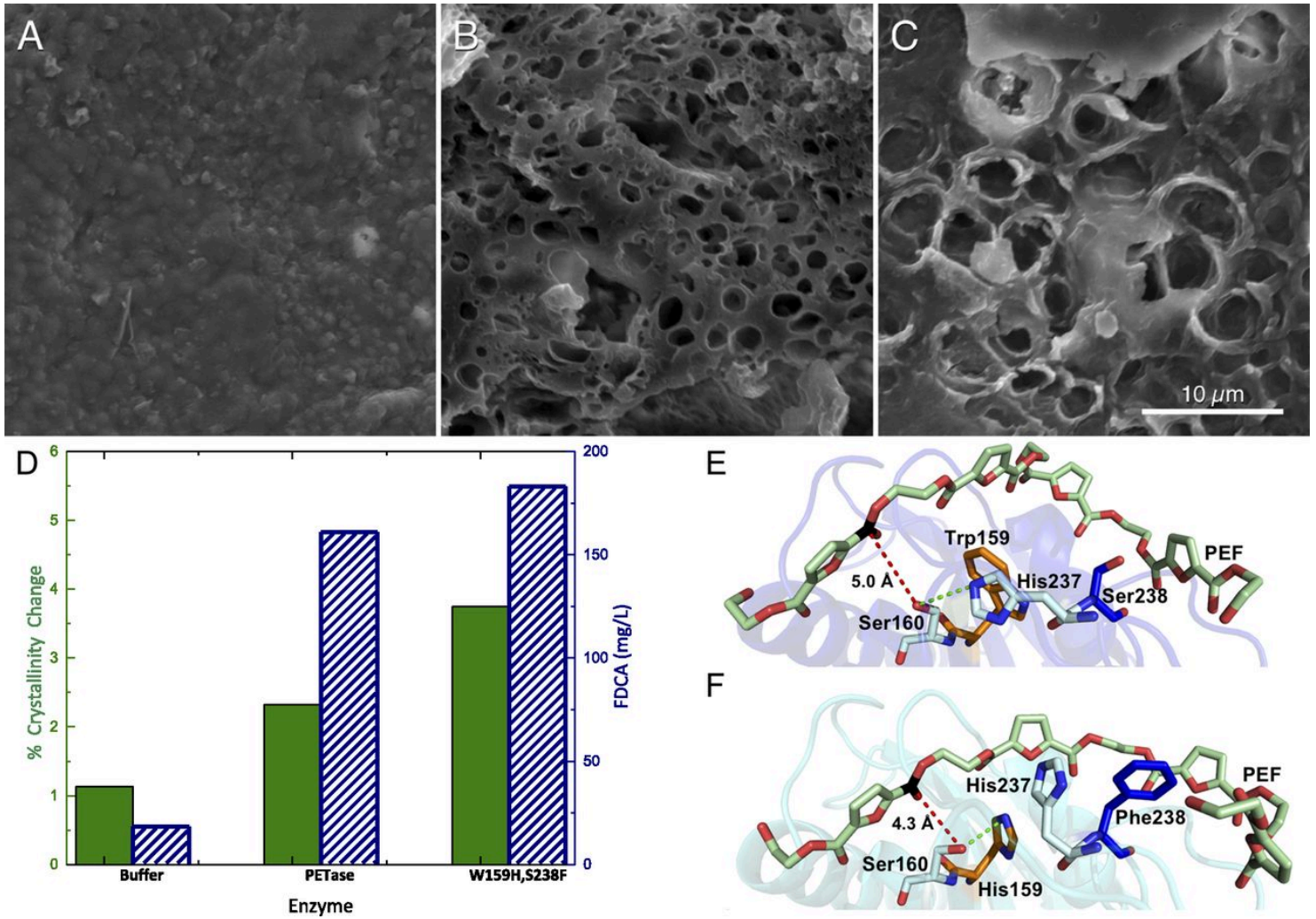
Comparison of PETase and the engineered enzyme S238F/W159H with PEF. (A) Buffer-only control of PEF coupon.



Harry P. Austin et al. PNAS 2018;115:19:E4350-E4357

3 Biotech

December 2016, 6:200 | Cite as

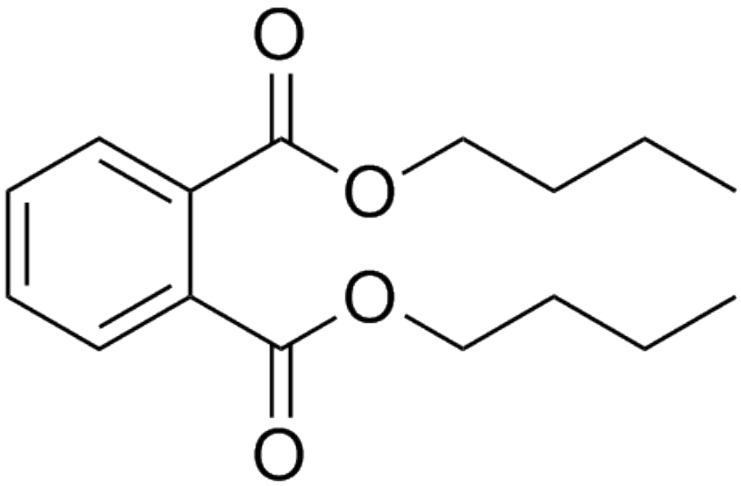
Biodegradation of endocrine disruptor dibutyl phthalate (DBP) by a newly isolated *Methylobacillus* sp. V29b and the DBP degradation pathway

Authors Authors and affiliations

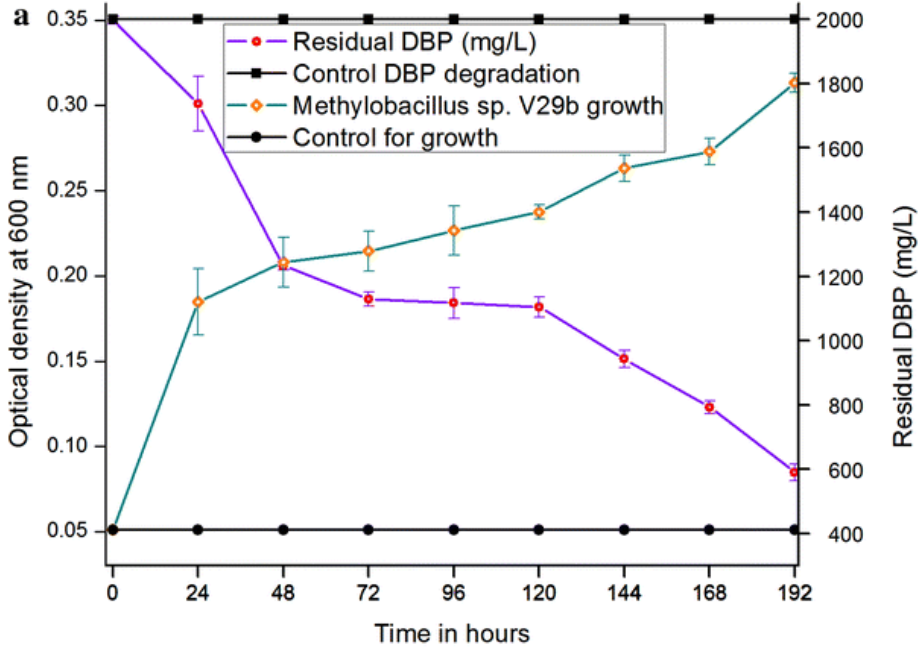
Vinay Kumar ✉, S. S. Maitra

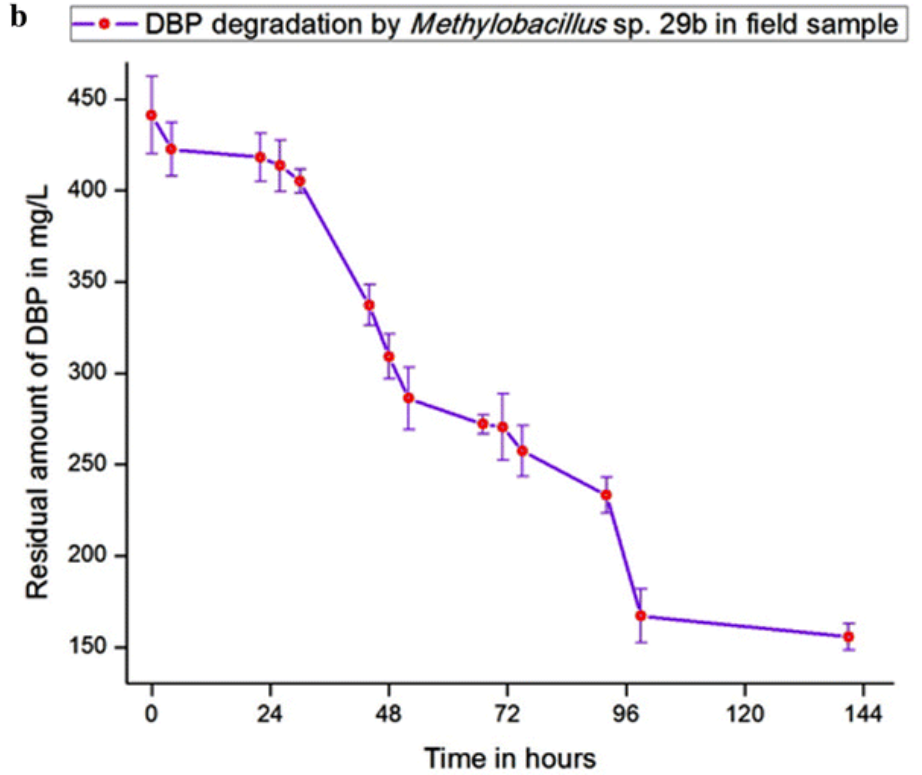
a) Degradation of DBP by *Methylobacillus* sp. V29b in MSM.

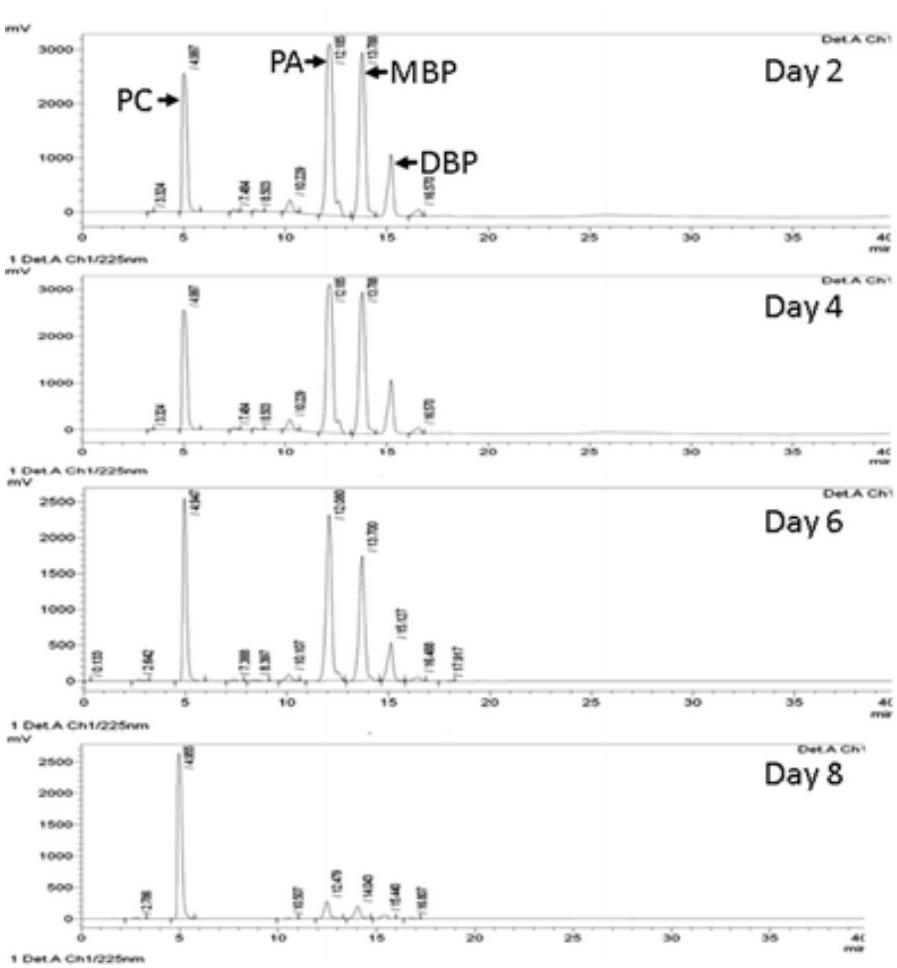
b) Degradation of DBP by *Methylobacillus* sp. V29b in the sample contaminated with DBP



DBP







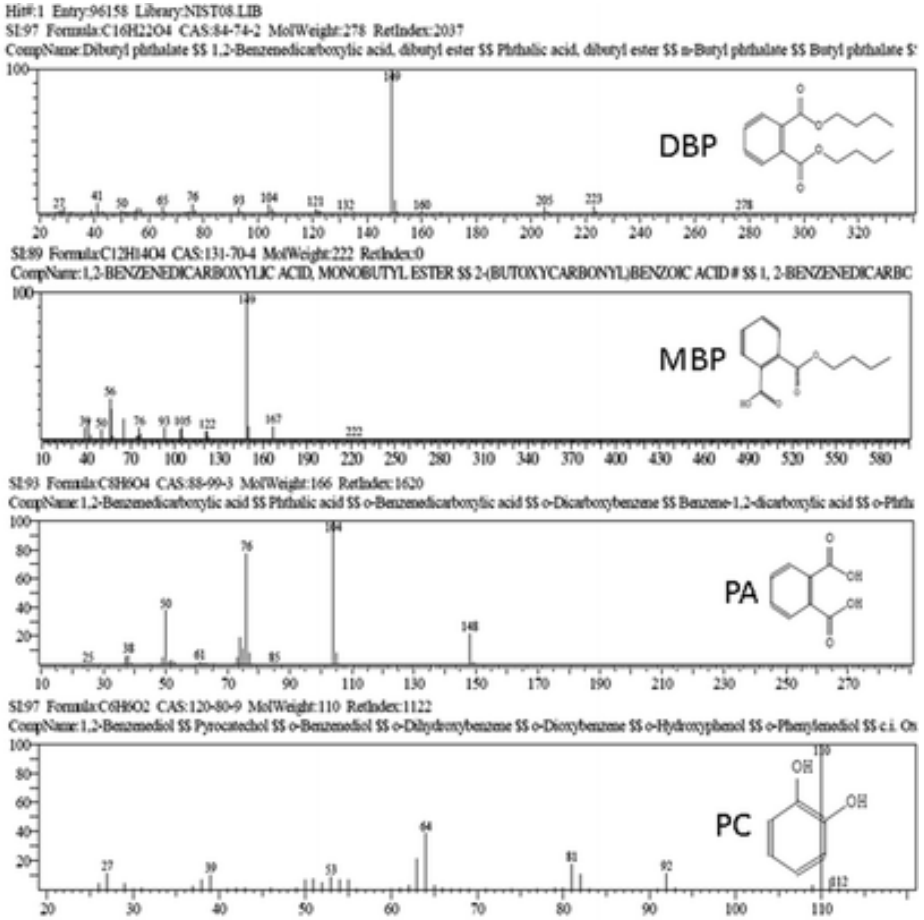


Fig. 5
DBP degradation metabolic intermediates identified by GC–MS. **a** HPLC chromatogram of the metabolic intermediates. **b** Structure and *m*/*z* of the identified metabolic intermediates. *DBP* dibutyl phthalate, *MBP* monobutyl phthalate, *PA* phthalic acid, *PC* pyrocatechol

A proposed biochemical pathway for DBP degradation by *Methylobacillus* sp. V29b. *DBP* dibutyl phthalate, *MBP* monobutyl phthalate, *PA* phthalic acid, *PC* pyrocatechol