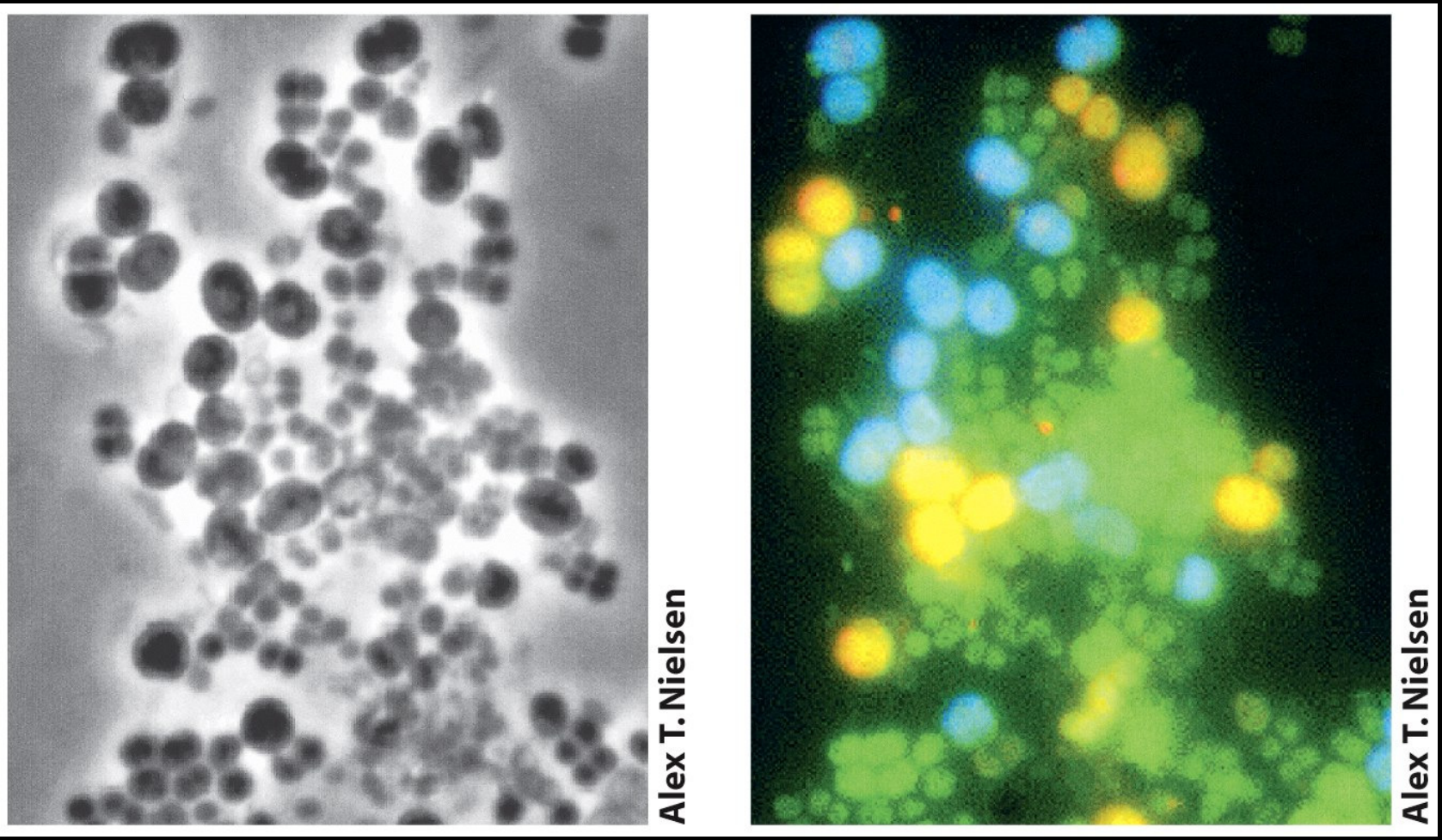
# Environmental Microbiology

## „Population analyses I“




Bettina Siebers

# Microbial ecology

- **Microbial ecology** deals with how microorganisms interact with one another and their biotic and abiotic environment.
- **Bacterial diversity**
  - Investigation of microbial ecology requires a system for identification, differentiation and organisation of microorganisms.
- **Bacterial numbers**
  - Methods for determining cell numbers
- **Spatial arrangement of bacteria**.
  - Methods for localising specific bacteria
- **Bacterial activity**
  - Gene expression and the role of the bacteria in the environment.
    - Biogeochemical cycles

# Microbial Diversity

Metabolic options for obtaining energy.

**„ Life styles“**

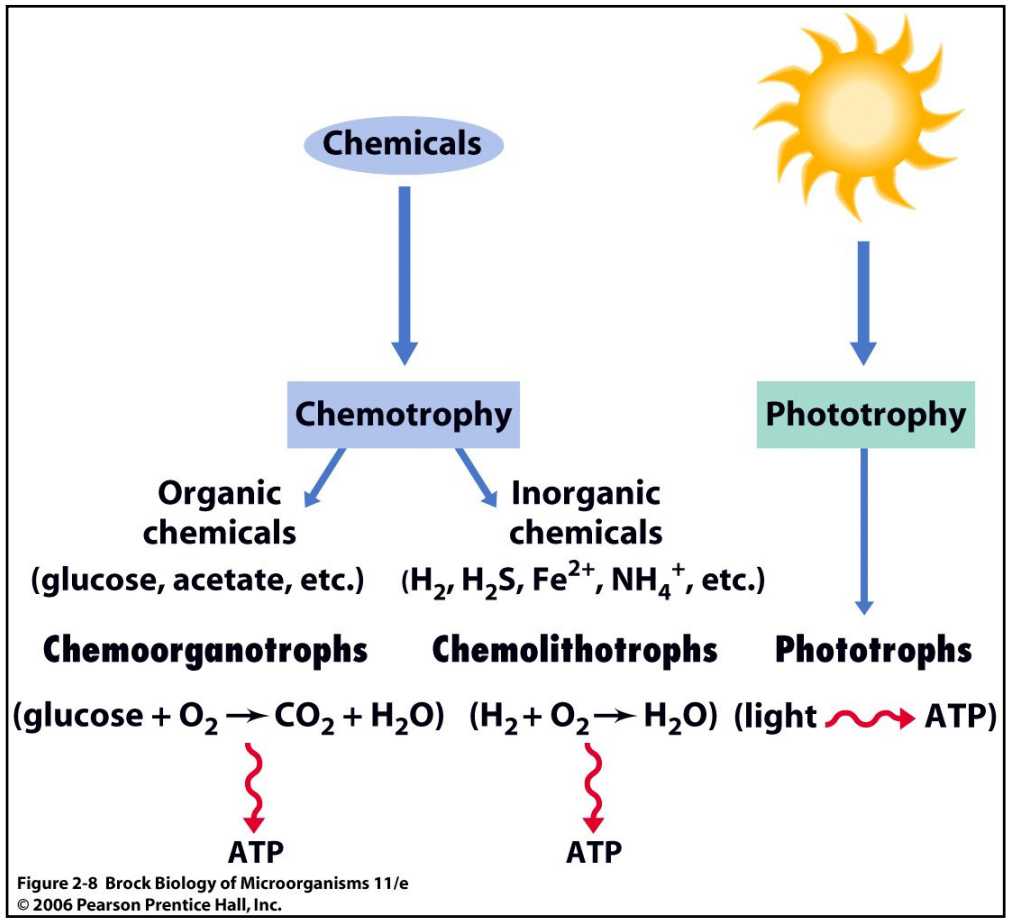
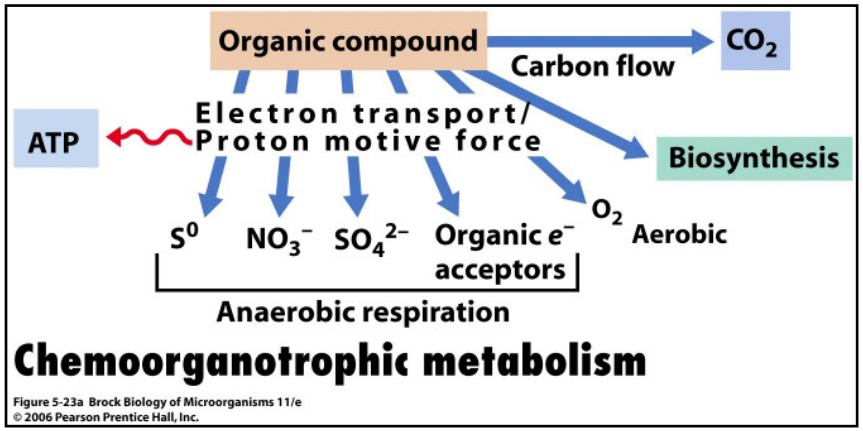



Figure 2-8 Brock Biology of Microorganisms 11/e

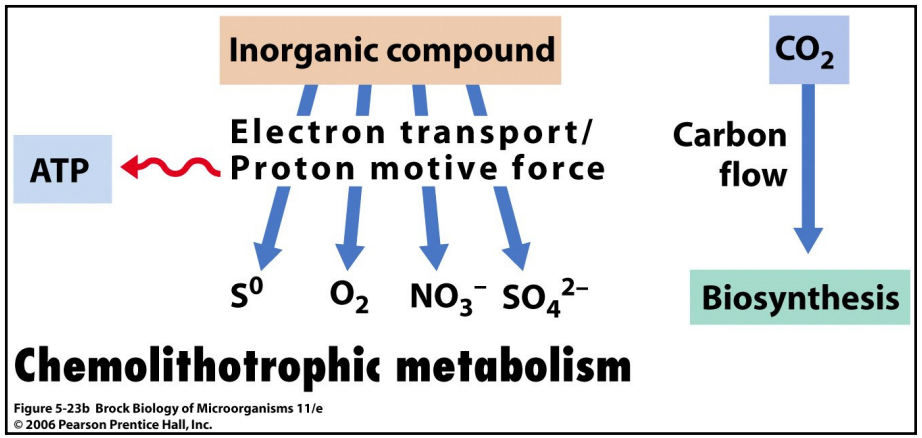
# Chemotrophic Metabolism

## Chemoorganotrophes




**Organic** substrates as energy- i.e. electron source.

## Chemolithotrophes




**Inorganic** substrates as energy- i.e. electron source.

## Differentiation according to Carbon source

**Heterotrophic**: Organic compounds as C-source

**Autotrophic**: Inorganic compounds; $CO_2$ as unique C-source.

# Chemolitho/autotrophy

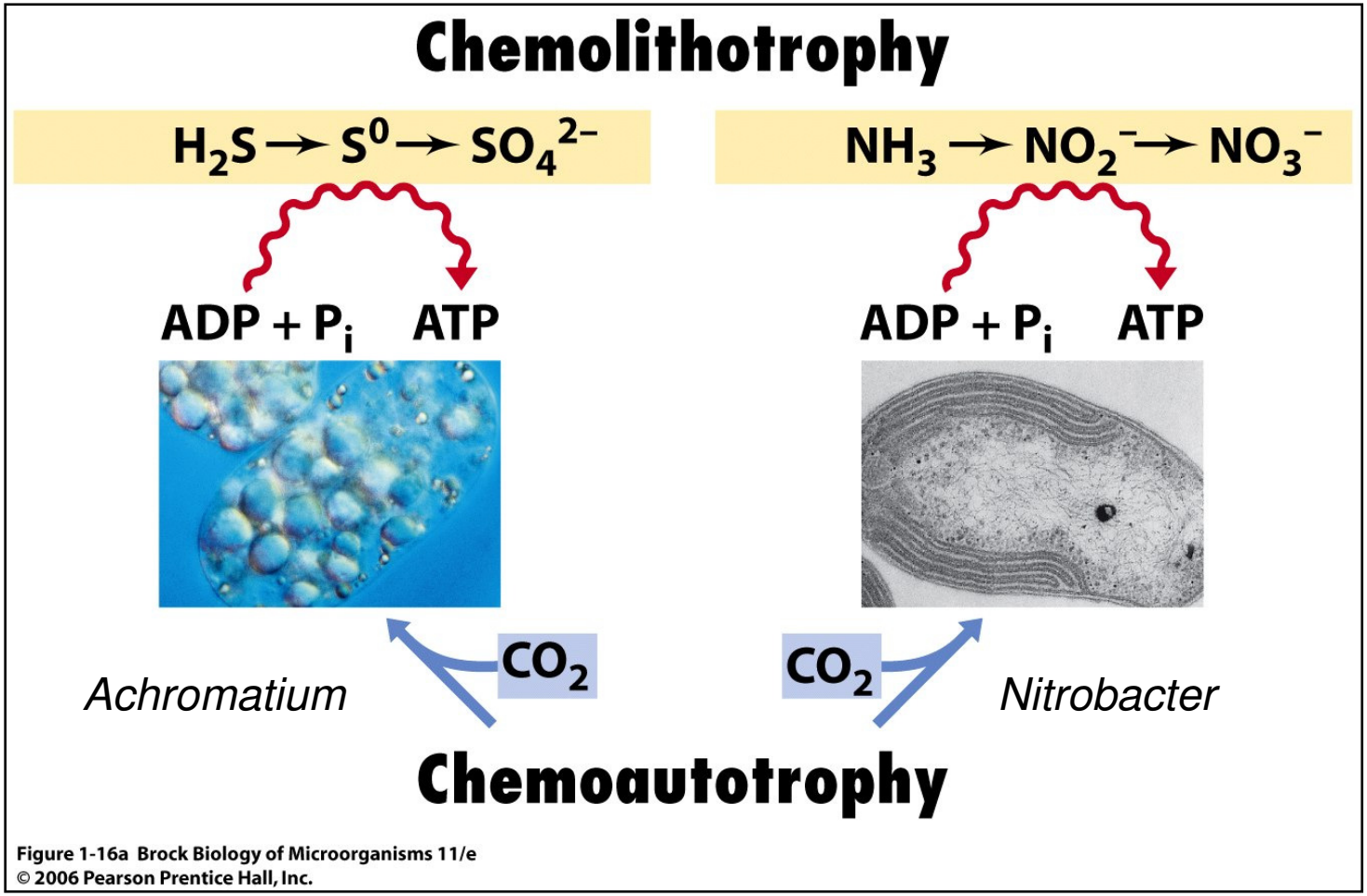



Figure 1-16a Brock Biology of Microorganisms 11/e

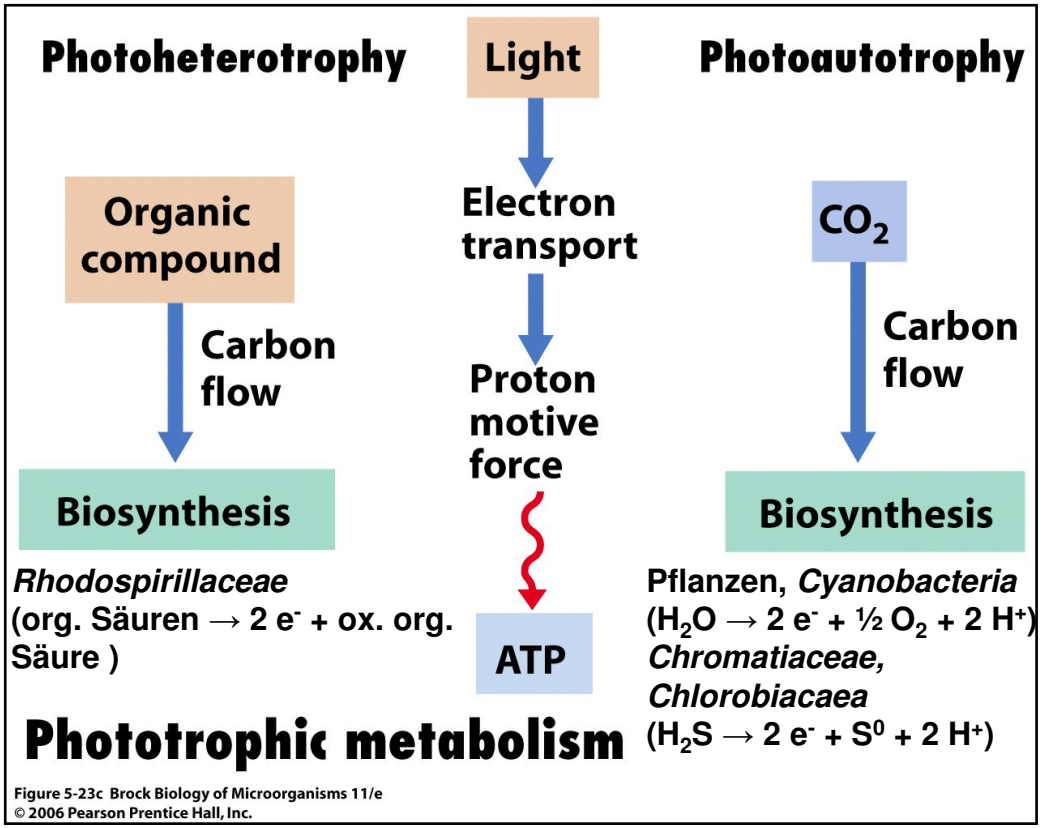
# Phototrophic Metabolism

**Photoorganotrophic**

**Organic** substrates as energy- i.e. electron source.

**Photolithotrophic**

**Inorganic** substrates as energy- i.e. electron source.




**Energetics and carbon flow.**

# Environmental Effects on Microbial Growth

Temperature
pH
Osmolarity
Oxygen

# Temperature

- Temperature is a major environmental factor controlling microbial growth.
- The **cardinal temperatures** are the minimum, optimum, and maximum temperatures at which each organism grows.

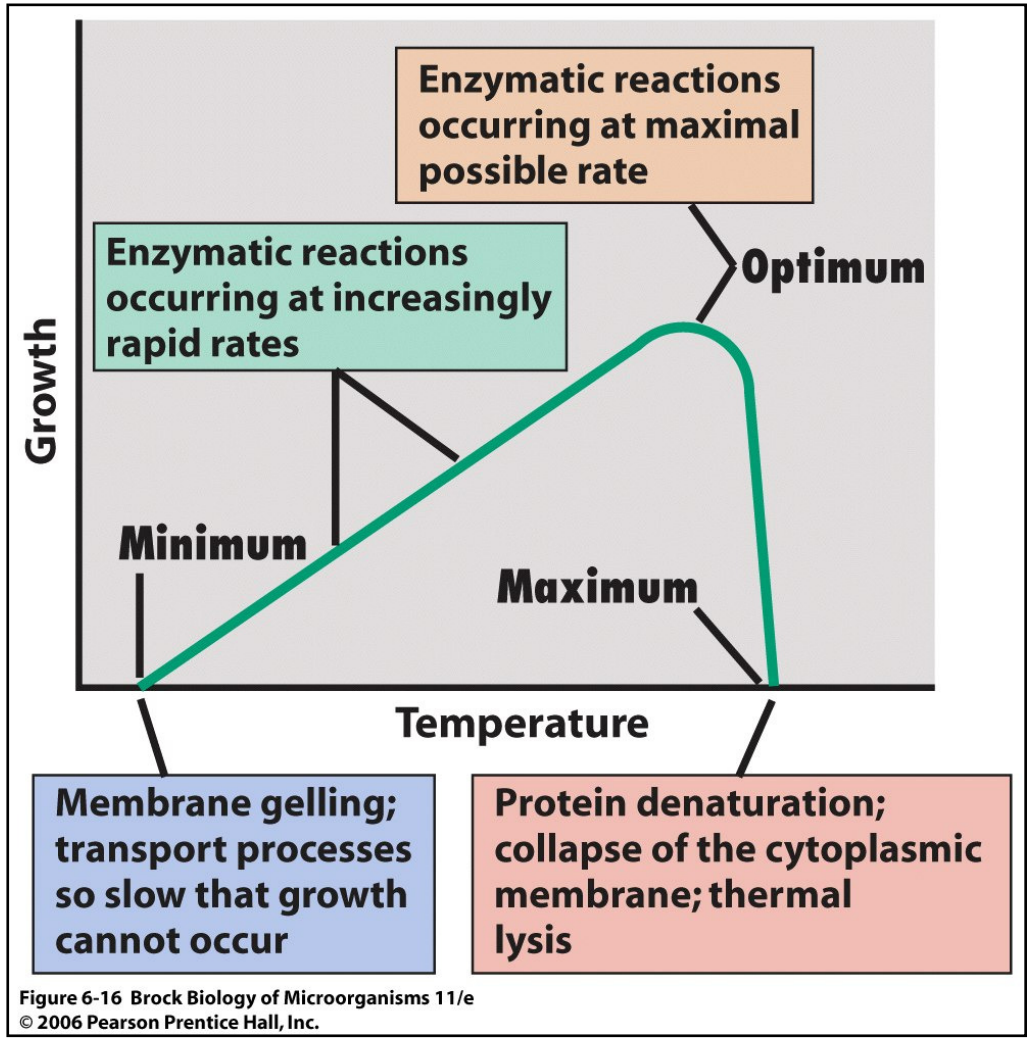



Figure 6-16 Brock Biology of Microorganisms 11/e

# Temperature Classes

- Microorganisms can be grouped by the temperature ranges they require.

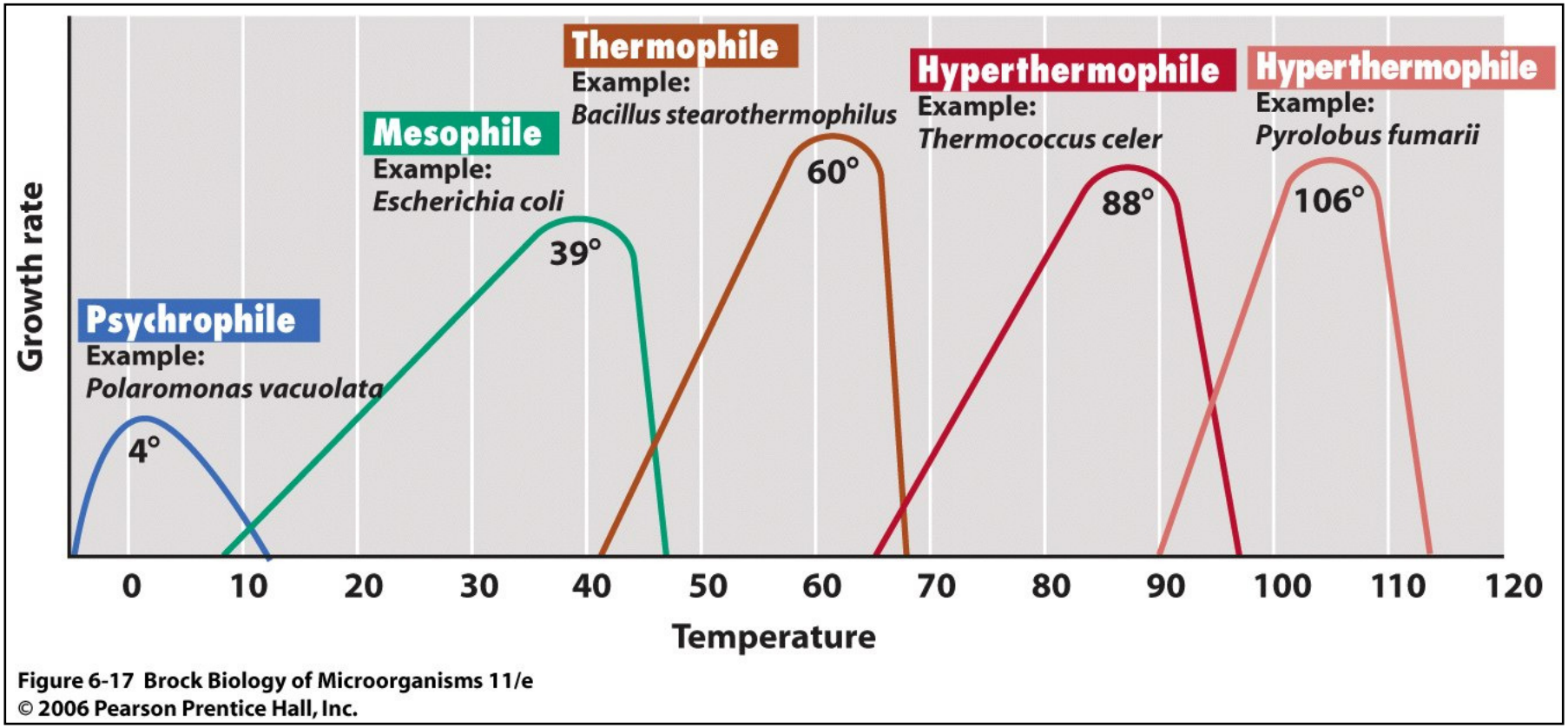



Figure 6-17 Brock Biology of Microorganisms 11/e

# Temperature Classes

- **Mesophiles**, which have midrange temperature optima, a re found in warm-blooded animals and in terrestrial and aquatic environments in temperate and tropical latitudes.
- **Extremophiles** have evolved to grow optimally under very hot or very cold conditions.

# pH

- The acidity or alkalinity of an environment can greatly affect microbial growth.

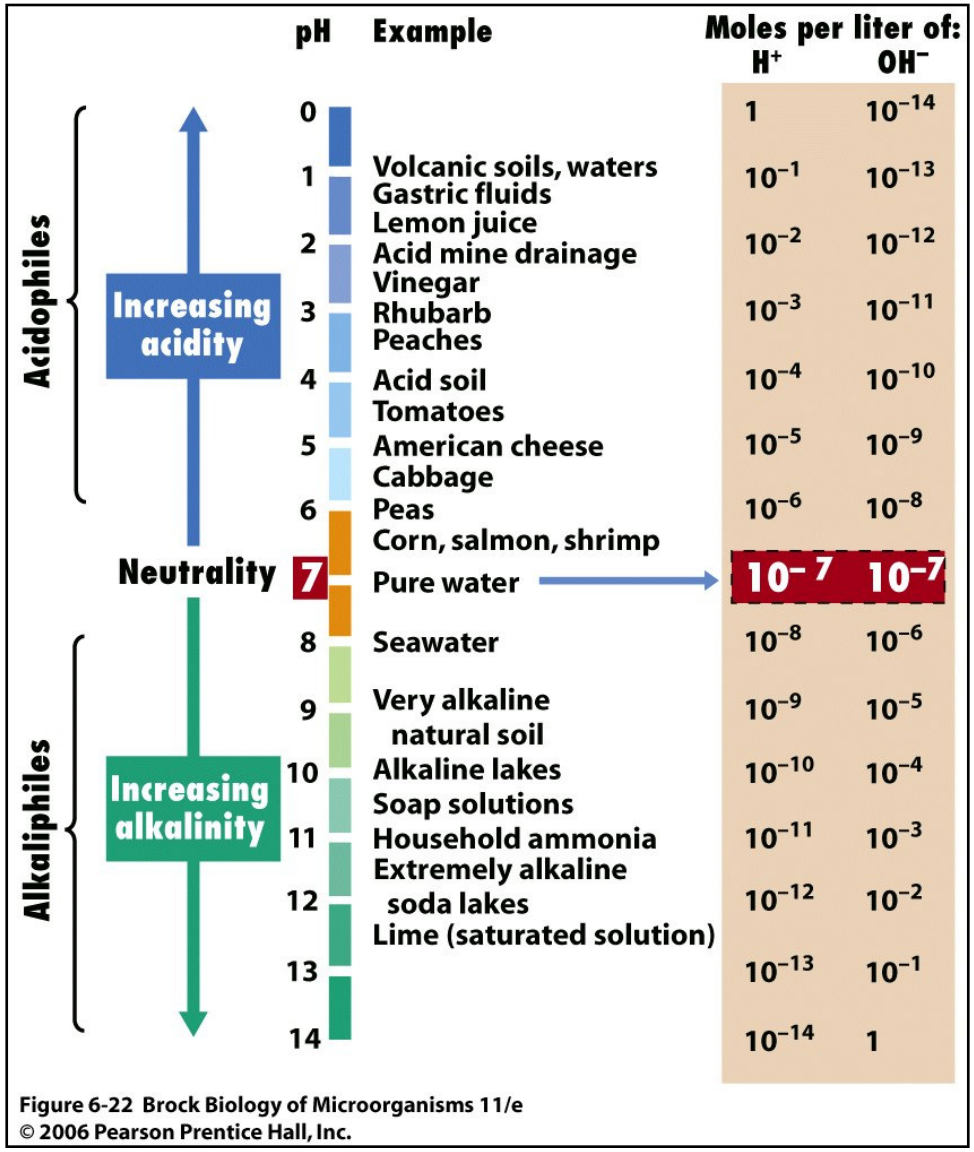



Figure 6-22 Brock Biology of Microorganisms 11/e

# pH

- Some organisms have evolved to grow best at low or high pH, but **most organisms grow best between pH 6 and 8**.
- The **internal pH** of a cell must stay relatively **close to neutral** even though the external pH is highly acidic or basic.
- Organisms that grow best at low pH are called **acidophiles**; those that grow best at high pH are called **alkaliphiles**

# Osmolarity

- Water is the solvent of life.
- Water availability is an important factor affecting the growth.
- Water activity ($a_w$) is the ratio of the vapor pressure of the air in equilibrium with a substance or solution to the vapor pressure of pure water.

**Table 6.2 Water activity of several substances**

| Water activity ($a_w$) | Material | Example organisms[a] |
|---|---|---|
| 1.000 | Pure water | *Caulobacter, Spirillum* |
| 0.995 | Human blood | *Streptococcus, Escherichia* |
| 0.980 | Seawater | *Pseudomonas, Vibrio* |
| 0.950 | Bread | Most gram-positive rods |
| 0.900 | Maple syrup, ham | Gram-positive cocci such as *Staphylococcus* |
| 0.850 | Salami | *Saccharomyces rouxii* (yeast) |
| 0.800 | Fruit cake, jams | *Saccharomyces bailii, Penicillium* (fungus) |
| 0.750 | Salt lakes, salted fish | *Halobacterium, Halococcus* |
| 0.700 | Cereals, candy, dried fruit | *Xeromyces bisporus* and other xerophilic fungi |

[a] Selected examples of prokaryotes or fungi capable of growth in culture media adjusted to the stated water activity.

Table 6-2 Brock Biology of Microorganisms 11/e

# Osmolarity

- Some microorganisms (**halophiles**) have evolved to grow best at reduced water potential, and some (**extreme halophiles**) even require high levels of salts for growth.
- **Halotolerant** organisms can tolerate some reduction in the water activity of their environment but generally grow best in the absence of the added solute.

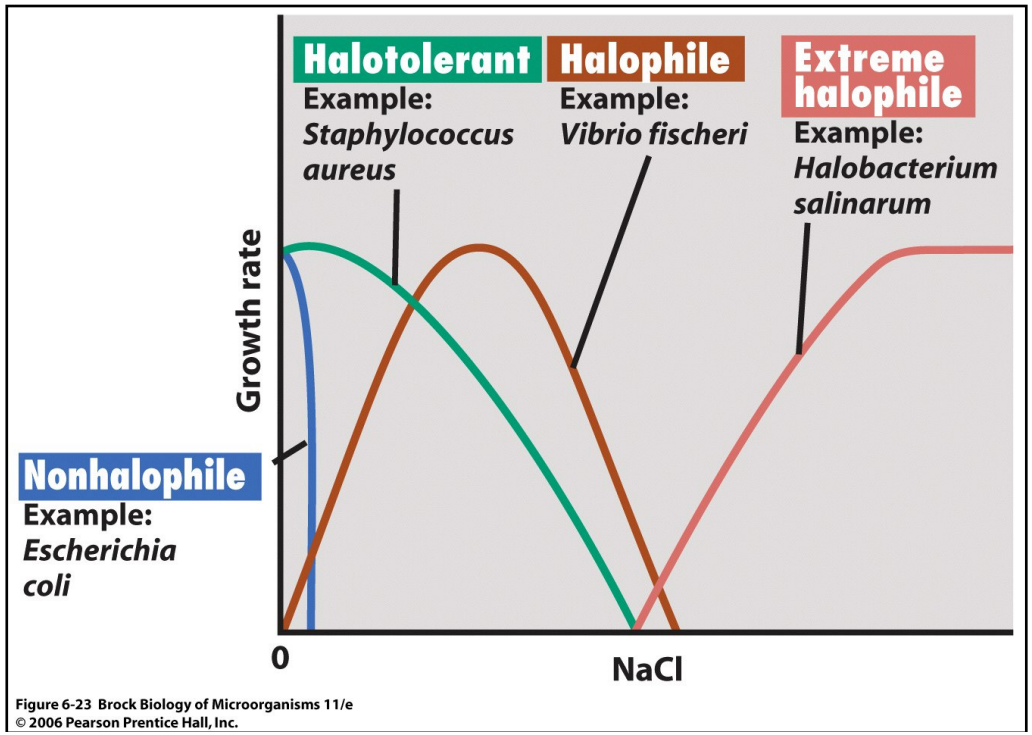


Figure 6-23 Brock Biology of Microorganisms 11/e

# Oxygen

- **Aerobes** require oxygen to live, whereas **anaerobes** do not and may even be killed by oxygen.
- **Facultative** organisms can live with or without oxygen. **Aerotolerant anaerobes** can tolerate oxygen and grow in its presence even though they cannot use it.
- **Microaerophiles** are aerobes that can use oxygen only when it is present at levels reduced from that in air.

# Oxygen

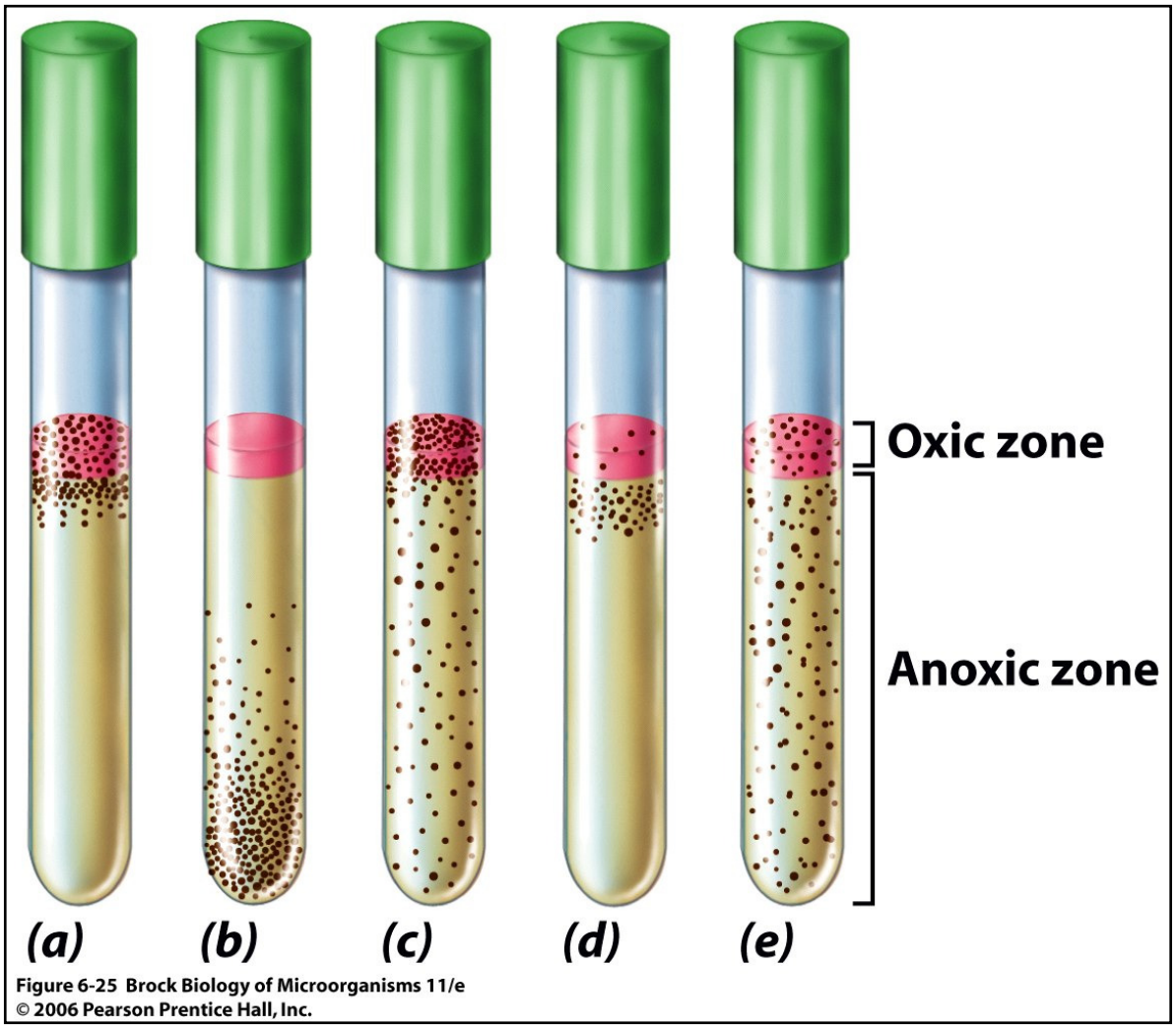



Figure 6-25 Brock Biology of Microorganisms 11/e


a) Obligat aerobe Org.
b) Anaerobe Org.
c) Fakultativ anaerobe Org.
d) Mikroaerophile Org.
e) Aerotolerante Org.

# Oxygen

- Relationships of some microorganisms to oxygen.

**Table 6.4 Oxygen relationships of microorganisms**

| Group | Relationship to $O_2$ | Type of metabolism | Example[a] | Habitat[b] |
|---|---|---|---|---|
| **Aerobes** | | | | |
| Obligate | Required | Aerobic respiration | *Micrococcus luteus* (B) | Skin, dust |
| Facultative | Not required, but growth better with $O_2$ | Aerobic respiration, anaerobic respiration, fermentation | *Escherichia coli* (B) | Mammalian large intestine |
| Microaerophilic | Required but at levels lower than atmospheric | Aerobic respiration | *Spirillum volutans* (B) | Lake water |
| **Anaerobes** | | | | |
| Aerotolerant | Not required, and growth no better when $O_2$ present | Fermentation | *Streptococcus pyogenes* (B) | Upper respiratory tract |
| Obligate | Harmful or lethal | Fermentation or anaerobic respiration | *Methanobacterium formicicum* (A) | Sewage sludge digestors, anoxic lake sediments |

[a] Letters in parentheses indicate phylogenetic status (B, *Bacteria*; A, *Archaea*). Representatives of either domain of prokaryotes are known in each category. Most eukaryotes are obligate aerobes, but facultative aerobes (for example, yeast) and obligate anaerobes (for example, certain protozoa and fungi) are known.

[b] Listed are typical habitats of the example organism.

Table 6-4 Brock Biology of Microorganisms 11/e

# Microbial Ecosystems

## Populations, Guilds, and Communities

# Microbial Ecosystems

- Microbial communities consist of populations of cells of various species.
- **Guilds** are populations of metabolically related organisms.
- The **Figure** shows an example of microbial community structure in a lake **ecosystem** (a community of organisms and their natural environment).

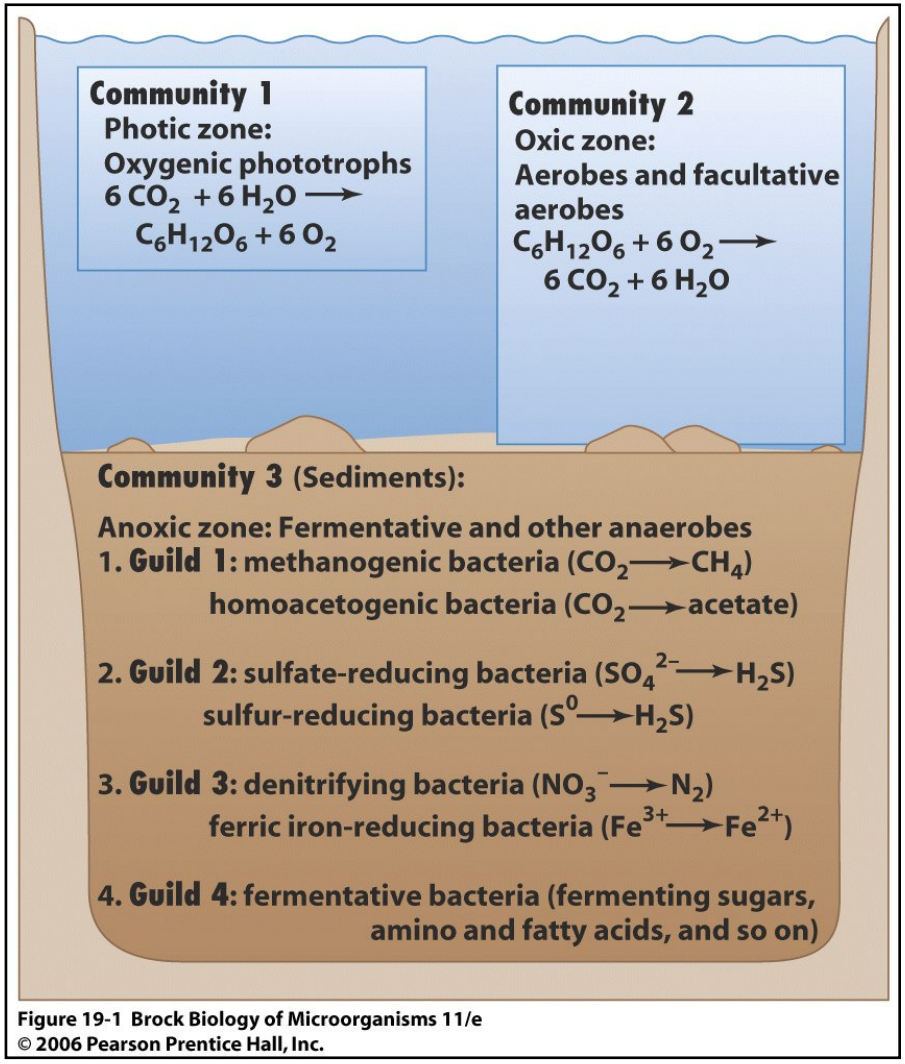



Figure 19-1 Brock Biology of Microorganisms 11/e

# Biogeochemistry

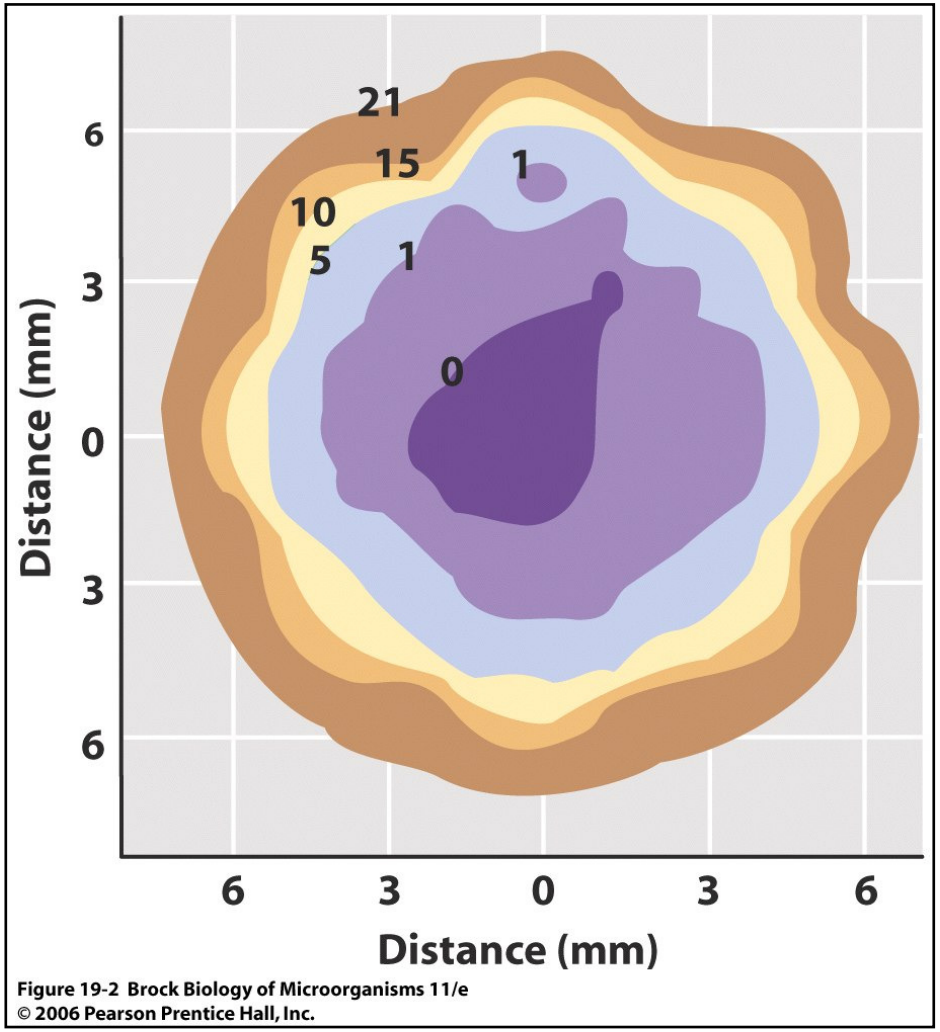
- Microorganisms play major roles in energy transformations and biogeochemical processes that result in the recycling of elements essential to living systems.
- The study of these chemical transformations is called **biogeochemistry**.

# Environments and Microenvironments

- Microorganisms are very small, and their habitats are likewise small. The **microenvironment** is the place where the microorganism actually lives.
- For a typical bacterium 3 µm the distance of 3 mm is huge (equivalent to that which a human experiences 2 km).

# Microenvironment

- For example, the outer zones of a small soil particle may be fully **oxic**, meaning that $O_2$ is present, whereas the center, only a very short distance away, can remain completely **anoxic** ($O_2$-free).




$O_2$ concentration (in %, air is 21%)

# Microenvironment

- The growth of microorganisms depends on the resources (nutrients) available and on the growth conditions.

**Table 19.1 Major resources and conditions that govern microbial growth in nature**

| Resources | Conditions |
|---|---|
| Carbon (organic, $CO_2$) | Temperature: cold → warm → hot |
| Nitrogen (organic, inorganic) | Water potential: dry → moist → wet |
| Other macronutrients (S, P, K, Mg) | pH: 0 → 7 → 14 |
| Micronutrients (Fe, Mn, Co, Cu, Zn, Mn, Ni) | $O_2$: oxic → microoxic → anoxic |
| $O_2$ and other electron acceptors ($NO_3^-$, $SO_4^{2-}$, $Fe^{3+}$, etc.) | Light: bright light → dim light → dark |
| Inorganic electron donors ($H_2$, $H_2S$, $Fe^{2+}$, $NH_4^+$, $NO_2^-$, etc.) | Osmotic conditions: freshwater → marine → hypersaline |

Table 19-1 Brock Biology of Microorganisms 11/e

# Microbial Ecosystems

- Microorganisms in nature often live a **feast-or-famine** (Festgelage-Hungersnot) existence such that only the best-adapted species thrive in a given niche.
- **Cooperation** among microorganisms is also important in many microbial interrelationships.

# Some examples

# Microbial Growth on Surfaces and Biofilms

- Biofilms can lead to the destruction of inert and living surfaces as a result of the products excreted by the bacterial cells.
- Biofilm formation is a complex process involving cell-to-cell communication.

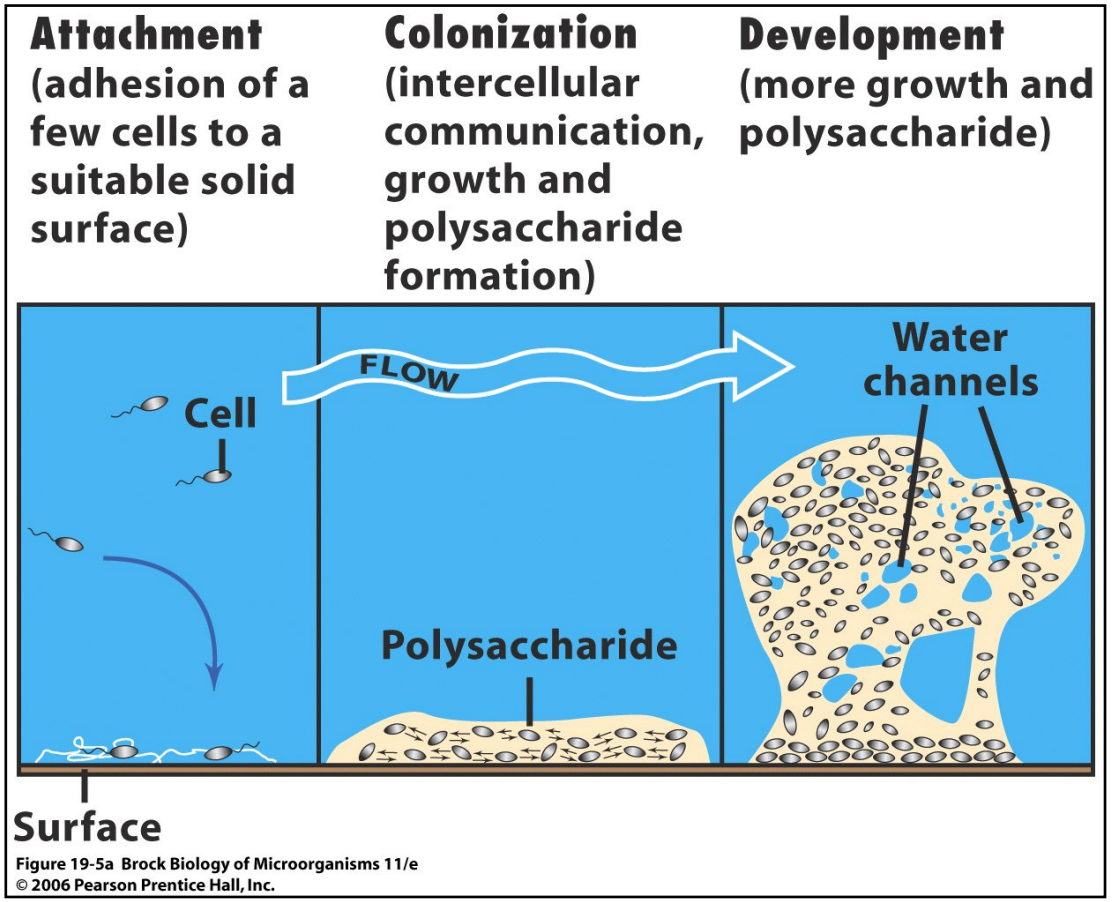


Figure 19-5a Brock Biology of Microorganisms 11/e

# Soil

- The soil is a complex habitat with numerous microenvironments and niches.

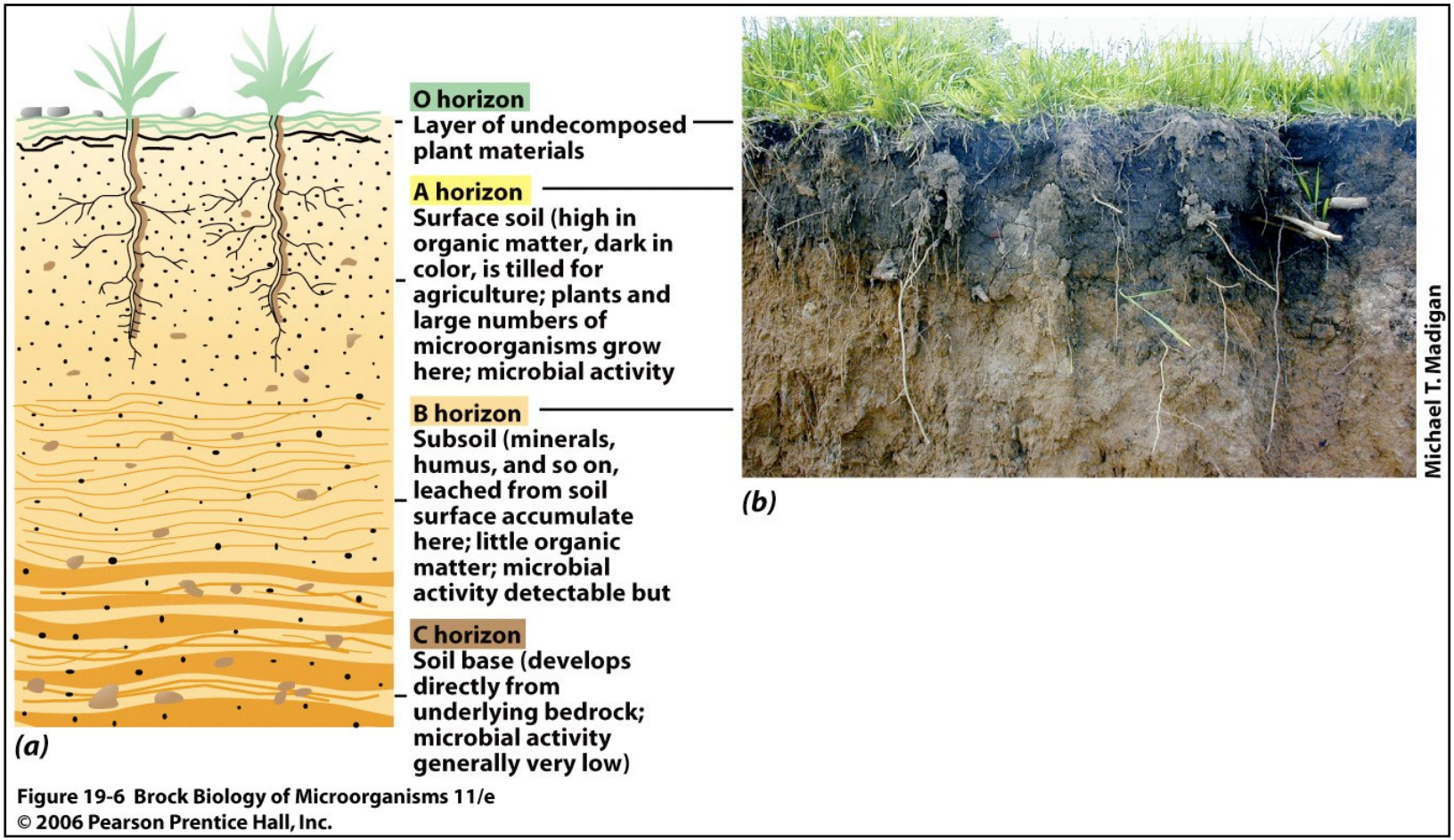
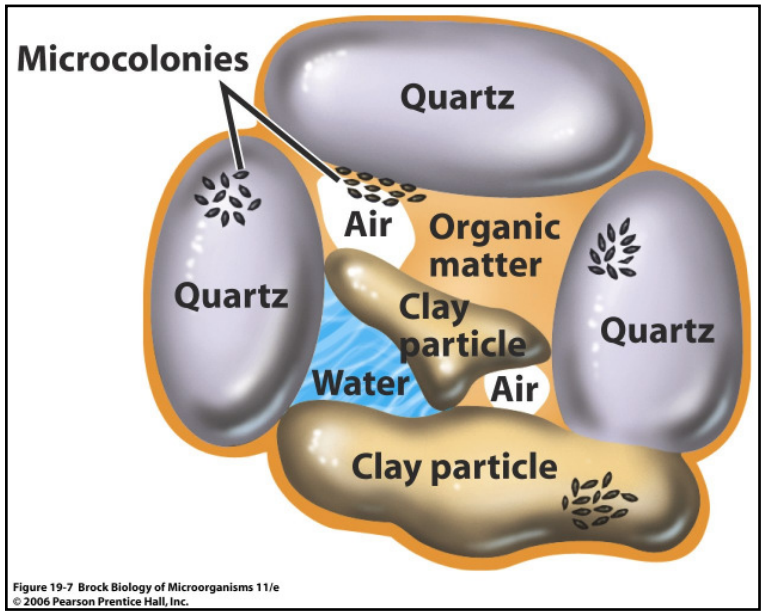



Figure 19-6 Brock Biology of Microorganisms 11/e

# Soil

- Microorganisms are present in the soil primarily attached to soil particles.
- The most important factor influencing microbial activity in **surface soil** is the **availability of water.**
- In **deep soil** (the subsurface environment), **nutrient** availability plays a major role.



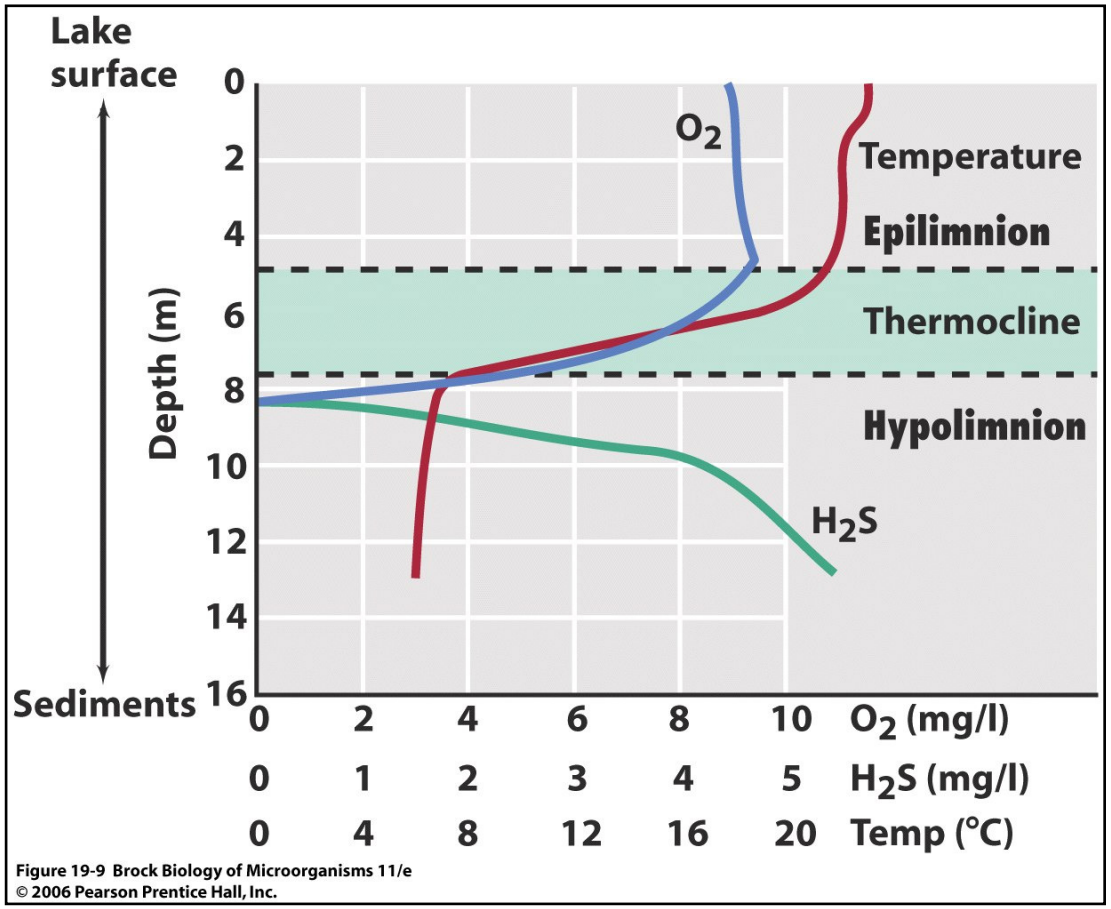

A soil aggregate composed of mineral and organic components, showing the localization of soil microorganisms.

# Freshwater Environments

- In aquatic ecosystems, the main **primary producers** are usually **phototrophic microorganisms**.
- **Bacteria** consume most of the **organic matter** produced, which can lead to **depletion of oxygen** in the environment.
- The **biochemical oxygen demand (BOD)** is a measure of the oxygen-consuming properties of a water sample.

# Freshwater Lake

- In many lakes in **temperate climates**, the water mass becomes **stratified** during the summer, with the warmer and less dense surface layers separated from the colder and denser bottom layers.




Development of anoxic conditions in the depths of a temperate lake as a result of summer stratification.

# Rivers

- Even though a river may be well mixed because of rapid water flow and turbulence, large amounts of added organic matter can lead to a marked oxygen deficit from bacterial respiration.

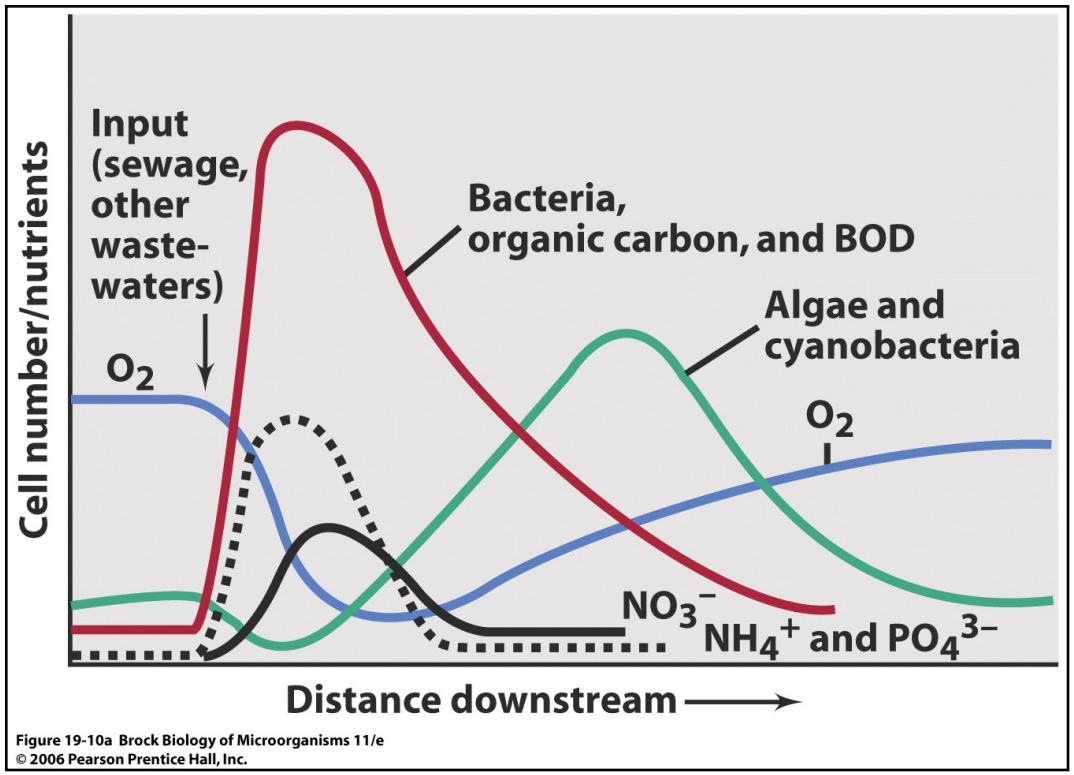



Figure 19-10a Brock Biology of Microorganisms 11/e

# Eutrophic (Nutritient-Rich) Lake

T. D. Brock

Figure 19-10b Brock Biology of Microorganisms 11/e

# Marine Habitats

- Marine waters have **less nutrients** than many freshwaters, yet **substantial numbers of microorganisms** exist there. Many of these use **light to drive ATP synthesis**.
- The form of rhodopsin (called **proteorhodopsin**) found in open ocean prokaryotes is very similar to bacteriorhodopsin but is present in cells that are phylogenetically *Bacteria*, not *Archaea*.

# Marine Habitats

- Numbers of prokaryotes decrease with depths (surface waters $10^5$-$10^6$; below 1000 m $10^3$-$10^5$ cells/ml)
- In terms of prokaryotes, species of the domain *Bacteria* tend to predominate in oceanic surface waters, whereas *Archaea* are more prevalent in deeper waters (phylogenetic stains).

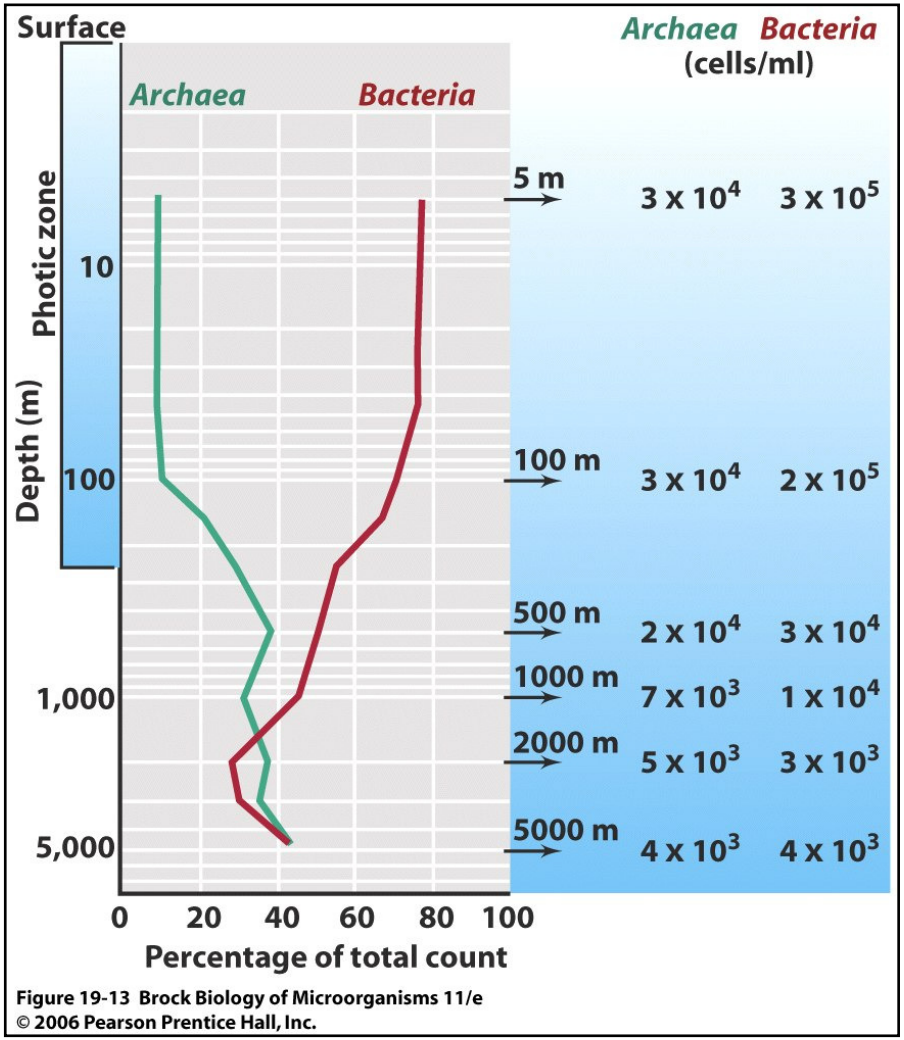


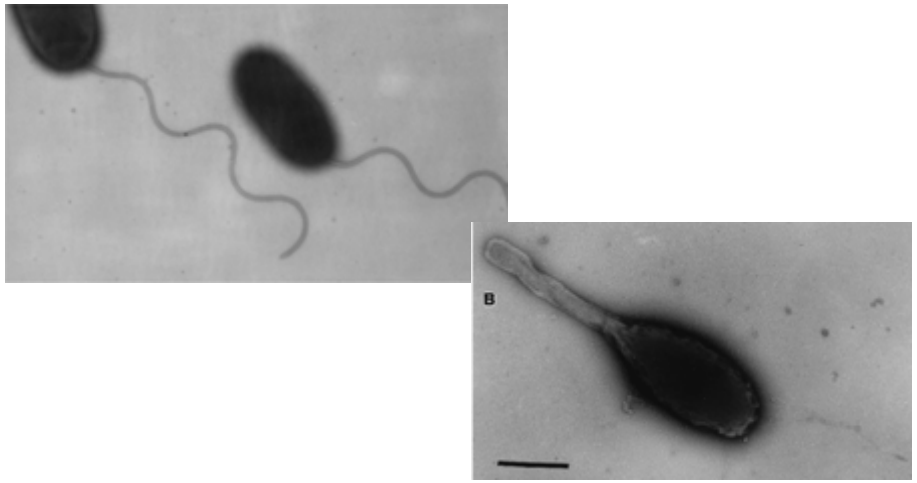
Figure 19-13 Brock Biology of Microorganisms 11/e

# I Culture-Dependent Analyses of Microbial Communities

# Enrichment and Isolation

# Classical methods for identification of microorganisms

- Phenotypic analysis
  - Morphology
    - Shape of bacterial cell
    - Aggregation
    - Colony morphology
    - Gram staining properties




  - Physiology
    - Aerobic or anaerobic
    - Energy production: respiration or fermentation or photosynthesis
    - Temperature and pH optima
    - Nutrient requirements (for example, carbon source)
    - Storage products & pigments

# Culture-Dependent Analyses of Microbial Communities

## •Enrichment and Isolation

•The **enrichment culture** technique is a means of obtaining microorganisms from natural samples. Hundreds of different enrichment strategies have been devised

# Culture Methods

**Table 18.1** Some enrichment culture methods for prokaryotes[a] (continues on next page)

**Light-phototrophic bacteria: main C source, $CO_2$**

| Incubation in air | Organisms enriched | Inoculum |
|---|---|---|
| $N_2$ as nitrogen source | Cyanobacteria | Pond or lake water; sulfide-rich muds; stagnant water; raw sewage; moist, decomposing leaf litter; moist soil exposed to light |
| $NO_3^-$ as nitrogen source, 55° C | Thermophilic cyanobacteria | Hot spring microbial mat |
| **Anoxic incubation** | | |
| $H_2$ or organic acids; $N_2$ as sole nitrogen source | Purple nonsulfur bacteria, heliobacteria | Same as above plus hypolimnetic lake water; pasteurized soil (heliobacteria) |
| $H_2S$ as electron donor | Purple and green sulfur bacteria | |

Table 18-1 part 1 Brock Biology of Microorganisms 11/e

# Culture Methods

**Table 18.1** Some enrichment culture methods for prokaryotes[a] (continues on next page)

**Dark-chemoorganotrophic bacteria and methanogens: main C source, organic compounds**

**Incubation in air: aerobic respiration**

| Electron donor and nitrogen source | Electron acceptor | Typical organisms enriched | Inoculum |
|---|---|---|---|
| Lactate + $NH_4^+$ | $O_2$ | *Pseudomonas fluorescens* | Soil, mud; lake sediments; decaying vegetation; pasteurize inoculum (80° C for 15 min) for all *Bacillus* enrichments |
| Benzoate + $NH_4^+$ | $O_2$ | *Pseudomonas fluorescens* | |
| Starch + $NH_4^+$ | $O_2$ | *Bacillus polymyxa*, other *Bacillus* spp. | |
| Ethanol (4%) + 1% yeast extract, pH 6.0 | $O_2$ | *Acetobacter, Gluconobacter* | |
| Urea (5%) + 1% yeast extract | $O_2$ | *Sporosarcina ureae* | |
| Hydrocarbons (e.g., mineral oil, gasoline, toluene) +$NH_4^+$ | $O_2$ | *Mycobacterium, Nocardia, Pseudomonas* (Figure 19.42) | |
| Cellulose + $NH_4^+$ | $O_2$ | *Cytophaga, Sporocytophaga* (Figure 12.90) | |
| Mannitol or benzoate, $N_2$ as N source | $O_2$ | *Azotobacter* | |

Table 18-1 part 3 Brock Biology of Microorganisms 11/e

# Classical Enrichment Culture

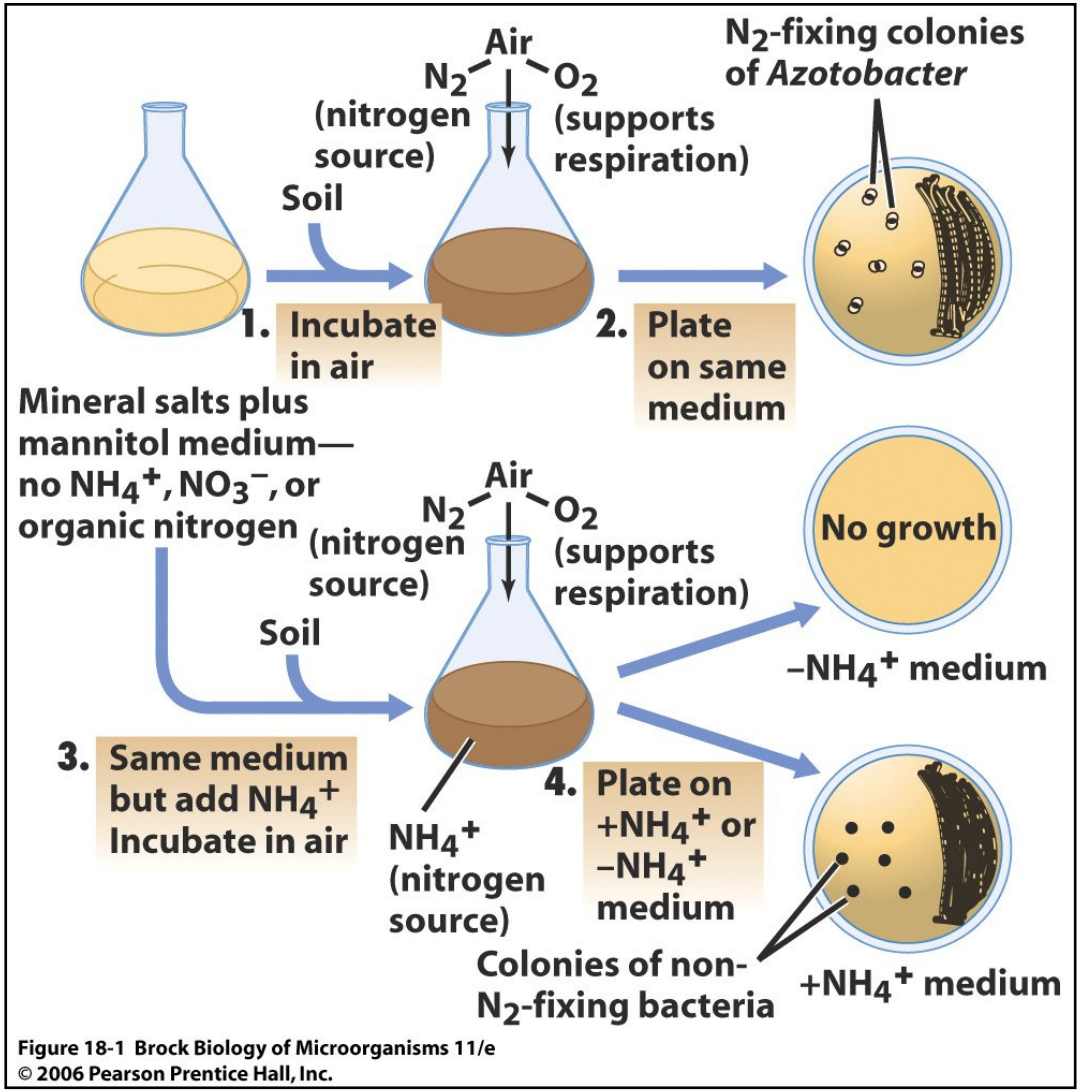



Figure 18-1 Brock Biology of Microorganisms 11/e


- Choosing media and incubation conditions selective for particular organisms enables specific reactions to be investigated by enrichment methods.

# The **Winogradsky Column**

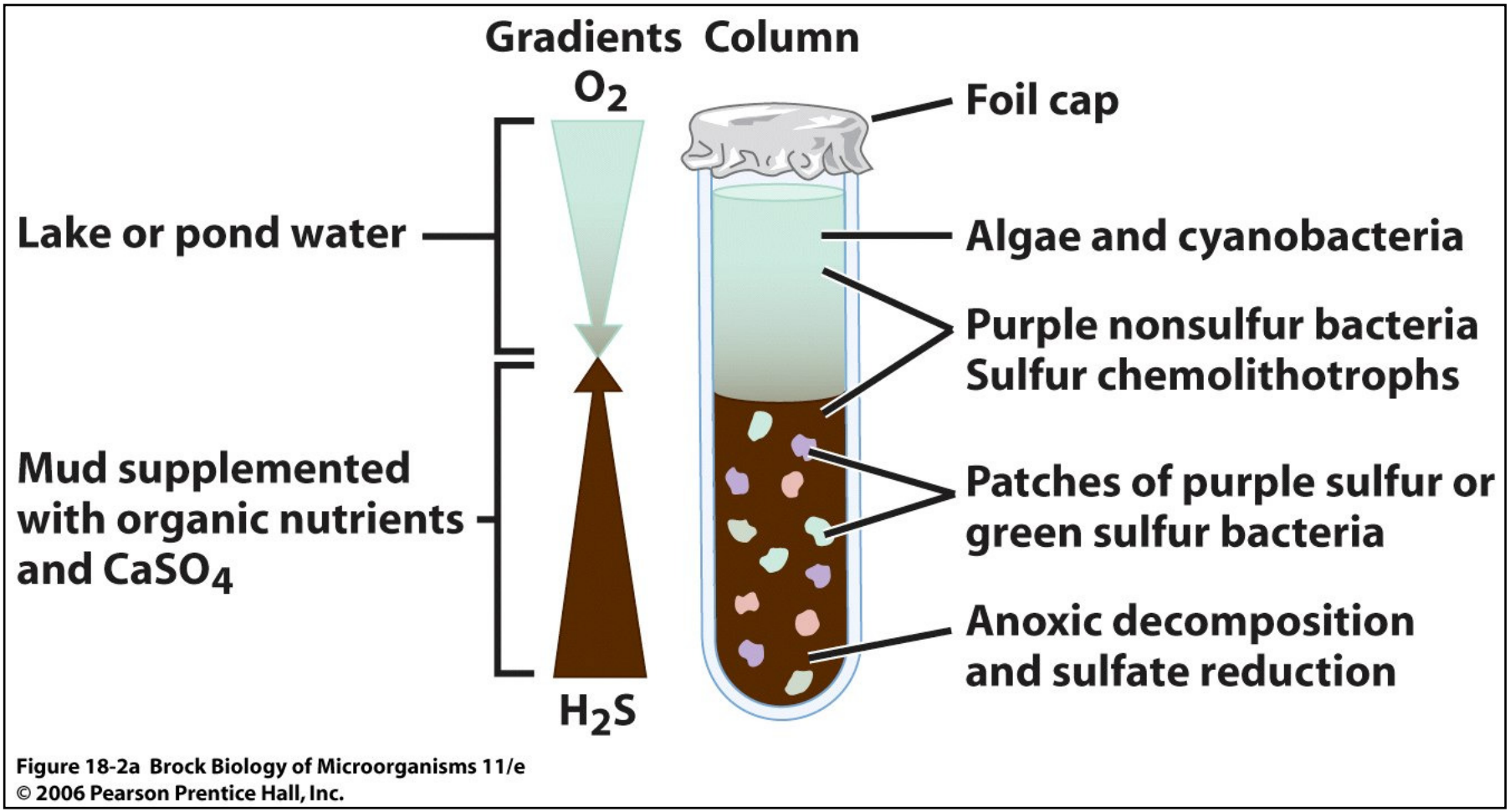



Figure 18-2a Brock Biology of Microorganisms 11/e

# The **Winogradsky column**

The **Winogradsky column** is a **miniature anoxic ecosystem** that can be used as a **long-term source** of bacteria for enrichment culture purposes

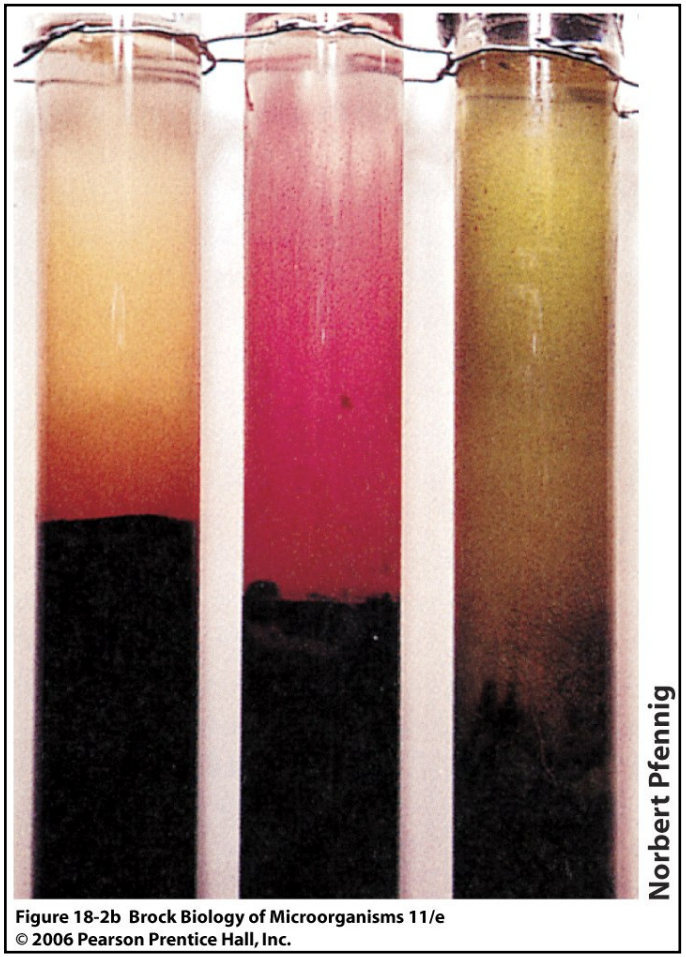


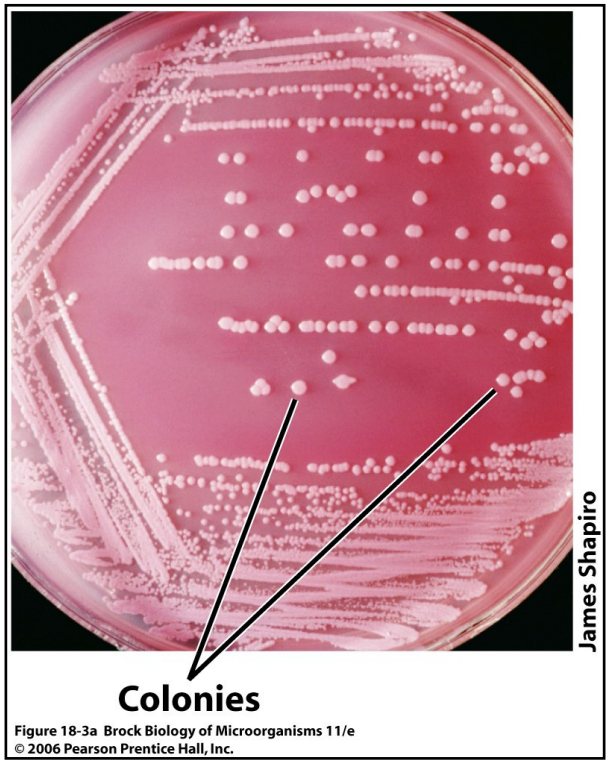

Figure 18-2b Brock Biology of Microorganisms 11/e

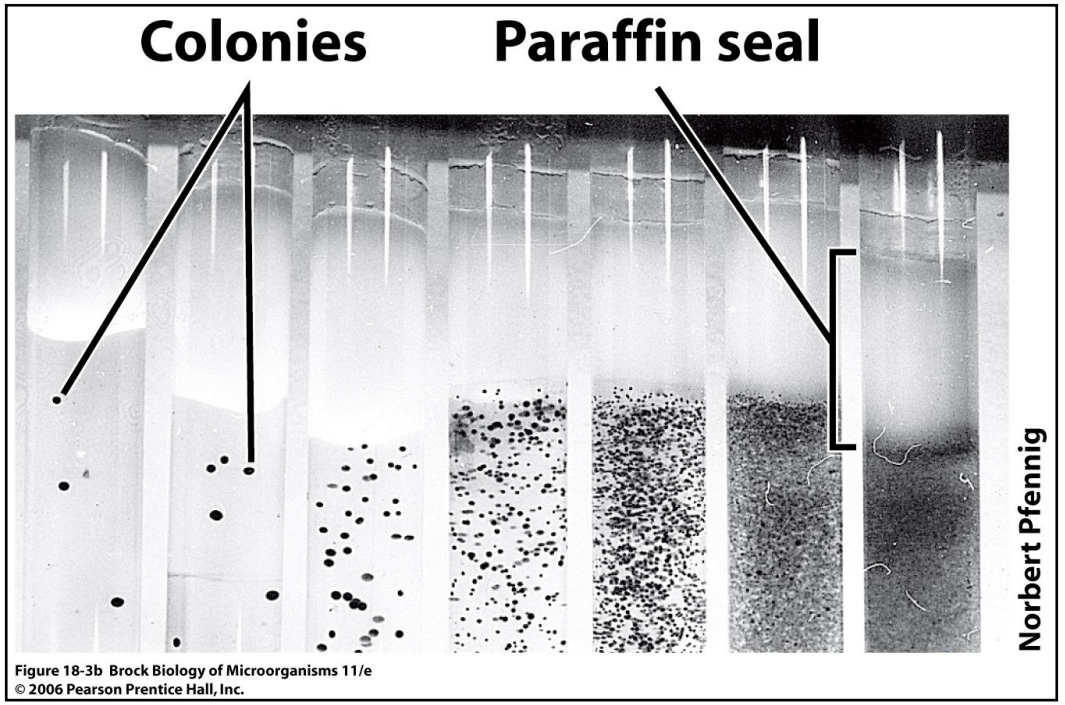
# The Enrichment Bias

- Although the enrichment culture is a powerful tool, in most enrichments there exists a bias.
- **Selection** for the most fit organism for laboratory culture.
- **Enrichment bias** can be demonstrated by comparing the results obtained in dilution cultures with classical liquid enrichment.

# Isolation in Pure Culture

• Once a successful enrichment culture has been established, a pure culture can be obtained by **conventional microbiological procedures**, including **streak plates**, **agar shakes**, and **dilution methods**.




Agar shake technique for oxygen-sensitive anaerobes

# Most probable number (MPN) technique

- Pure cultures can be obtained from repeated serial dilutions

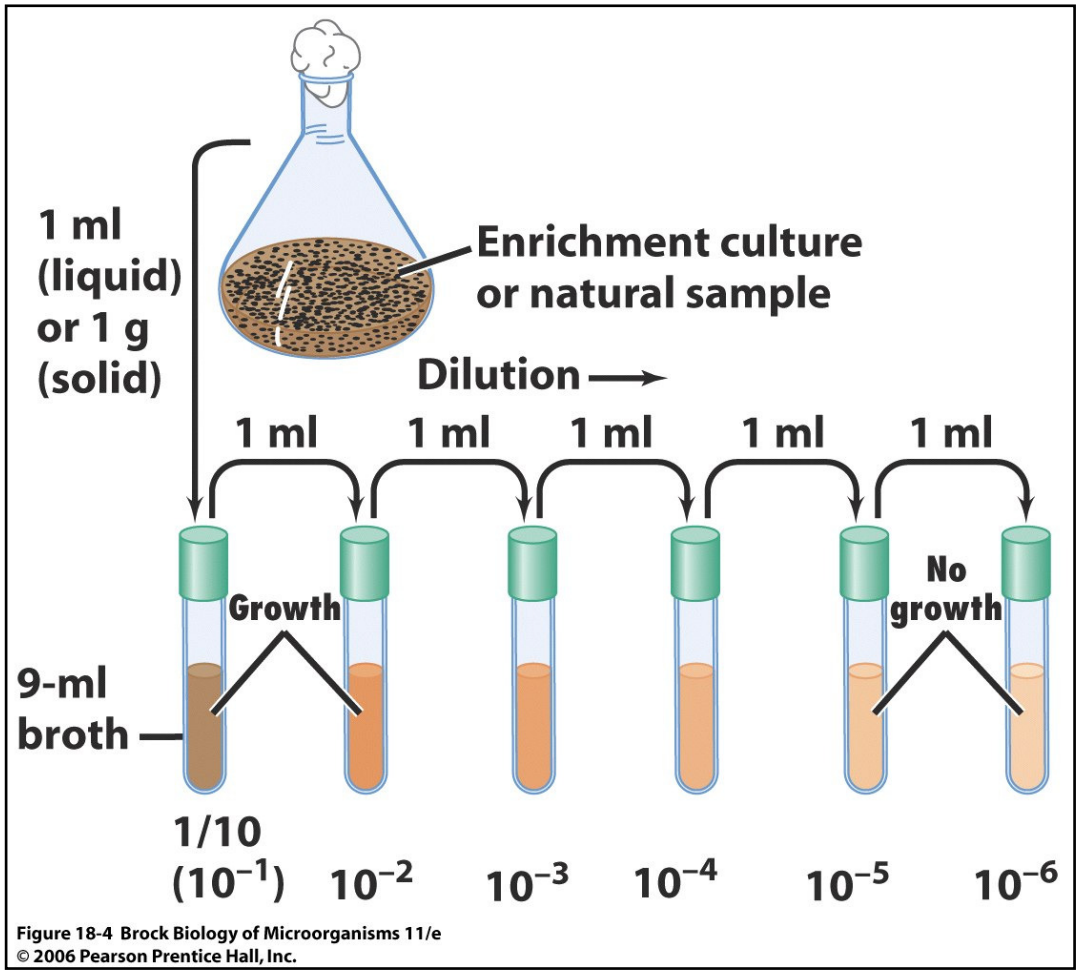
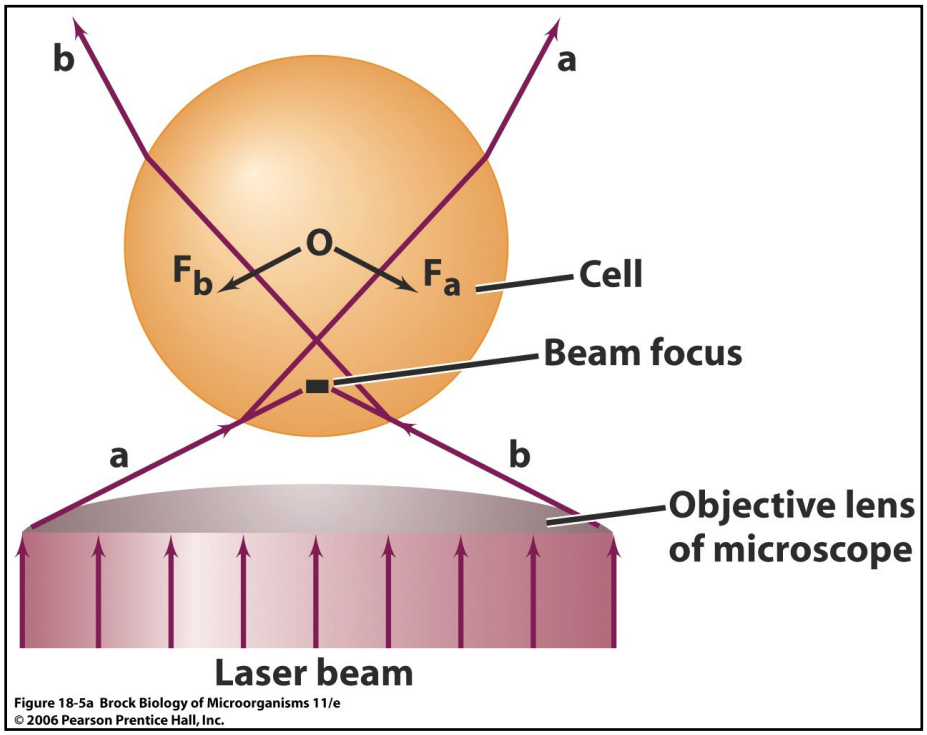



Figure 18-4 Brock Biology of Microorganisms 11/e

# Laser tweezers

- **Laser tweezers** allow one to "pick" a cell from a microscope field and literally move it away from contaminants.




- Focussed laser beam creates downwards radiation forces ($F_a$, $F_b$).
- Cell can be dragged in any direction.

# Laser tweezers

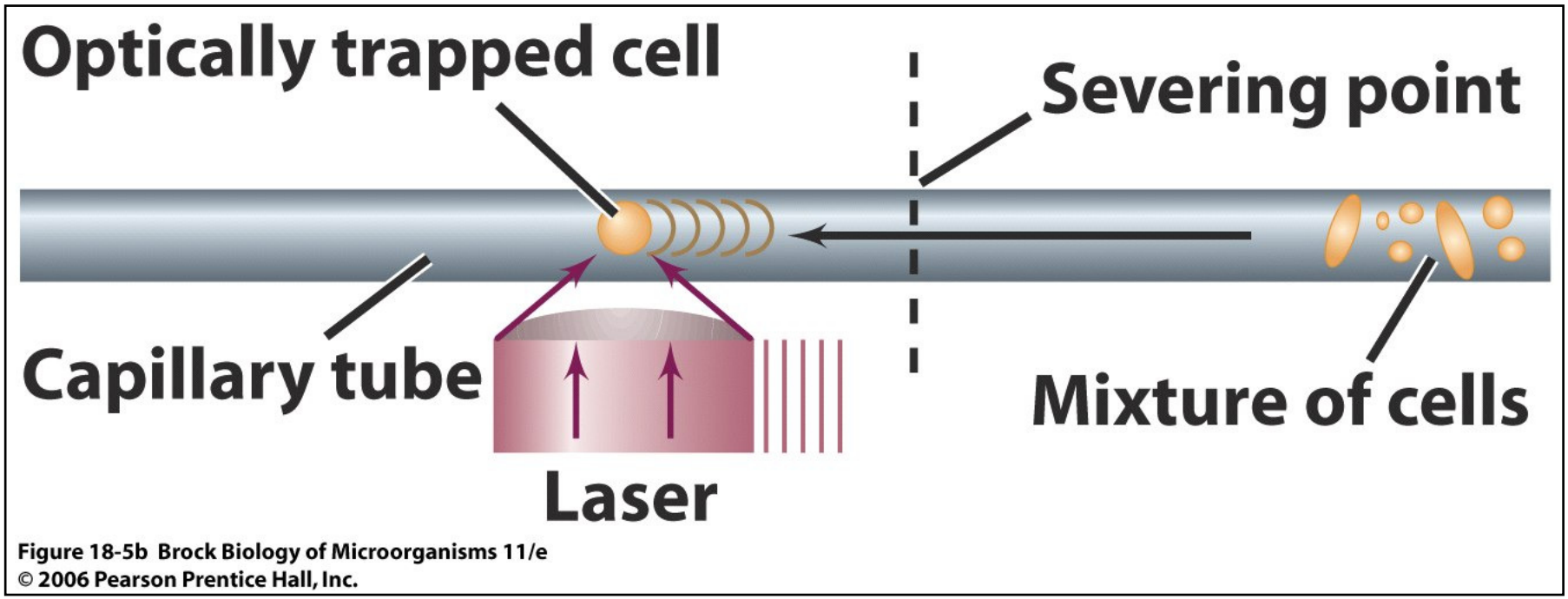



Figure 18-5b Brock Biology of Microorganisms 11/e

# But…….


**Less than 1% of bacteria in environmental samples can be cultivated**
**0.1-1% of bacterial diversity known!!**

# Limitations

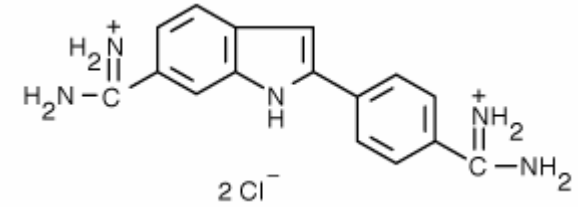
- Classical criteria, cellular morphology, for bacterial differentiation underestimates bacterial diversity.
- Difficult to obtain monocultures
  - Microorganisms normally exist as mixed communities - biofilms.
  - Cultivation and analysis of monocultures provides limited information about the actual role of that microorganism in its natural habitat.
- Not possible to culture all the microorganisms present in an environmental sample
  - Some microorganisms have special growth requirements which cannot be met in the laboratory.
  - Microorganisms can exist in a viable but non-culturable (VBNC) state.

# II Molecular (Culture-Independent) Analyses of Microbial Communities

# IIA Viability and Quantification Using Staining Techniques

# Staining Techniques

- **Fluorescent Staining**



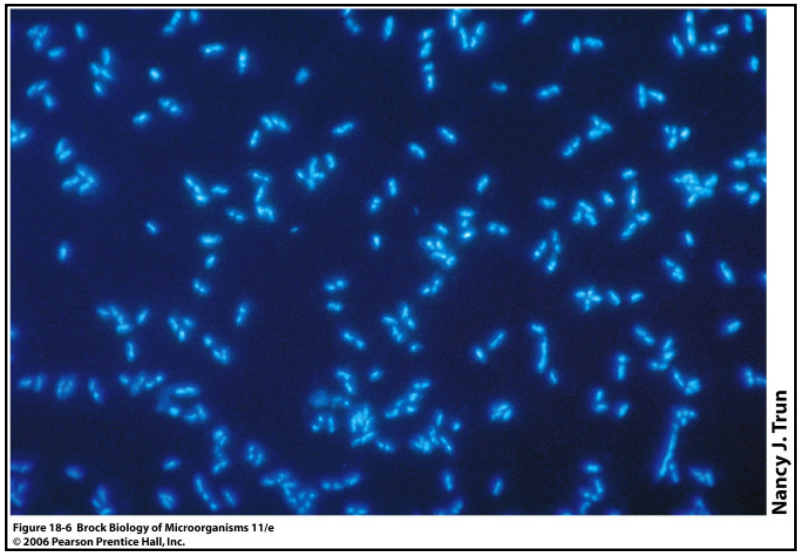

• **DAPI** (4',6-diamido-2-phenylindole) is a general stain for identifying microorganisms in natural samples.

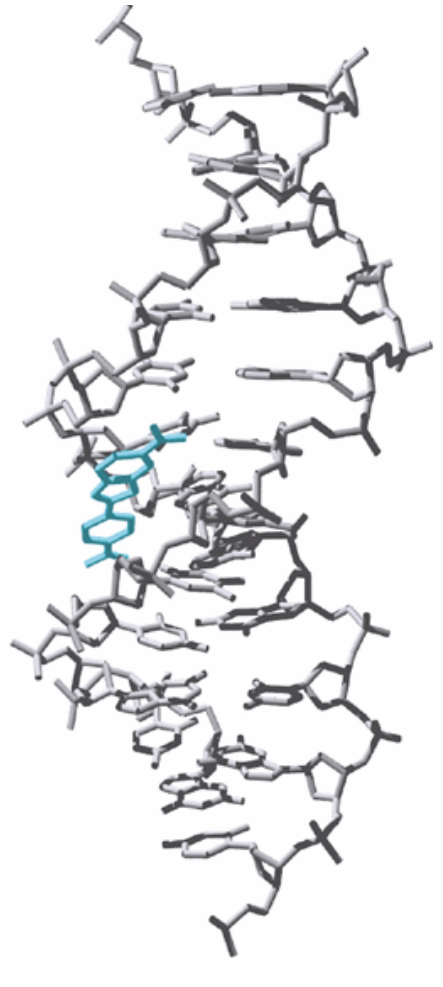
-Cells stained with DAPI fluoresce bright blue.

-Stains **nucleic acid**.

-Nonspecific background-staining sometimes a problem.

-**No differentiation living or dead cells (Quantification)!!**



# Staining Techniques

- **Fluorescent Staining**
- Differentiation whether cytoplasmic membrane intact or not.

-Two dyes added to cells.

-Green fluorescent dye penetrates all cells, viable or not.

-Red dye (propidium iodide) penetrates only cells without intact membrane (dead).

-Nonspecific background-staining great problem

(not ueseful for environmental samples).

-**Differentiation living or dead cells (Quantification & viability)!!**

**Green** cells = alive

**Red** cells = dead

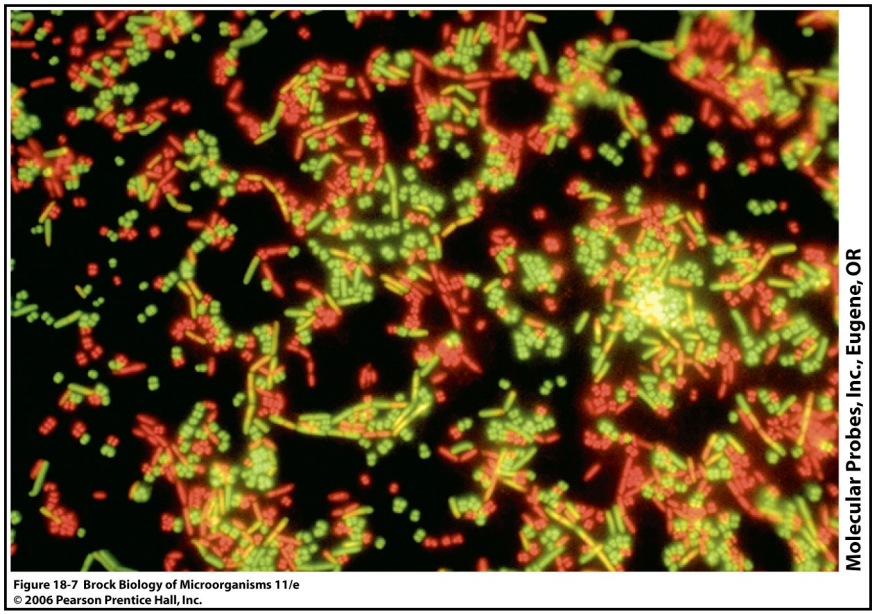
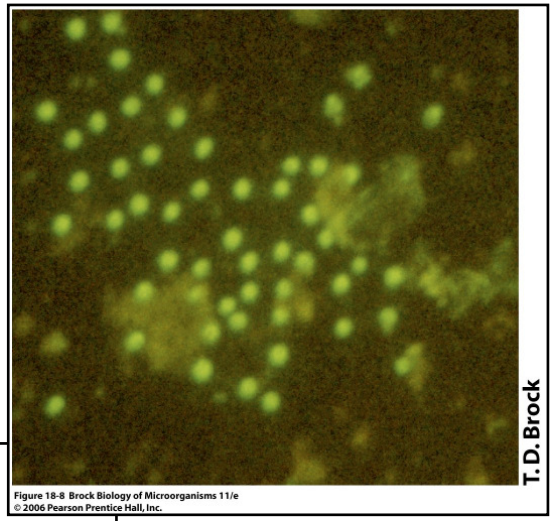


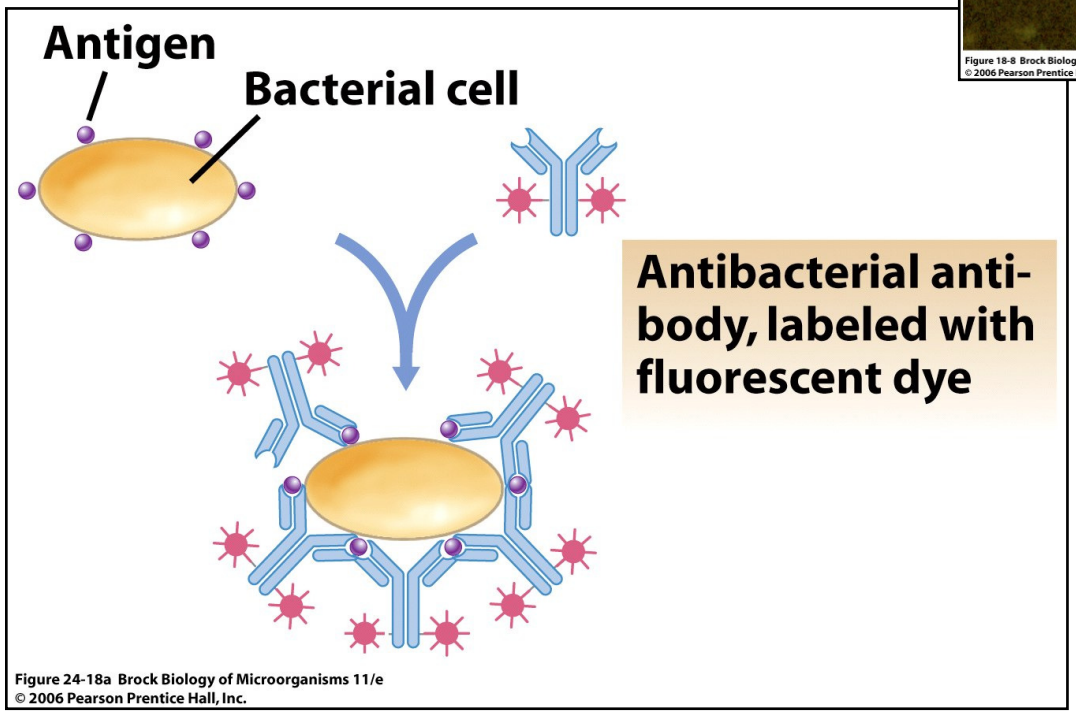

Figure 18-7 Brock Biology of Microorganisms 11/e

# Fluorescent Antibodies

- Great specificity against surface constituents of a particular organism.
- Allows tracking an organism in a complex habitat (e.g. soil, clinical sample)




*Sulfolobus acidocaldarius* (ARCHAEA) on solfatara soil particles



# Fluorescent Antibodies

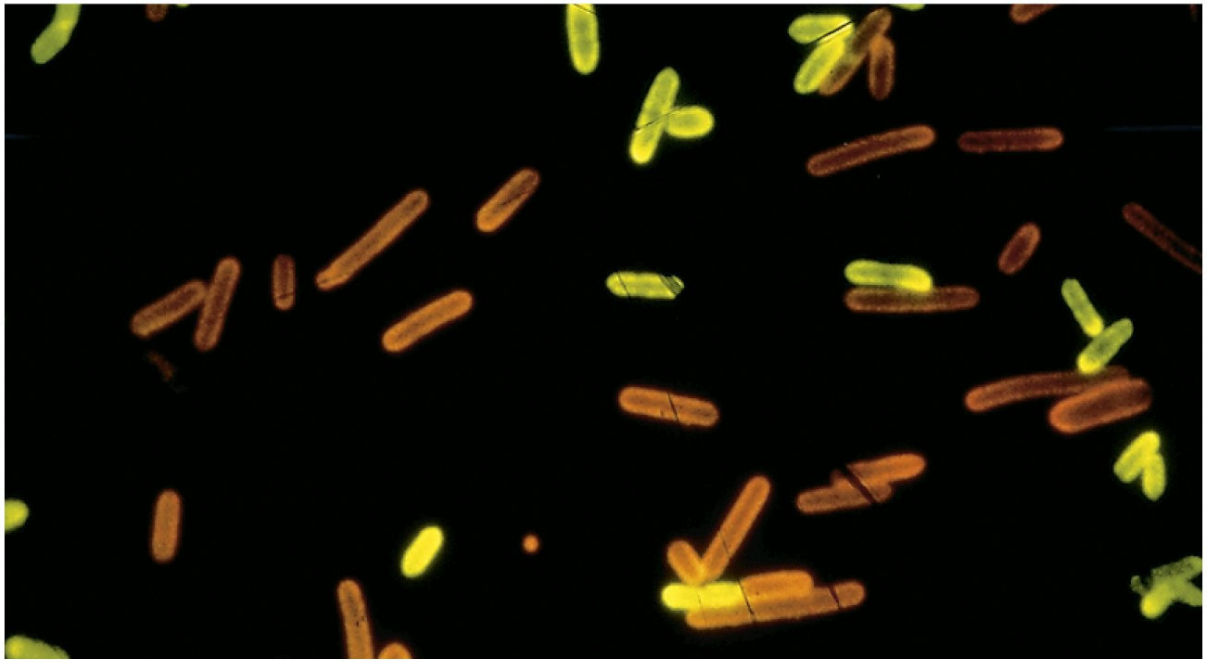



Figure 24-17 Brock Biology of Microorganisms 11/e


*Clostridium septicum*: stained with antibody conjugated to fluorescein isothiocyanate (yellow-green fluorescence)

*Clostridium chauvoei*: stained with antibody conjugated to rhodamine B (red-orange fluorescence)

# Green Fluorescent Protein (cell tag)

The **green fluorescent protein** makes cells autofluorescent and is a means for tracking cells introduced into the environment.

**Marker system**

- The ideal molecular marker system:
  - The *gfp* gene is found naturally in ***Aequoria victoria***, a jellyfish (Eukaryote!)

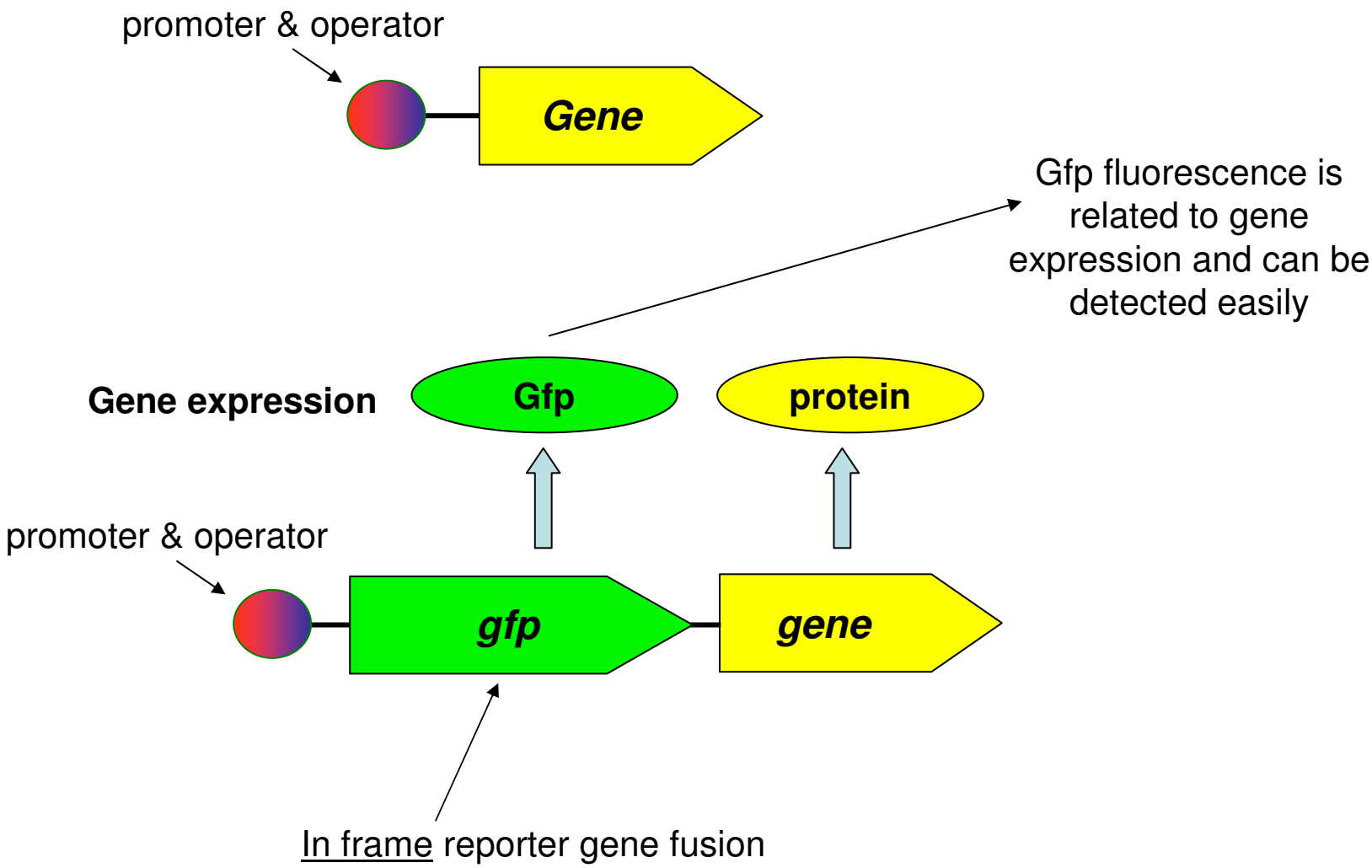
  - GFP emits green light at 508 nm when excited with UV light at 296 nm.
  - Does not require addition of substrates.
  - Is not species specific.
  - All that is required is gene expression in the host cell, and post-translational modification.

Lowder, AEM (2000) 66:3160 - 3165

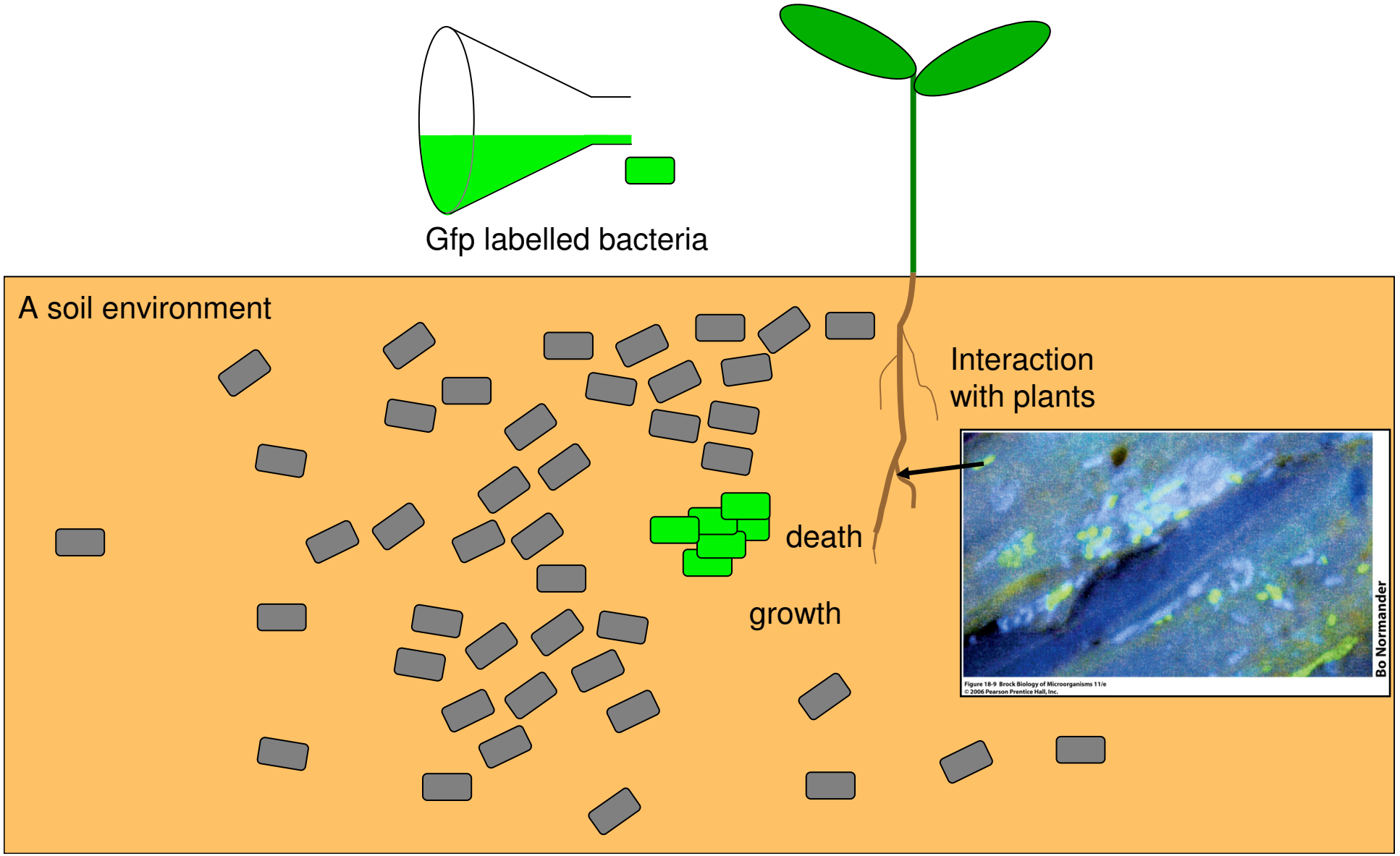
# Use of *gfp*

- There are 2 main possible uses of the *gfp* reporter gene:
  - It can be under the control of a constitutively expressed promoter.
    - This means that the bacteria constantly produce Gfp all the time.
  - It can be fused with a gene which is only expressed under a specific set of conditions
    - The bacteria will only fluoresce when the conditions are suitable for expression of the gene under investigation.

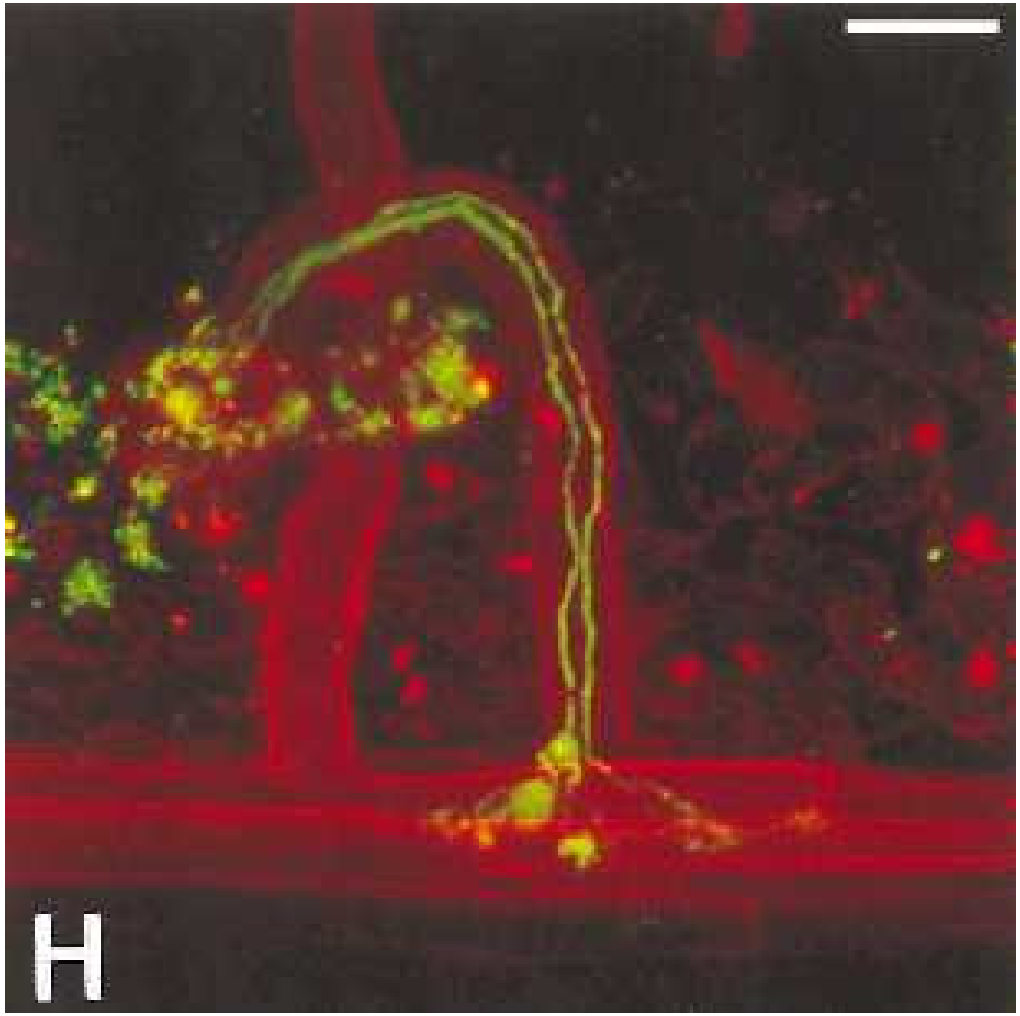
# Visualising gene regulation using GFP reporter gene fusions



# Green Fluorescent Protein (cell tag)



# Applications of GFP

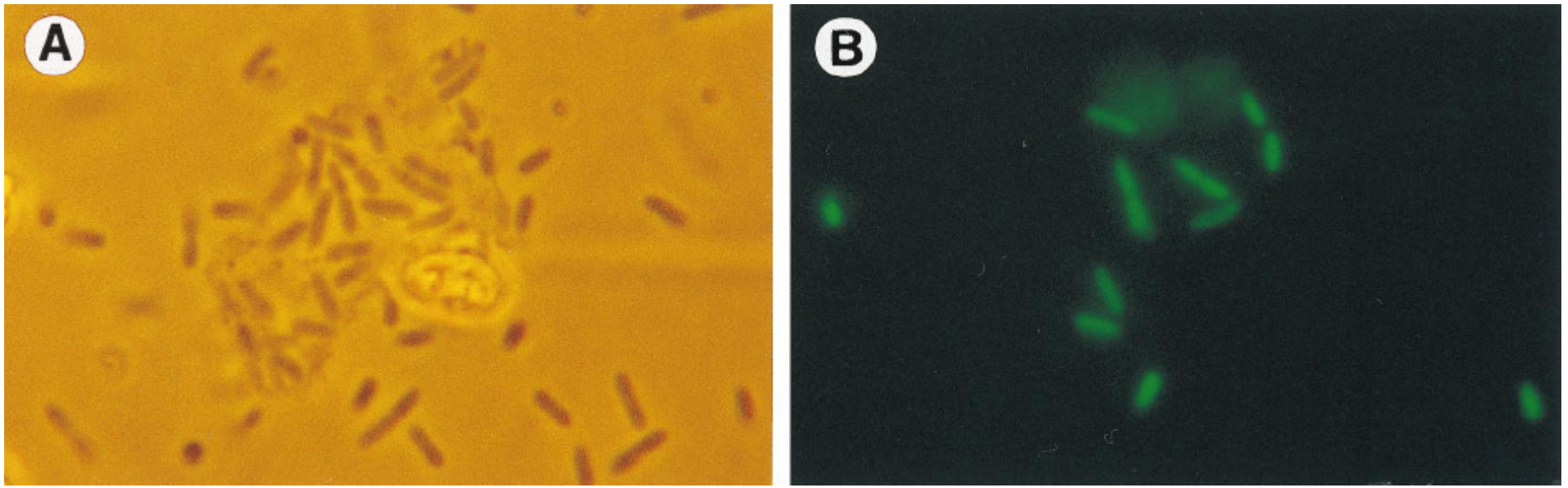



- Investigation of the early stages in the symbiosis between rhizobia and their host plant.
- GFP labelled rhizobia travelling down an infection thread into a plant root

Gage *et al*., 1996. J. Bacteriology 178:7159-7166

# Applications of GFP

- GFP as a marker for *Pseudomonas* spp.



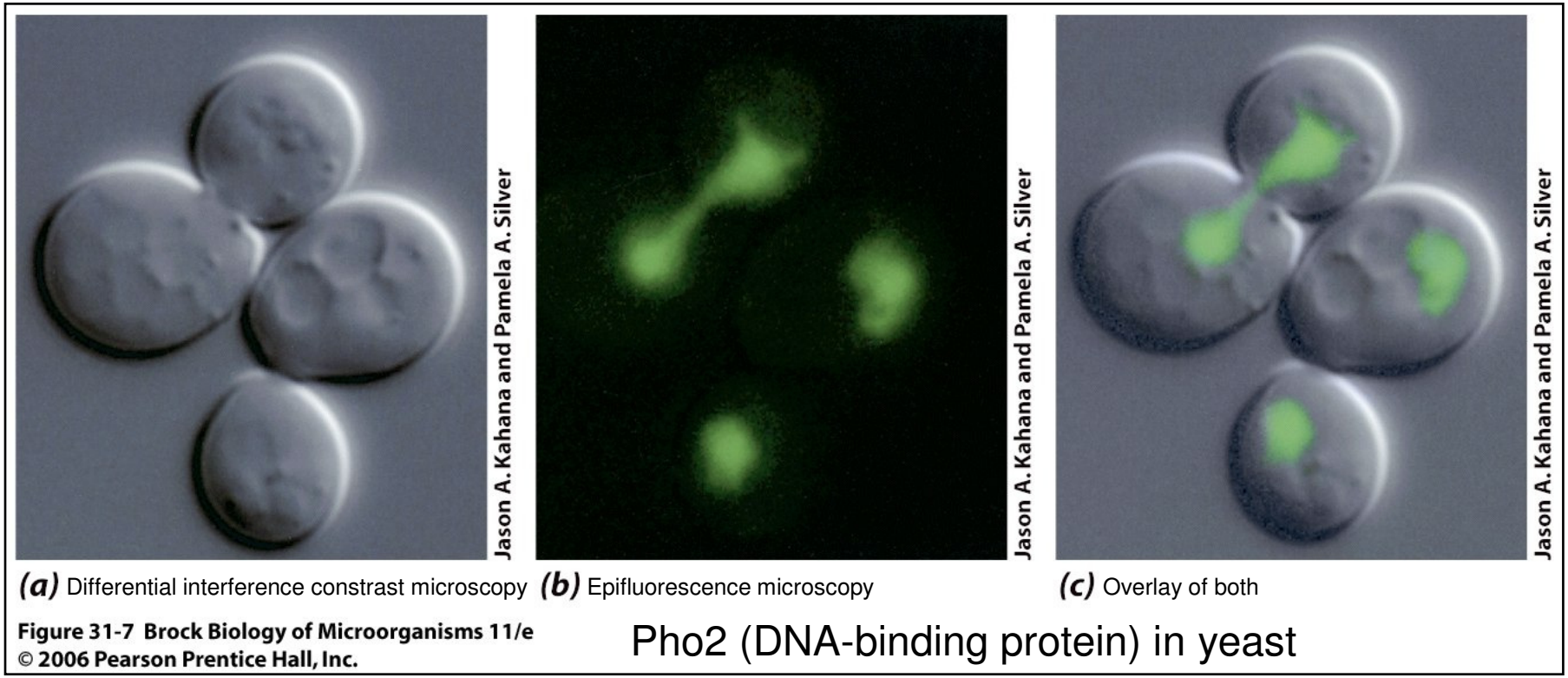

- Detection of marked *Pseudomonas* spp. in mixed cultures.

Bloemberg *et al.*, 1997 AEM 63:4543-4551

# GFP „reporter gene“

GFP as a tag for **protein localization** *in vivo.*

**GFP-Fusionsprotein**




Pho2 (DNA-binding protein) in yeast