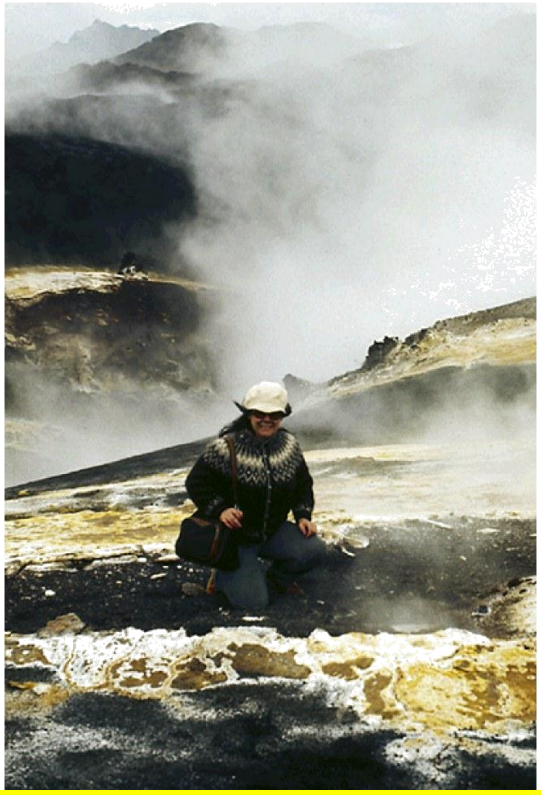
GROWTH AND CULTURING OF BACTERIA

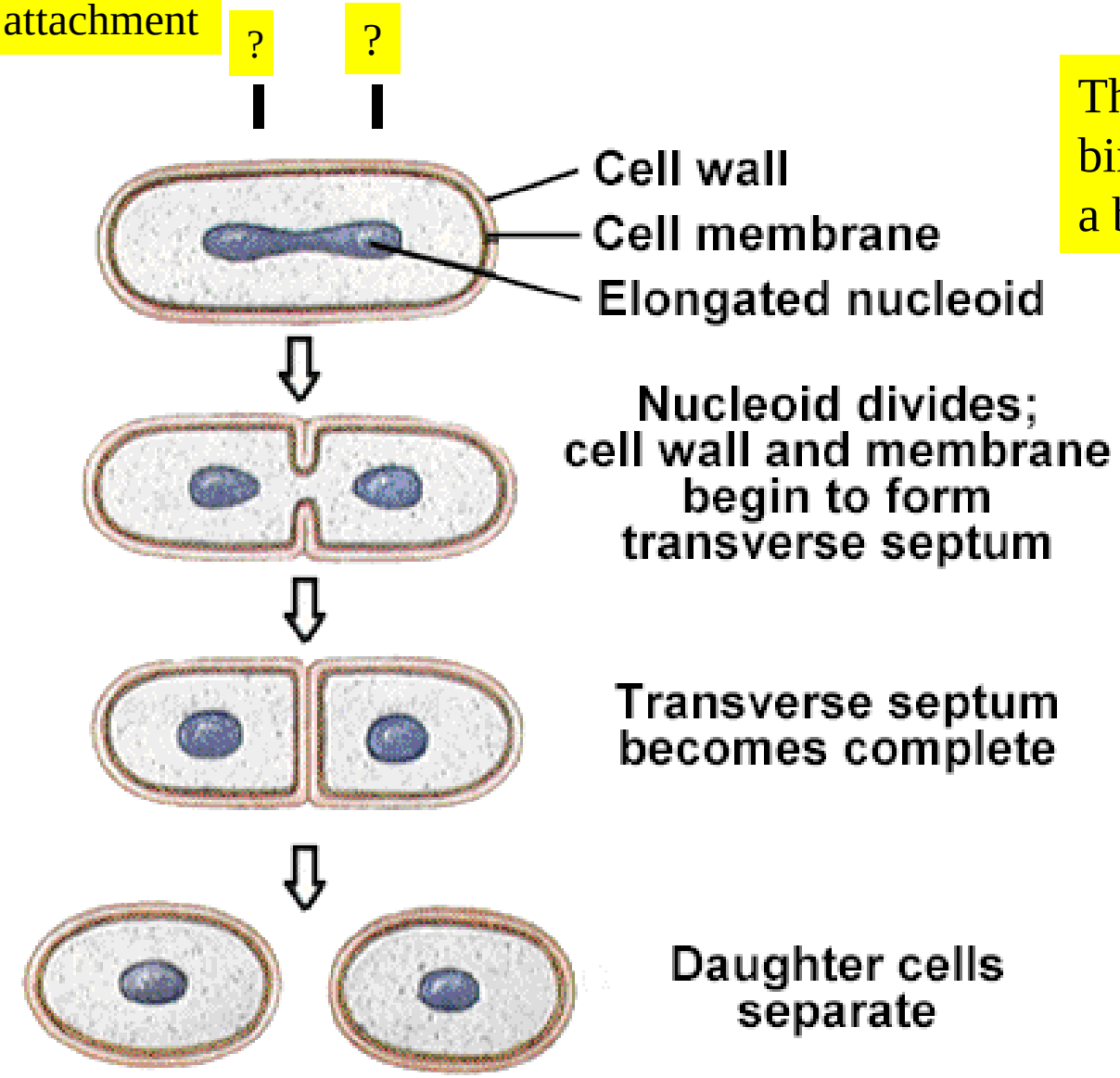
CHAPTER 6



Some of the material in this chapter will also be covered in the laboratory

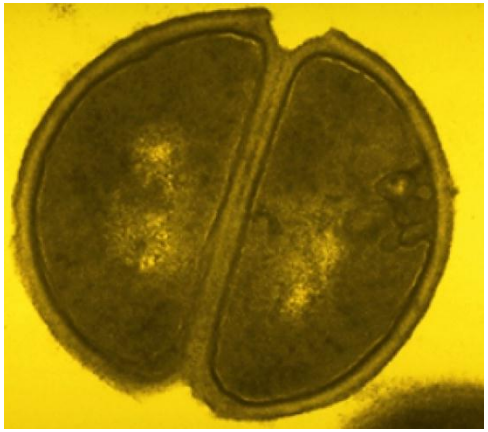
? = Membrane attachment

The stages of binary fission in a bacterial cell



http://www.youtube.com/watch?v=hOyUcjqcGpQ&feature=related

Fig. 6.1 **Binary Fission**



Staphylococcus undergoing binary fission

Nucleoid region of bacterium

Figure 6.1- Binary fission

GROWTH AND CULTURING OF BACTERIA

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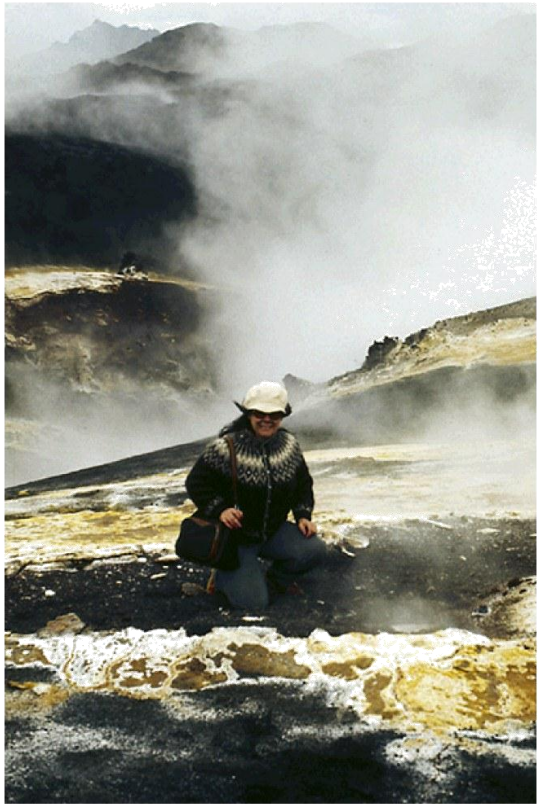
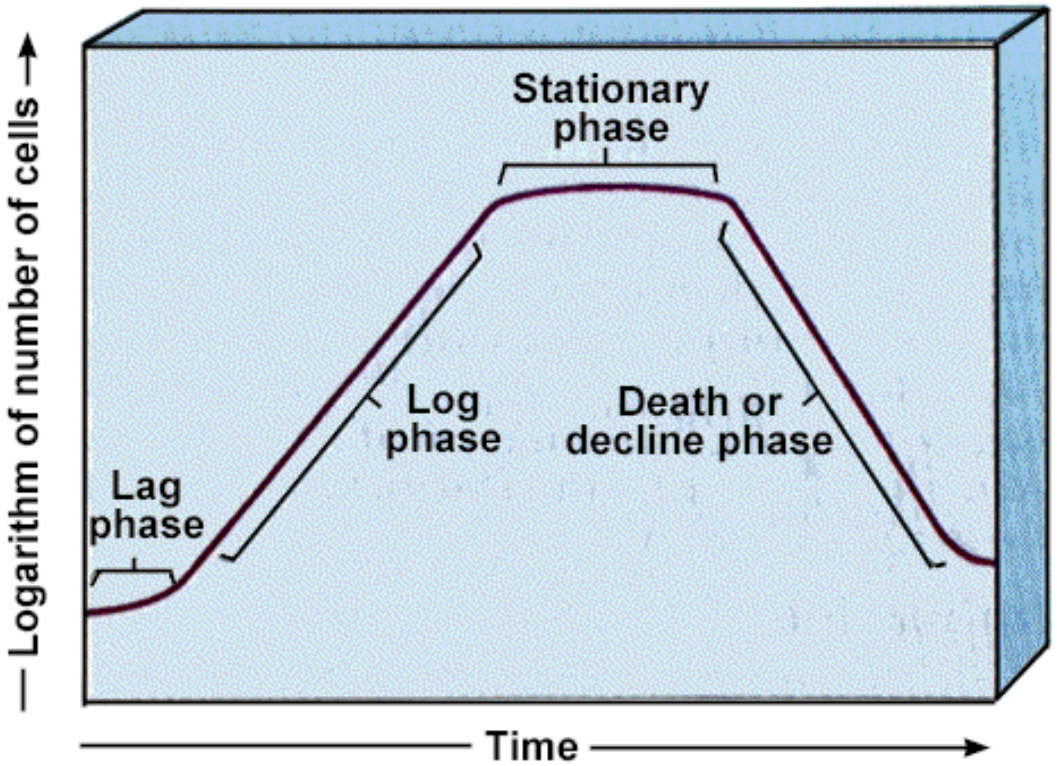


Courtesy Jacquelyn G. Black

Phases of Bacterial Growth Cycle



http://www.youtube.com/watch?v=SuvGpMevLPU

Fig. 6.3 **A standard bacterial growth curve**

The lag phase: organisms do not increase significantly in number but they are often larger in size and are very metabolically active-synthesizing enzymes, and incorporating need nutrients from the medium. Older cultures usually stay in lag phase longer than organism transferred from a "fresh" starter culture.

The log phase: During the log phase, the organisms divide at their most rapid rate- a regular genetically determined interval called the generation time.

Figure 6.4 Logarithmic growth- log to the base 2 rather than to the base 10 as is most common.

To maintain organisms in log growth a chemostat is often used. A chemostat constantly renews nutrients in a culture making it possible to grow organisms continuously in the log phase.

The stationary phase: when the rate of production new cells equals the number of cells that die that is known as stationary phase.

The decline or death phase- when dying cells outnumber the dividing cells.

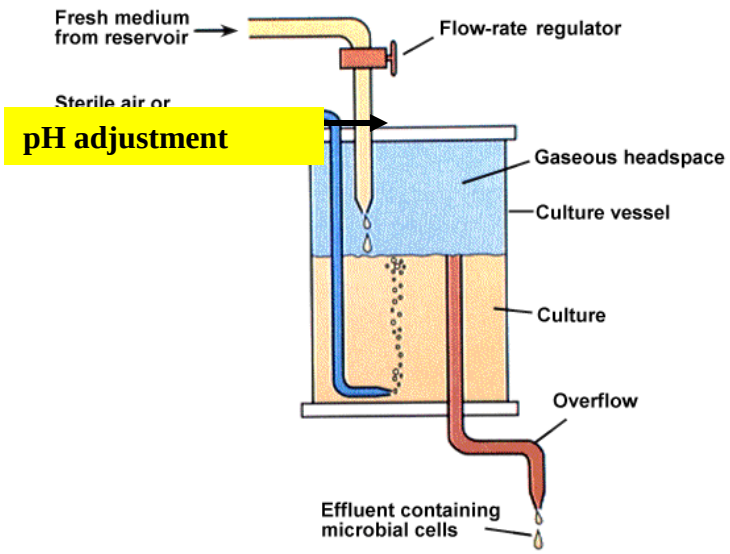


Figure 6-5 Microbiology, 7/e
Courtesy Sartorius BBI Systems, Inc.

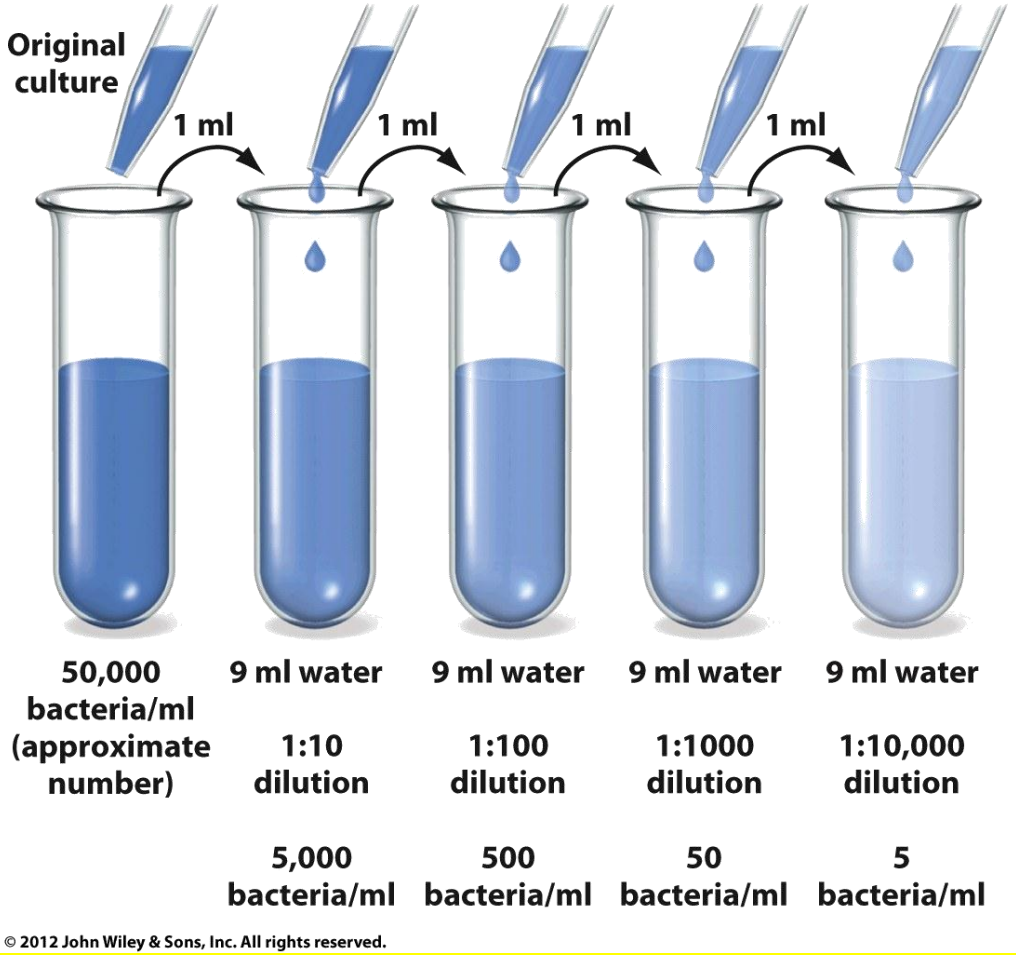
Nutrients are added, wastes are removed, pH is maintained, appropriate O_2 level is maintained automatically. Chemostat is a way to produce lots of log cells in a relatively small area.

Fig. 6.5 **Microbes growing in a chemostat**

Measuring Bacterial Growth

Counting Large Populations

- Serial Dilutions/ Standard plate counts



If this number of orgs was to be plated directly the number of colonies would be too great and so one needs to dilute the organisms

Fig. 6.6 **Serial dilution**

In this example the spread method or pour plate method have the same concentration of cells (i.e., cell number)

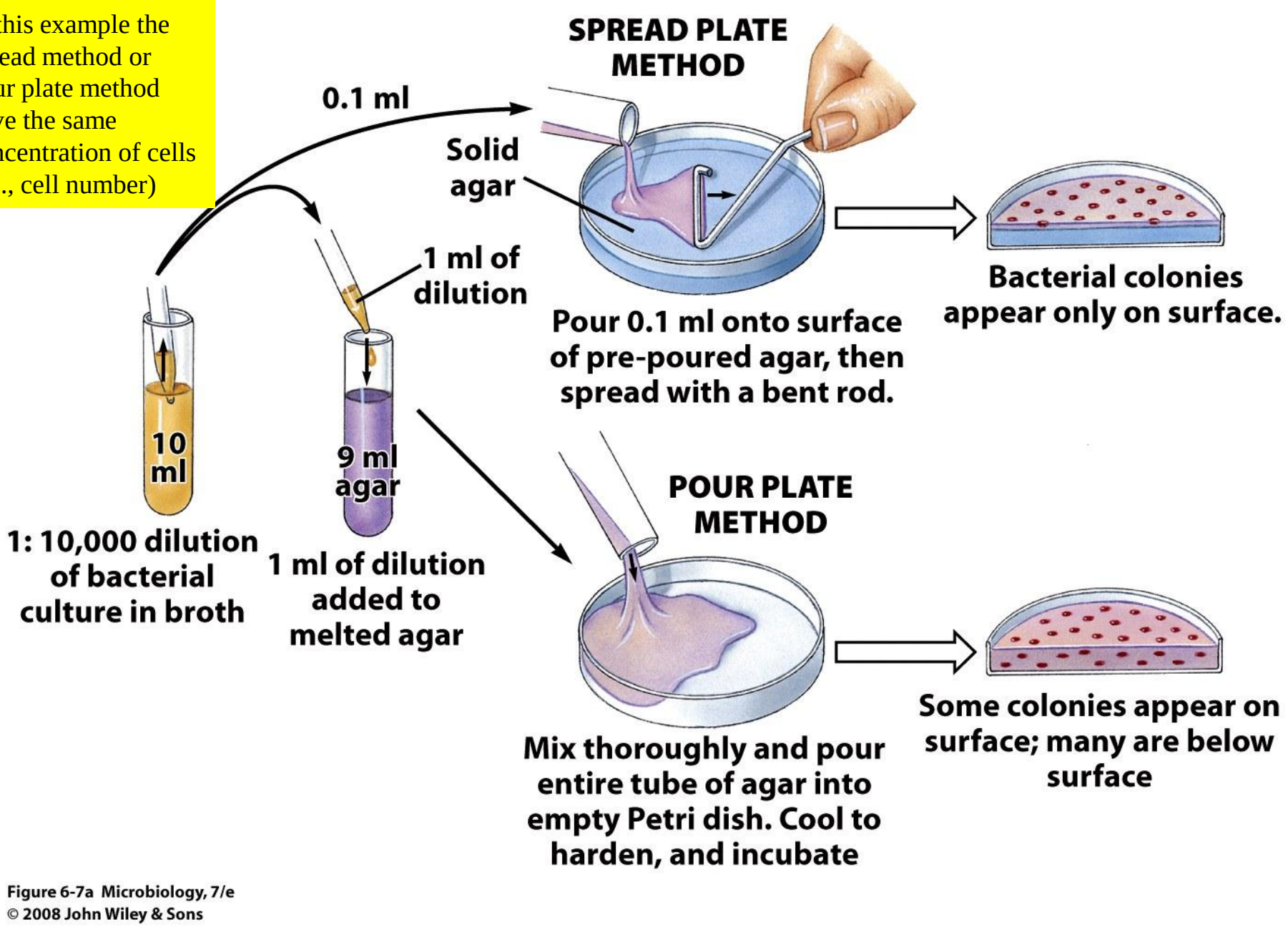


Figure 6-7a Microbiology, 7/e

Fig. 6.7 **Calculation of the number of bacteria per milliliter of culture using serial dilution.**

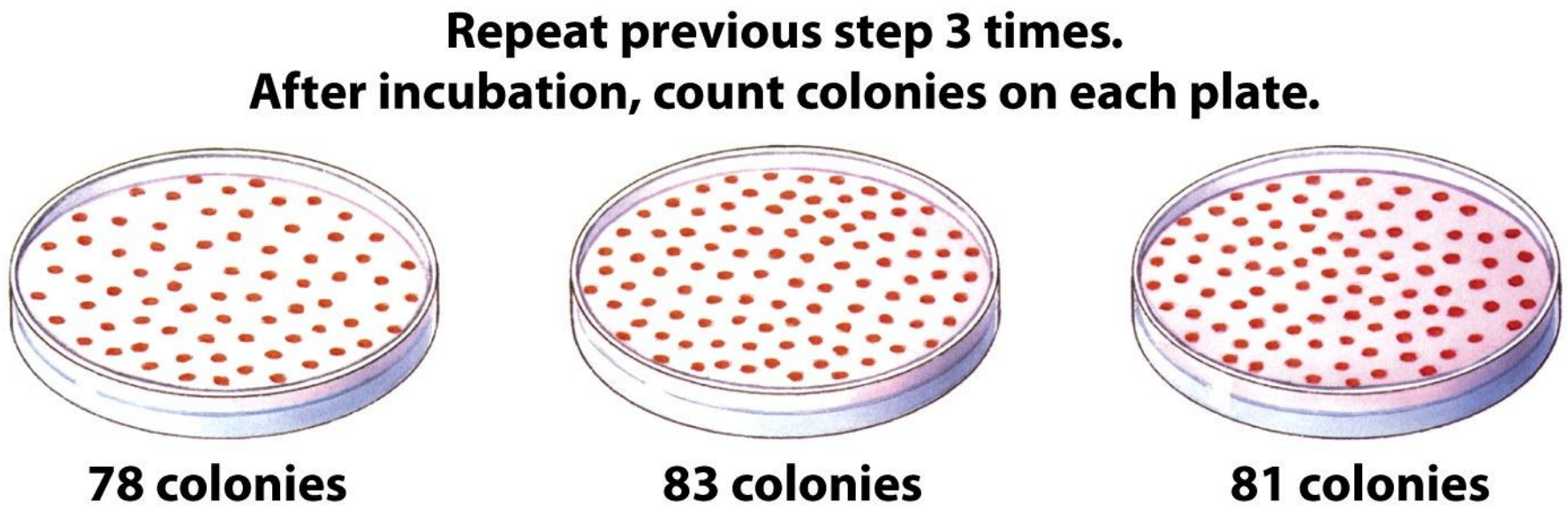


Figure 6-7b Microbiology, 7/e

Fig. 6.7 **Calculation of the number of bacteria per milliliter of culture using serial dilution.**

Direct Counts

Figure 6-8a Microbiology, 6/e
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Figure 6-8c Microbiology, 6/e
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Top using a bacterial
Colony counter.

Bacterial colonies viewed
Through a magnifying glass
Against a colony-counting grid

Which of the plates to the left
would be the best one to count?
Why?

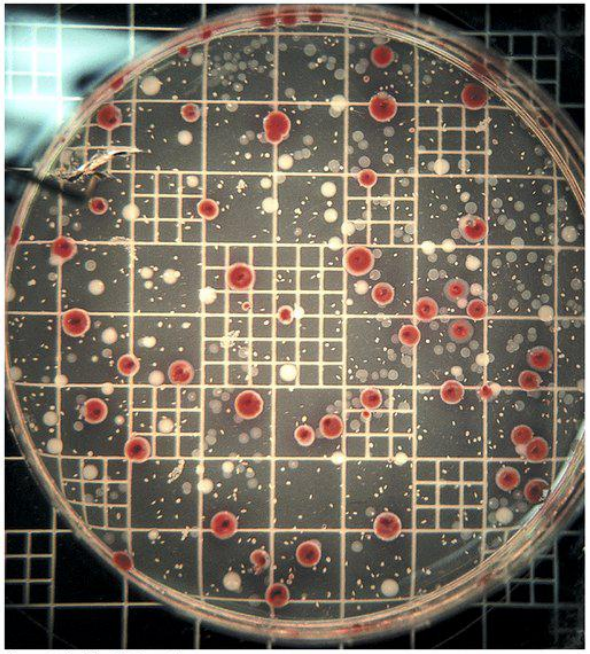

Figure 6-8b Microbiology, 6/e
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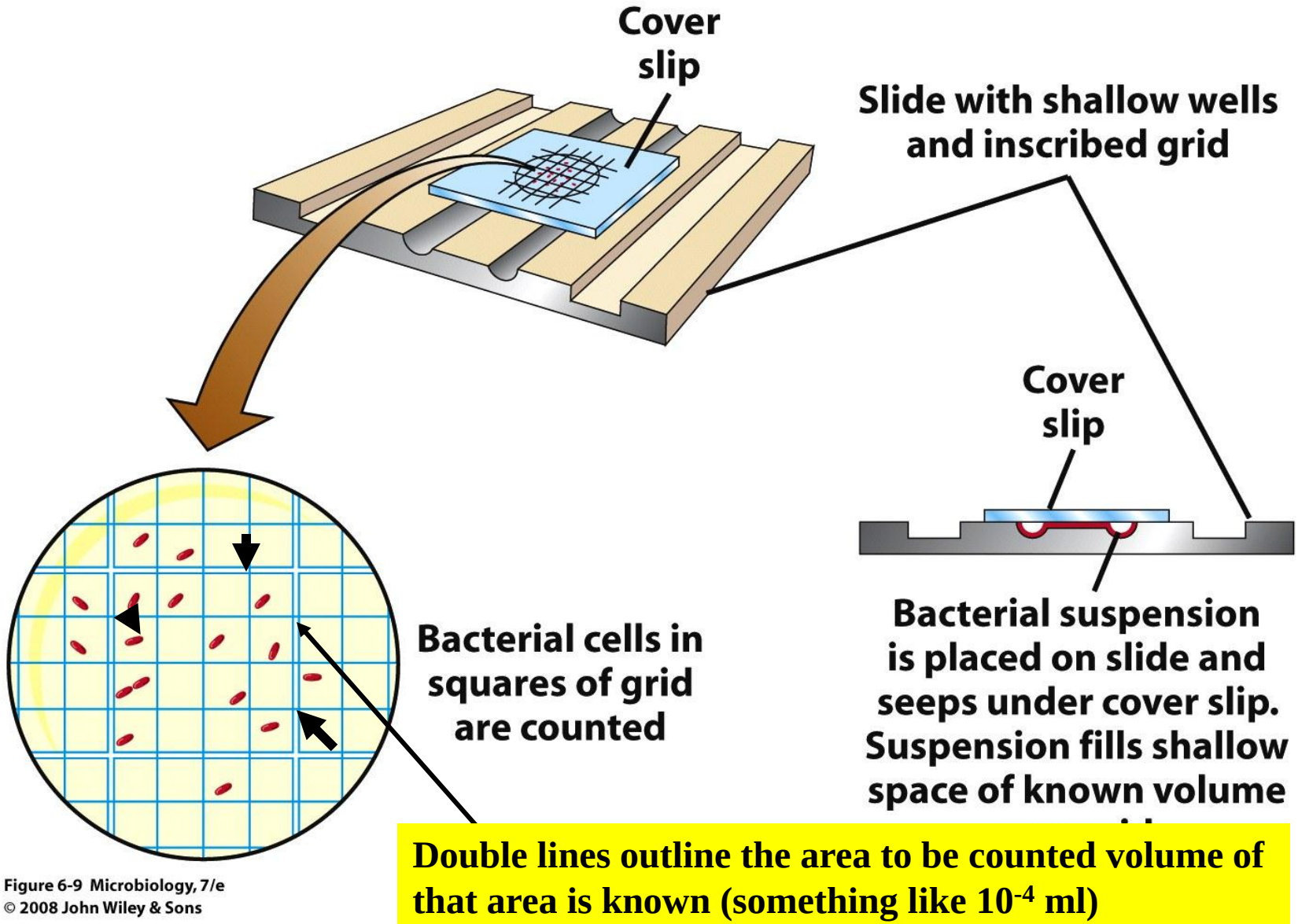
Fig. 6.8 Counting colonies

Direct microscopic counts: advantages and disadvantage



Double lines outline the area to be counted volume of that area is known (something like 10^{-4} ml)

Fig. 6.9 **The Petroff-Hausser counting chamber**

Tuberculosis: Taking High Doses of Vitamin D Speeds Recovery, Scientists ReportHigh doses of vitamin D speed the recovery of tuberculosis patients, according to a new study. The inspiration for testing the idea, scientists from Queen Mary University of London and other British hospitals said, was that 19th-century tuberculosis patients were sent to the mountains to lie in the sun. Ultraviolet B rays in sunshine convert cholesterol in the skin into vitamin D. In the decades before antibiotics, doctors knew that TB patients sometimes recovered, or at least lived longer, at high altitudes. Spas for wealthy patients were built in the Alps, the Rockies and other mountain ranges. Some doctors thought alpine air was the reason TB patients fared better, but others believed in "heliotherapy." Even bedridden patients were wheeled out onto sun decks. **Vitamin D seems to prevent lung damage by slowing down inflammatory responses to the TB bacterium**. Since it does not interfere with the action of antibiotics, it may be useful in other illnesses, like pneumonia, according to the authors of the study, published online by Proceedings of the National Academy of Sciences last week**. In a clinical trial of 95 patients on antibiotics, those who also got vitamin D had less inflammation, and the mycobacteria in their lung phlegm cleared up 13 days earlier on average. Tuberculosis kills 1.5 million people a year and is a common co-infection in people with AIDS. Drug-resistant strains are becoming more common.**

Alzheimer's Drug Shows No Benefit in Large Study A highly anticipated experimental drug for Alzheimer's disease was shown to be ineffective in its first large clinical trial. The phase 3 trial of 1,100 patients with mild to moderate Alzheimer's found that bapineuzumab provided no benefits, *The New York Times* reported.

The findings will be presented at a medical meeting in September, according to Pfizer, one of the three companies behind the drug.

All the patients in the study had the ApoE4 gene, which increases the risk that a person will develop Alzheimer's and can make the disease worse. Findings from a phase 2 study suggest that bapineuzumab may be more effective in patients without the gene, *The Times* reported.

The drug was designed to target beta-amyloid, a protein that has toxic effects in the brain and is widely believed to be a

Failed J&J/Pfizer Alzheimer's drug was hitting target : studiesCHICAGO (Reuters) - Data from two large studies of Pfizer Inc and Johnson & Johnson's Alzheimer's drug, bapineuzumab, show the treatment reduced **underlying markers of the disease in some patients, suggesting the failed medication might work at an earlier stage.** The findings, presented at a European neurology meeting in Stockholm, followed the companies' announcement last month that they were scrapping large-scale clinical trials of the drug after it failed to improve memory or thinking skills in patients with mild to moderate Alzheimer's. **Many researchers had long expected bapineuzumab to fail this test because they believe Alzheimer's starts years before memory problems become apparent. These biomarker results show that compared with the subjects who were give a placebo, bapineuzumab significantly reduced the amount of the protein beta amyloid on the brain scans of patients with a gene mutation that increases their risk of Alzheimer's. The drug also significantly reduced the amount of a toxic form of the protein tau in spinal fluid, a sign of brain cell death, compared with patients who were given a placebo.**

However, MRI tests showed patients in the treatment and placebo groups had a similar loss of brain volume.

Counting Small Populations

Volume of Dilution Added	Culture Results	Number of Positive Tubes
10 ml	+ + + + +	5
1 ml	+ – – – +	2
0.1 ml	– – – – –	0

Figure 6-10a Microbiology, 7/e

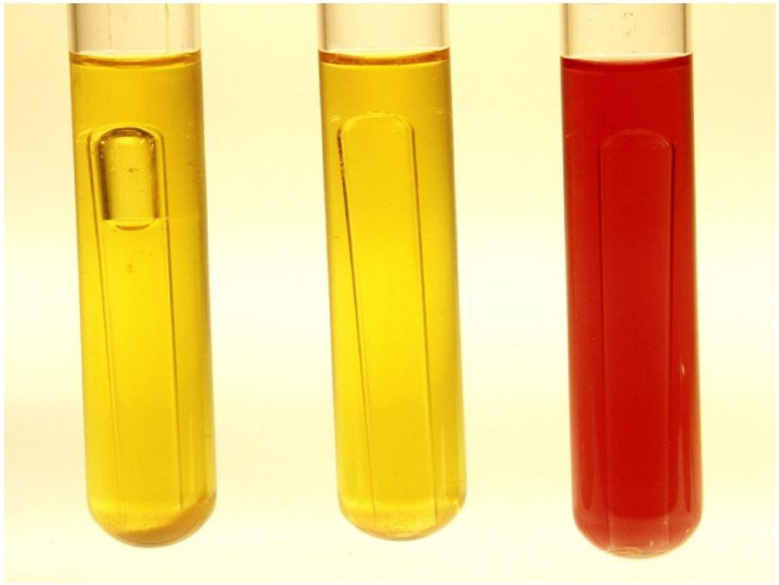


Figure 6-10b Microbiology, 7/e
Courtesy Jacquelyn G. Black

1 is a positive in which acid (yellow color) and gas are produced, 2, only acid is produced and 3 no reaction (neither acid or gas is produced).

Fig. 6.10 Most Probable Number: Method for testing very dilute samples such as water samples can have very few organisms. The method is based on a statistical probabilities

Most Probable Number (/) Index for Combinations of Positive and Negative Results When Five Tubes Are Used per Dilution (Five Each of 10 ml, 1 ml, and 0.1 ml)

Number of Tubes with Positive Results							
10 ml	1 ml	0.1 ml	MPN Index/100 ml	10 ml	1 ml	0.1 ml	MPN Index/100 ml
0	0	0	<2	4	3	1	33
0	0	1	2	4	4	0	34
0	1	0	2	5	0	0	23
0	2	0	4	5	0	1	30
1	0	0	2	5	0	2	40
1	0	1	4	5	1	0	30
1	1	0	4	5	1	1	50
1	1	1	6	5	1	2	60
1	2	0	6	5	2	0	50
2	0	0	4	5	2	1	70
2	0	1	7	5	2	2	90
2	1	0	7	5	3	0	80
2	1	1	9	5	3	1	110
2	2	0	9	5	3	2	140
2	3	0	12	5	3	3	170
3	0	0	8	5	4	0	130
3	0	1	11	5	4	1	170
3	1	0	11	5	4	2	220
3	1	1	14	5	4	3	280
3	2	0	14	5	4	4	350
3	2	1	17	5	5	0	240
4	0	0	13	5	5	1	300
4	0	1	17	5	5	2	500
4	1	0	17	5	5	3	900
4	1	1	21	5	5	4	1600
4	1	2	26	5	5	5	≥1600
4	2	0	22				
4	2	1	26				
4	3	0	27				

Table 6-1 Microbiology, 7/e

5 tubes used at all concentrations

10 mls of sample added to 5 tubes yields 5 positive tubes, 1 ml of sample added to 5 tubes yields 2 positives tubes and 0.1ml of sample yields 0 out of 5 tubes. The most probable number of organisms per 100 mls. is 50.

Fig. 6.10 Most Probable Number: Method for testing very dilute samples such as water samples can have very few organisms. The method is based on a statistical probabilities

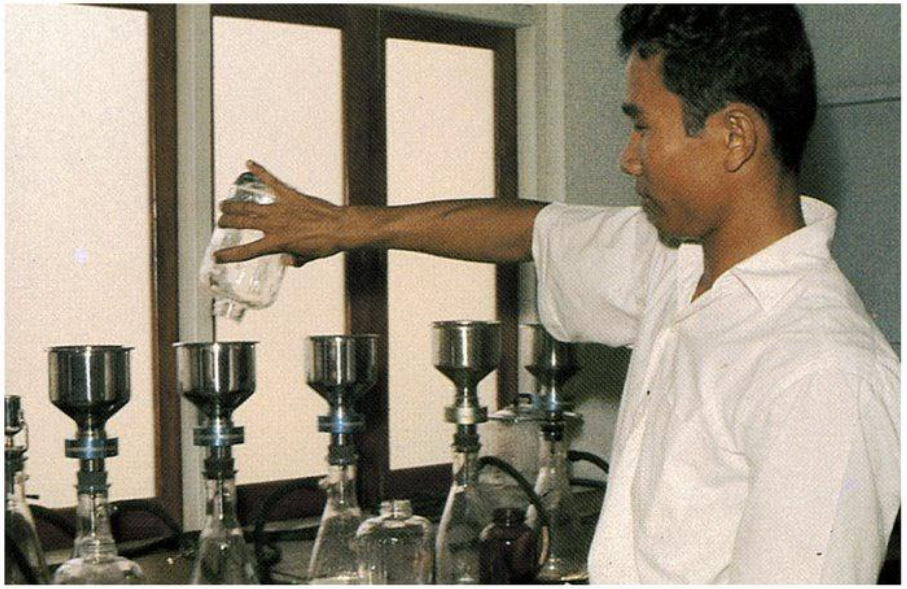


Figure 25-20a Microbiology, 6/e

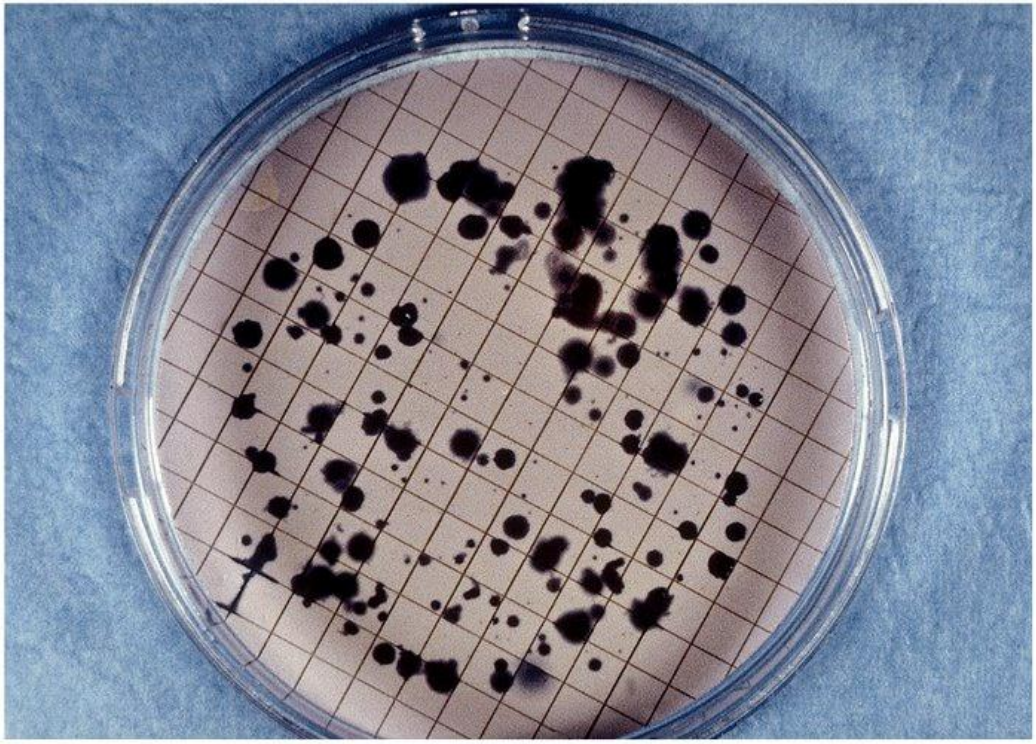


Figure 25-20b Microbiology, 6/e

What do you think the major Advantage of this approach to measuring the number of bacteria versus streak or pour plate methods?

Fig.25.20 The membrane filter test for water purity

Other Measurements

- Spectrophotometer

Courtesy Thermo Electron Corporation

- Tube turbidity

Richard Megna/Fundamental Photographs

- Dry Weight Measurement

Physical Factors Affecting Bacterial Growth

Temperature According to their growth temperature range, bacteria can be classified as: psychrophilic, mesophilic, and thermophilic bacteria

a. **psychrophiles-** (cold loving) grow best at temperature of 15C to 20C. Can be subdivided into obligate (e.g., ***Bacillus globisporus)***, which cannot grow above 20C and facultative psychrophiles (e.g., ***Xanthomonas pharmicola***) which grows best below 20C but also can grow above that temperature. Hence, they do not grow in the human body but can be important pathogens associated with food spoilage of refrigerated or even frozen foods (e.g., ice cream) *(e.g,* ***Listeria sp.*****(facultative to psychrotolerant))**

b. **mesophiles-** includes most bacteria and growth is best between 25C and 40C. Most human pathogens are included in this category. ***Thermoduric*** organisms ordinarily live as mesophiles but can withstand short periods of exposure to high temperatures.

c. **thermophiles-** (heat loving**)** grow best at temperatures from 50-60-C. They can be further classified as obligate thermophiles, which can only grow at temperatures above 37C,, or facultative thermophiles which can grow both above or below 37C. *Bacillus stearothermophilus*, which is usually considered an obligate thermophile, grows at its maximum rate at 65 to 75C but can display minimal growth and cause food spoilage at temperatures as low as 30C.

You will have to know examples, i.e., specific organisms.

Listeria Outbreak Traced to Cantaloupe Packing Shed Government investigators said that workers had tramped through pools of water where listeria was likely to grow, tracking the deadly bacteria around the shed, which was operated by Jensen Farms, in Granada, Colo. The pathogen was found on a conveyor belt for carrying cantaloupes, a melon drying area and a floor drain, among other places. The outbreak, which began in late July, is the deadliest caused by foodborne disease since 1985. A total of 123 people in 26 states have fallen ill, including those who died, according to the Centers for Disease Control and Prevention. The farm had passed a food safety audit by an outside contractor just days before the outbreak began. Eric Jensen, a member of the family that runs the farm, said in an e-mail that the auditor had given the packing plant a score of 96 points out of 100. The food industry increasingly has come to rely on what it calls third-party audits of farms or processing plants to ensure the safety of food. But the auditors are hired by the companies being inspected, and their procedures are largely unregulated. In several recent food safety lapses, the facilities involved had passed third-party audits. Listeria is frequently found in soil or manure, but tests of the soil on the farm did not turn up the bacteria. Officials said that a dump truck used to take culled melons to a cattle farm was parked near the processing shed and could have brought bacteria to the facility**. Listeria is frequently found in soil or manure, but tests of the soil on the farm did not turn up the bacteria**. Officials said that a dump truck used to take culled melons to a cattle farm was parked near the processing shed and could have brought bacteria to the facility.

Factors affecting bacterial growth- obligate means must grow under a certain set of conditions (e.g., obligate acidophile must grow under acidic conditions) whereas, a facultative organism has the ability to grow under a certain set of conditions but typically grows under more temperate conditions). Most organisms do not grow more than 1 pH unit above or below their optimum pH. Depending on their pH optimum they are classified as:

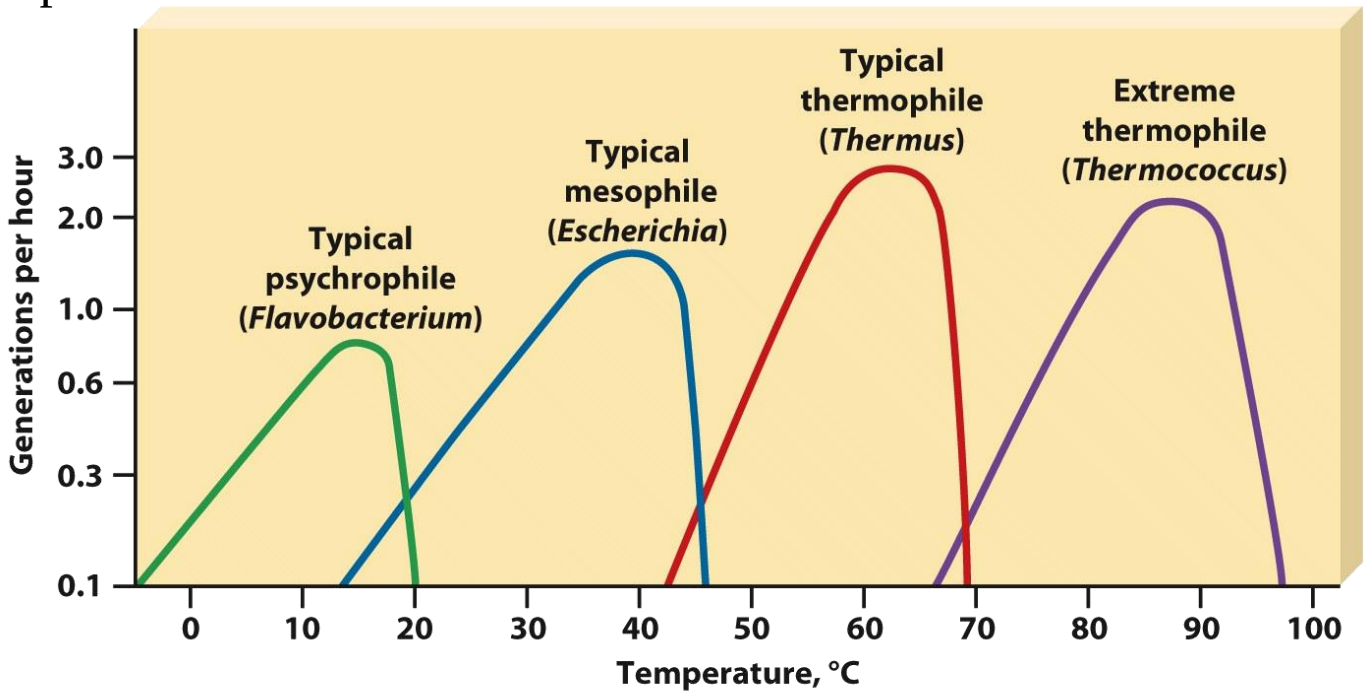
acidophiles (pH 0.1 to 5.4)- acid loving *(Thiobacillus sp.)*

neutrophiles (pH 5.4-8.0), or- neutral (most disease organsims in this group.

alkaliphiles (pH7.0-11.5)- alkali-loving (soil bacterium *Agrobacterium sp.* Grows in alkali soil pH 12.0)

Physical Influences on Growth

- pH
- Temperature



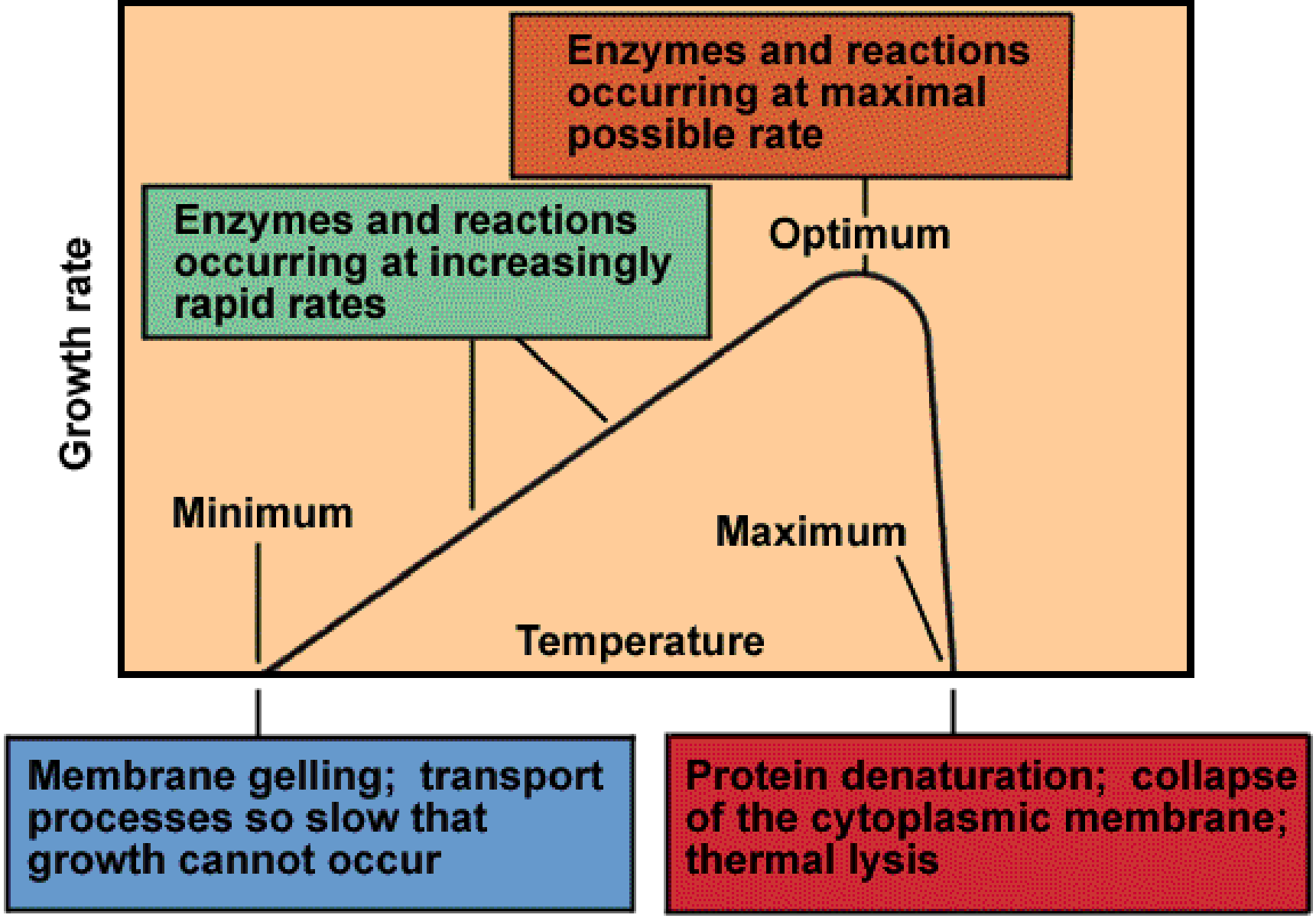
*** Thermoduric organisms typically grow as mesophiles but can withstand exposure to short periods of high temperature that can lead to spoilage of canned goods or milk products**

Fig. 6.14 **Growth rates of psychrophilic, mesophilic and themophilic bacteria**

Minimal growth temperatures, the lowest temperature at which cells can divide

Maximum growth temperature, the highest temperature at which cells can divide

Optimum growth temperature, the temperature at which cells divide most rapidly-that is, have the shortest generation time



Effect of temperature on different properties of bacterial cells

Growth positions in Thioglycollate medium

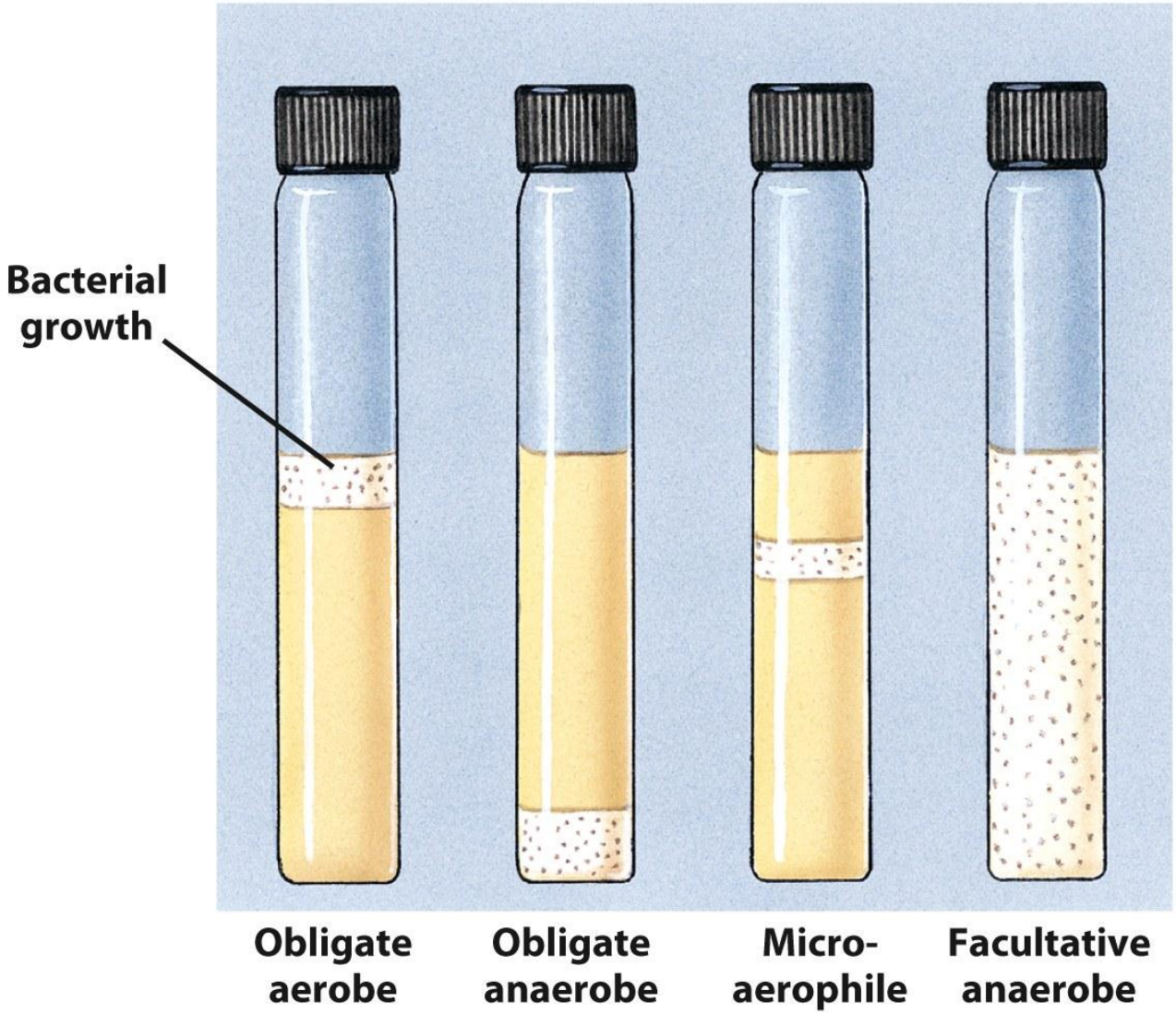


Fig. 6.15 **Patterns of oxygen use**

Oxygen's Influence on Growth

- Aerobes
  - Use O_2 in their metabolic pathways
  - Obligate Aerobes
  - Microaerophiles
- Anaerobes
  - Do not use O_2 in their metabolic pathways
  - Obligate Anaerobes
  - Facultative Anaerobes
  - Aerotolerant Anaerobes

Oxygen

obligate aerobes, e.g., *Pseudomonas sp.* common cause of nosocomial infections

obligate anerobes e.g, *Bacteroides sp*.-killed by free oxygen-a prominent microganism in the gut

microaerophiles e..g, *Camplyobacter sp. require small but definite amounts of oxygen (Camplybacter is also* a capnophiles or carbon dioxide loving organism (require CO_2 at levels higher than is normally present in atmosphere)).

facultative anaerobes- ordinarily grow fine in oxygen but can grow under anaerobic conditions, e.g., *Escherichia coli*

Aerotolerant anaerobes can survive in the presence of oxygen but do not use it in their metabolism. *Lactobacillus sp.* is always fermentative whether or not oxygen is present.

Whooping cough vaccine loses punch too fastNEW YORK (AP) — As the U.S. wrestles with its biggest whooping cough outbreak in decades, researchers appear to have zeroed in on the main cause: **The safer vaccine that was introduced in the 1990s loses effectiveness much faster than previously thought**. A study published in Wednesday's New England Journal of Medicine found that the protective effect weakens dramatically soon after a youngster gets the last of the five recommended shots around age 6. The protection rate falls from about 95 percent to 71 percent within five years, said researchers at the Kaiser Permanente Vaccine Research Center in Oakland, Calif. The U.S. has had more than 26,000 whThe substantial majority of the cases are explained by this waning immunity," said Dr. William Schaffner, an infectious-disease specialist at Vanderbilt University. In light of the findings and earlier, similar research, health officials are considering recommending another booster shot for children, strengthening the vaccine or devising a brand new one. Whooping cough, or pertussis, is a highly contagious bacterial disease that can strike people of any age but is most dangerous to children. Its name comes from the sound youngsters make as they gasp for breath. It used to be common, causing hundreds of thousands of illnesses annually and thousands of deaths. Cases dropped after a vaccine was introduced in the 1940s, and for decades, fewer than 5,000 a year were reported in the U.S. Because of **side effects that included pain and swelling at the injection site, fever and apparently, in rare cases, brain damage, the vaccine was replaced in the 1990s. The newer version used only parts of the bacterium instead of the whole thing and carried fewer complications. But cases of whooping cough began to climb, sometimes topping 25,000 a year during the past decade. Also disturbing: The proportion of cases involving children ages 7 to 10 — most of them vaccinated — rose from less than 10 percent before 2006 to nearly 40 percent this year, according to the CDC. The researchers found that the risk of getting whooping cough increases by about 42 percent a year after a child's last dose of vaccine.** Health officials have long recommended that children get vaccinated in five doses, with the first shot at 2 months and the final one between 4 and 6 years, and receive a booster shot at 11 or 12.

Now there's a growing consensus that something more needs to be done. Ideas include somehow pumping up the effectiveness of the vaccine or developing a new one.

A Dose of 'Good' Bacteria Could Help Your Sinus Problems Some chronic sinus problems may be caused by a depletion of "good" bacteria and the presence of "bad" bacteria in the sinuses, a new study says. The findings suggest adding back some of these good bacteria to the sinuses may help treat the condition, analogous to the way probiotics may treat certain intestinal problems. In the study, the researchers analyzed the populations of bacteria present in the sinuses of 10 people with chronic sinus problems, known as chronic rhinosinusitis, and 10 healthy people. They identified the bacteria species by looking at their genes. People with chronic sinus problems had fewer types of bacteria in their sinuses compared with healthy people, and a significant reduction in bacteria that produce lactic acid. In addition, they had an increase in a bacteria species called *C. tuberculostearicum*. (*Corynebacterium*). **When the researchers gave mice antibiotics to eliminate the normal bacteria in their sinuses, and then gave them C. tuberculostearicum, the mice developed symptoms of chronic rhinosinusitis**. However, mice that received the lactic acid **bacteria L. sakei** in addition to C. tuberculostearicum did not develop symptoms of chronic rhinosinusitis. **The findings add to a growing body of research showing that the bacterial community in the human body as a whole, and not the presence of a single harmful species, is responsible for the development of certain diseases, the researchers said. The findings also suggest L. sakei could be used to treat or prevention of chronic sinus problems, the researchers said.**

The following slide addresses the question of why obligate anaerobic bacteria are killed in the presence of oxygen.

(a) Catalase:

$$H_2O_2 + H_2O_2 \longrightarrow 2\,H_2O + O_2$$

(b) Peroxidase:

$$H_2O_2 + NADH + H^+ \longrightarrow 2\,H_2O + NAD^+$$

(c) Superoxide dismutase:

$$O_2^- + O_2^- + 2\,H^+ \longrightarrow H_2O_2 + O_2$$

(d) Superoxide dismutase/catalase in combination:

$$4\,O_2^- + 4\,H^+ \longrightarrow 2\,H_2O + 3\,O_2$$

Obligate aerobes and facultative organisms have both catalase and SOD; some facultative and aerotolerant anaerobes have SOD but lack catalase. Most obligate anaerobes lack both enzymes

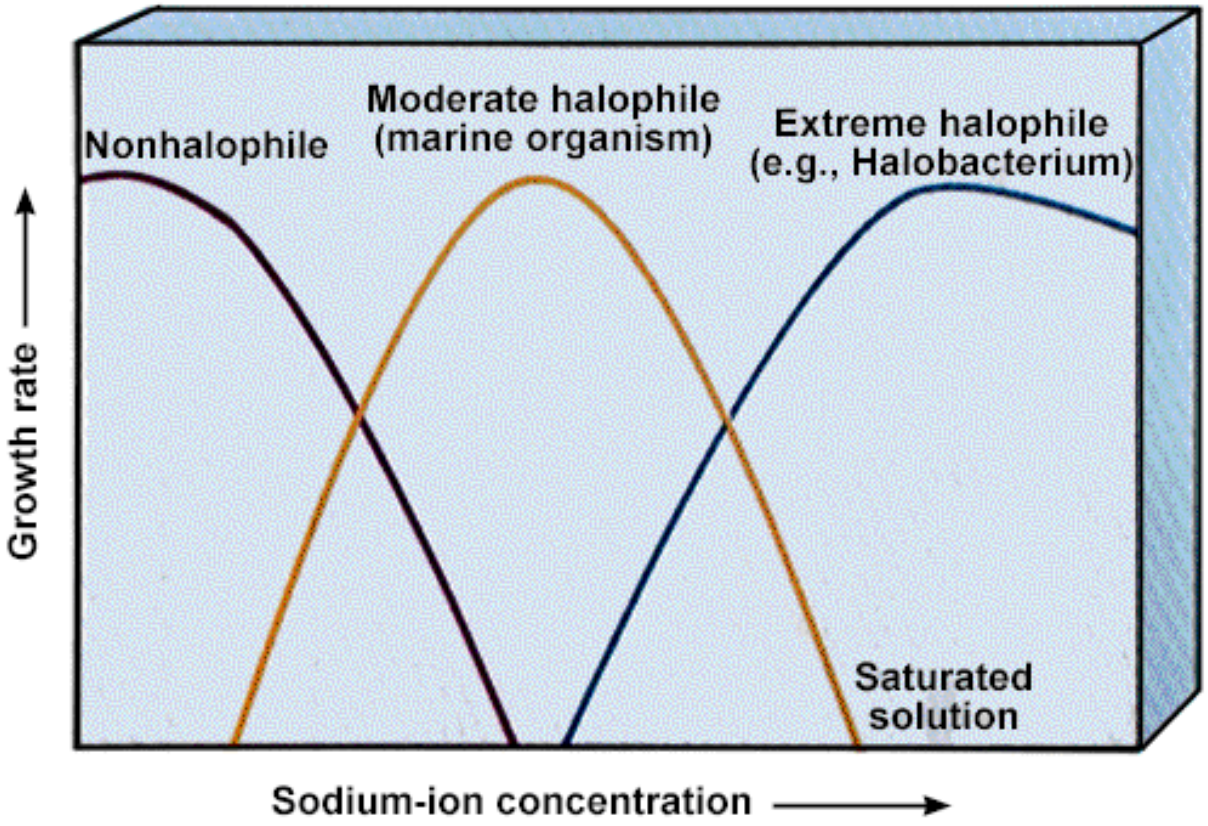
Peroxisome

Superoxide is a highly toxic product- as is hydrogen peroxide- Hence, if they are not removed the cell dies.

Obligate anaerobes are killed by a toxic form of oxygen termed **superoxide (O_2^-)**. Superoxide is converted to O_2 and toxic hydrogen peroxide (H_2O_2) by an enzyme called **superoxide dismutase**. H_20_2 is converted to water and molecular oxygen by an enzyme termed **catalase.**

Role of catalase, peroxidase and superoxide dismutase in anaerobiosis

Halophiles are High Sodium requiring organisms

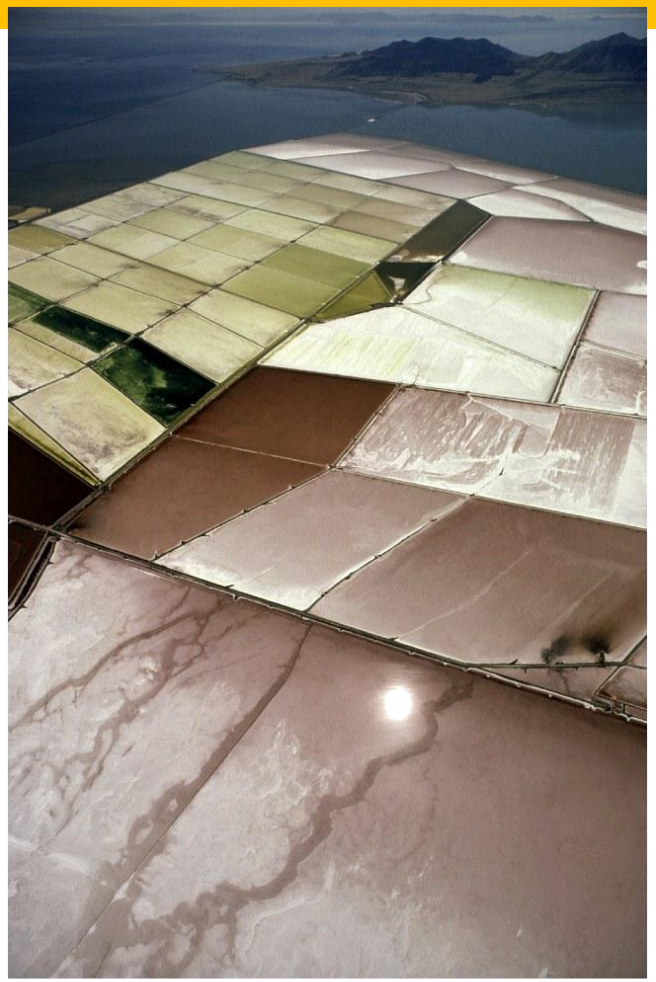


Extreme halophiles require salt concentrations of 20-30% (dead sea (approx. 32% salt). Most halophiles are found in the sea where the salt is around 3.5%. These organisms actively pump sodium out of the cell and retain potassium. It is thought that the high potassium is needed for their enzyme function and the high salt many contribute to the structural integrity of their cell wall

Fig. 6.16 **Responses to salt-** growth rates of halophilic (salt-loving) and non halophilic organisms are related to sodium ion concentration.

Physical Influences on Growth

- Moisture
- Hydrostatic pressure
- Osmotic pressure
- Radiation



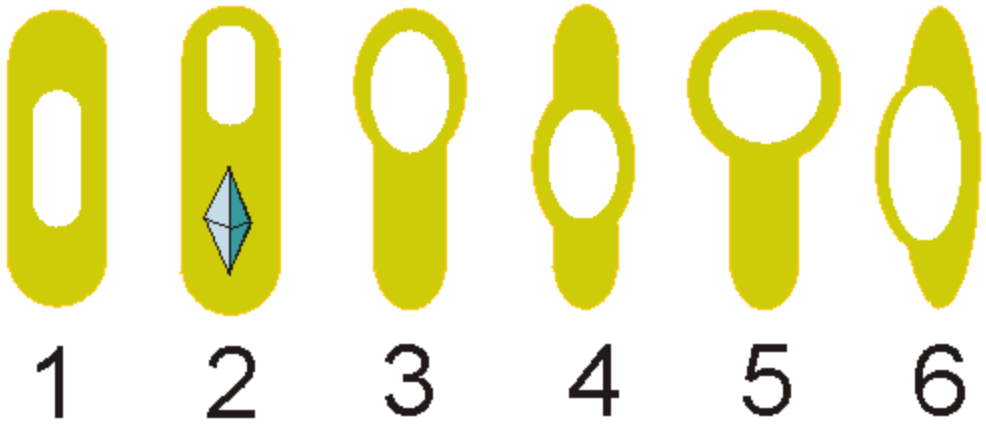
James L. Amos/National Geographic/Getty Images

Hydrostatic Pressure- Organisms, termed Barophiles, that live at the bottom of lakes or deep in the ocean.

It is thought that the hydrostatic pressure is necessary to maintain the proper three dimensional configuration of their proteins, i.e., enzymes.

Most of these organisms can live only a short time at standard atmospheric pressure. Hence, when they are studies it must be done under high pressure

Sporulation- The formation of endospores. Occurs in *Bacillus, Clostridium* and other *Gram-positive genera e.g., Sporosarcina* and *Gram negative species.* When nutrients such as *carbon or nitrogen* become *limiting*, highly resistant endospores form inside the mother cell. Although endospores are not metabolically active, they can survive long periods of drought and are resistant to killing by extreme temperatures, radiation, and some toxic chemicals.



Variations in endospore morphology:
(1, 4) central endospore; (2, 3, 5) terminal endospore; (6) lateral endospore

http://student.ccbcmd.edu/courses/bio141/lecguide/unit1/prostruct/sporeform_an.html

Sporulation

- Axial nucleiod
- Core structure
- Endospore septum
- Cortex
- Spore coat

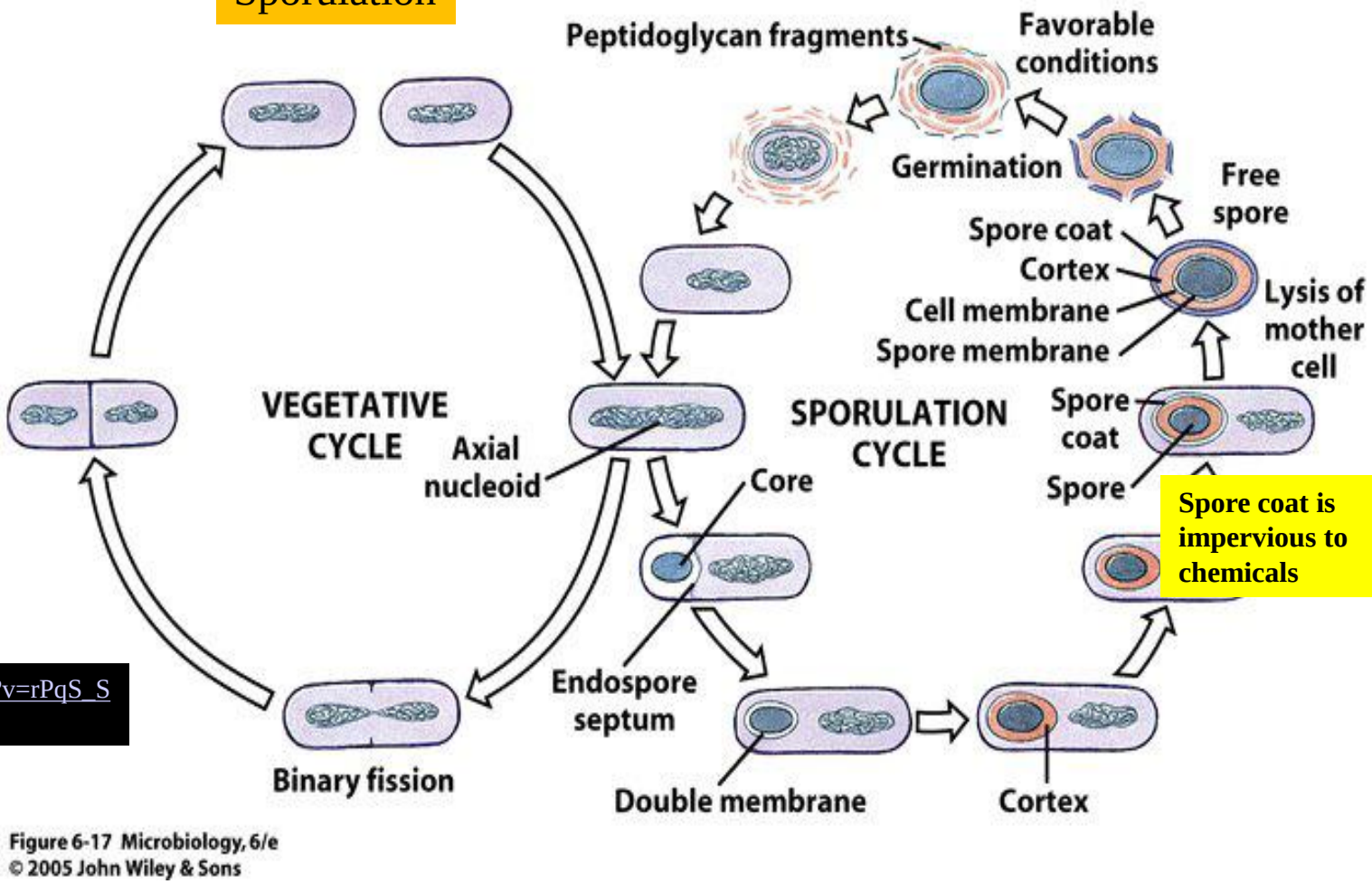


Figure 6-17 Microbiology, 6/e
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http://www.youtube.com/watch?v=rPqS_Sg-1W8&feature=related

Spore coat is impervious to chemicals

Spores are largely produced by species of ***Clostridium*** **and** ***Bacillus.*** A few other Gram positive organisms also produce spores (e.g., ***Sporosarcinae).***

Peptidoglycan is synthesized between the two layers of the double membrane to produce the cortex which protects the spore against changes in osmotic pressure

Fig. 617 **The vegetative and sporulation cycles in bacteria capable of sporulation.**

Majors steps in sporulation(spore formation):

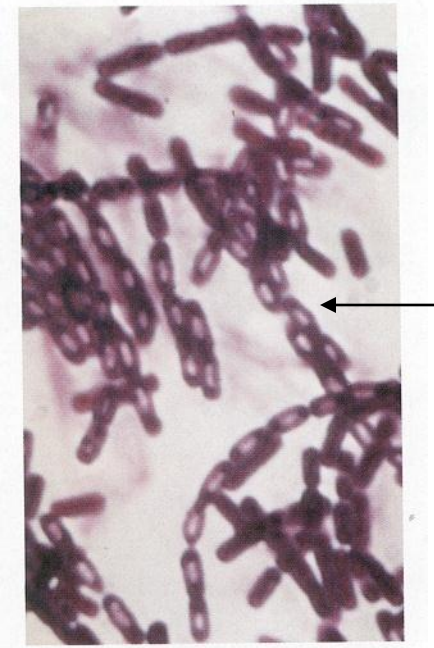
1. Formation of an axial nucleoid (DNA synthesis)
2. Separation of DNA to different locations in the cell
3. The DNA where the endospore willform directs endospore formation
4. Most of the cells RNA and some cytoplasmic protein molecules gather around the DNA to make the **core or living part of the endospore. The core contains dipicolinic acid and calcium which likely contributes to the spores heat resistance by stabilizing proteins- reminder that DPA mutants retain heat resistance.**
5. An **endospore septum, consisting of a cell membrane but lacking a cell wall, grows around the core, enclosing it in a double thickness of cell membrane.**
6. Both layers of his membrane synthesize peptidoglycan and release it into the space between the membranes
7. Thus, a laminated layer called the **cortex is formed. The cortex protects the core against changes in osmotic pressure, such as those that result from drying**
8. A **spore coat of keratin like protein (nails are made of keratin), which is impervious to many chemicals, is laid down around the cortex by the mother cell**
9. Finally in some endospores an exosporium, a lipid-protein membrane, is formed outside the coat by the mother cell.
10. Sporulation takes about 7 hours.

Steps in spore germination-

1. Activation: usually requires some traumatic agent such as low pH or heat, which damages the coat

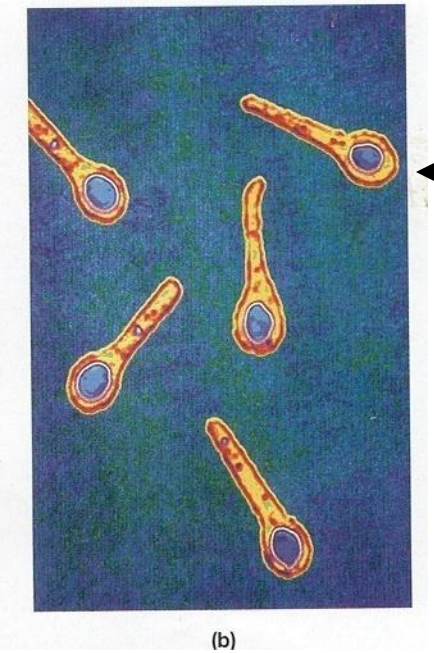
2. **Germination proper, requires water and a germination agent(such as the amino acid alanine or certain inorganic ions) the penetrates the damaged coat. During this process much of the cortical peptidoglycan is broken down, and its fragments are released into the medium. The living cell which occupied the core now takes in large quantities of water and loses its resistance to heat and staining as well as it *refractility.***

3. **Outgrowth occurs in a medium with adequate nutrients.**



(a)

Spore diameter not greater Than the cell diameter and Spore is located centrally



(b)

Spore is terminal and has a greater diameter than the vegetative cell. This drumstick appearance is typical of *Clostridum tetanus.*

Fig. 6.18 **Bacterial endospores in two *Clostridium* species**

Bacterial Interactions

- Quorum sensing
- Biofilms
- Toxin production to limit competitor "groups"
- Sociomicrobiology

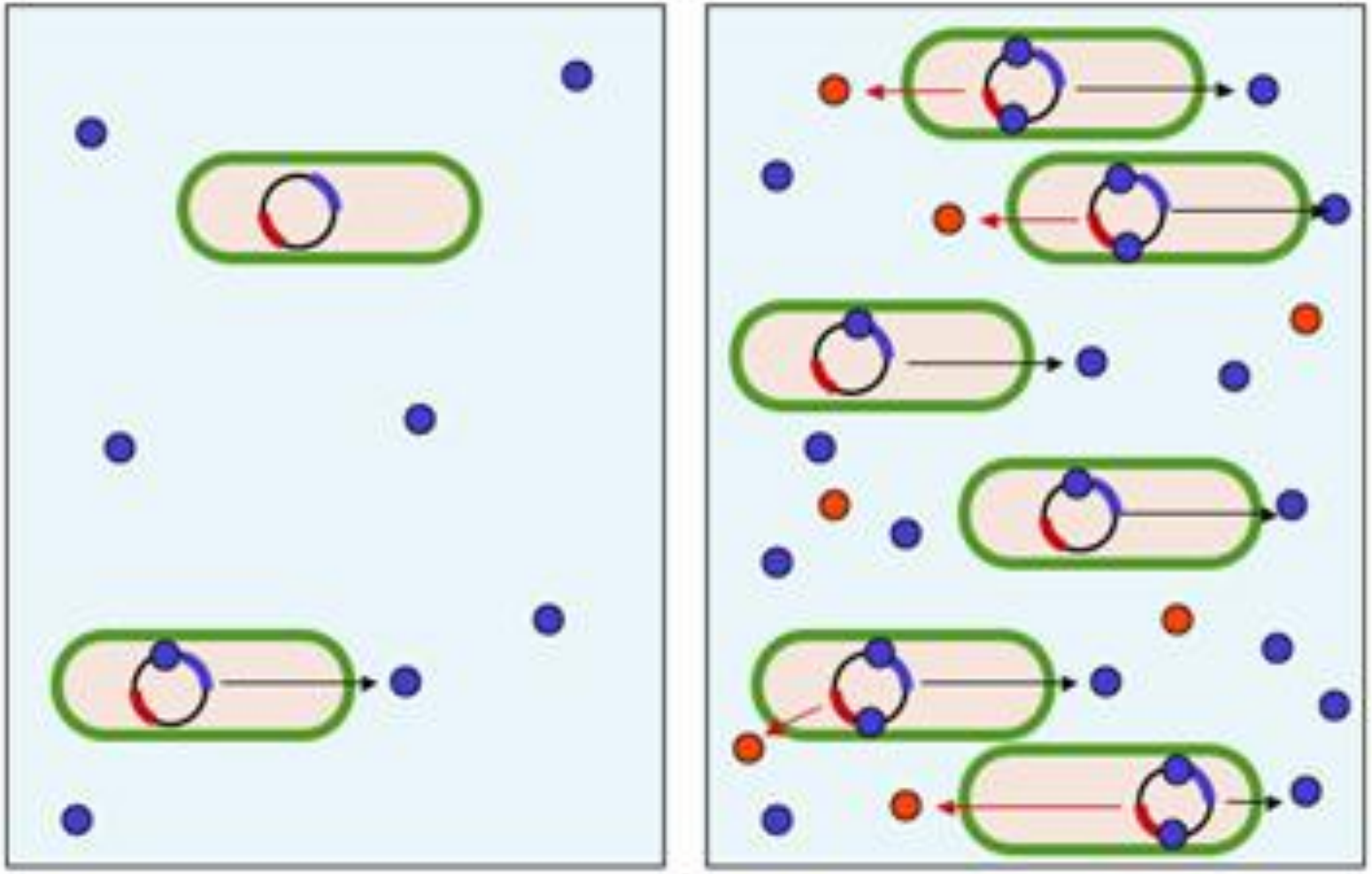


Diagram of quorum sensing. (left) In low density, the concentration of the autoinducer (blue dots) is relatively low and the substance production is restricted. (right) In high density, the concentration of the autoinducer is high and the bacterial substances (red dots) are produced

Microarray Technology for differentiating between organisms or metabolic states

http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter16/animations.html

The above microarray shows how to identify specific genes