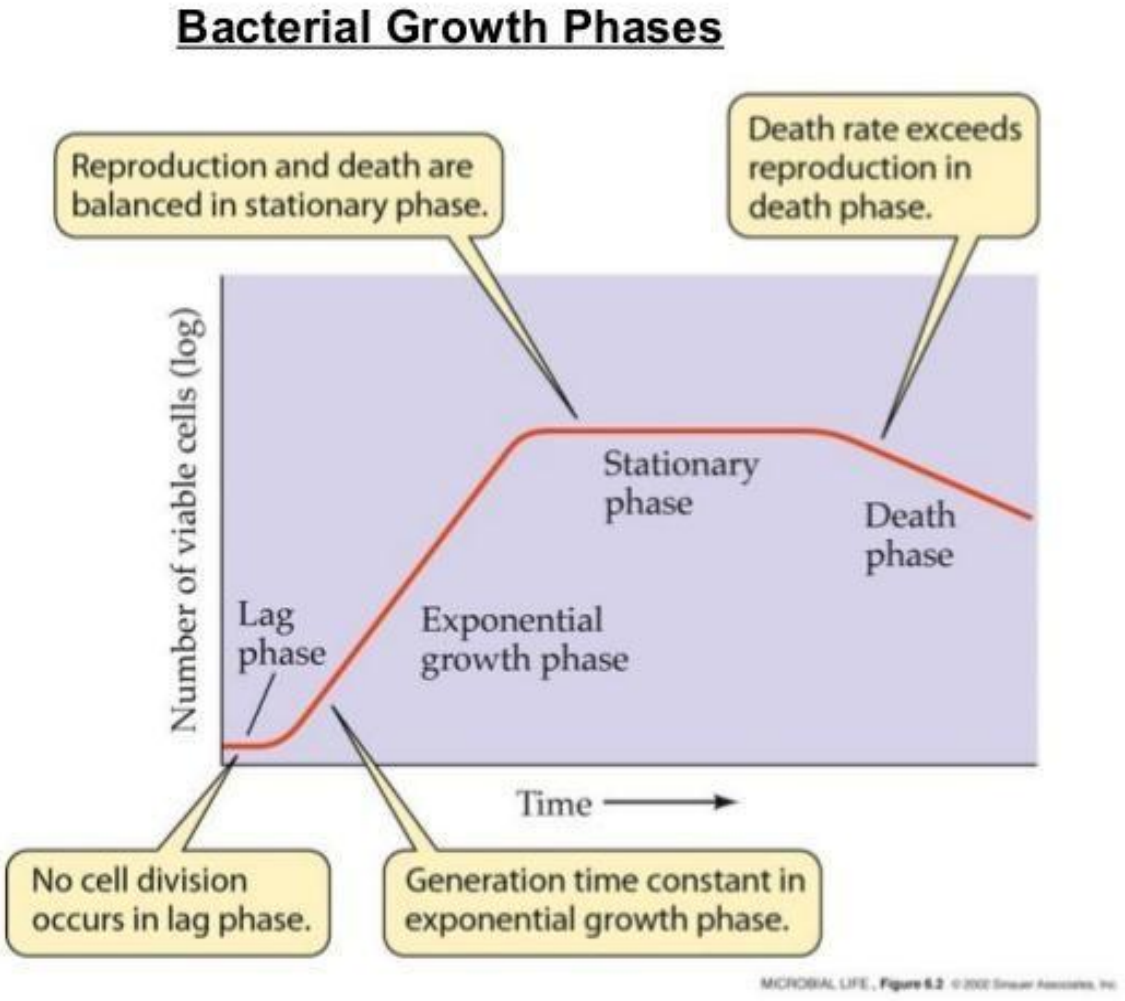
Lec. 1

The Bacterial Growth Curve

In the laboratory, under favorable conditions, a growing bacterial population doubles at regular intervals. Growth is by geometric progression: 1, 2, 4, 8, etc. or 2^0, 2^1, 2^2, 2^3.....2^n (where n = the number of generations). This is called **exponential growth**

- When a fresh medium is inoculated with a given number of cells, and the population growth is monitored over a period of time, plotting the data will yield a **typical bacterial growth curve**


Bacterial Growth Phases
Reproduction and death are balanced in stationary phase.
Death rate exceeds reproduction in death phase.
Number of viable cells (log)
Stationary phase
Death phase
Lag phase
Exponential growth phase
Time
No cell division occurs in lag phase.
Generation time constant in exponential growth phase.

Four main characteristic phases of the growth cycle are recognized:

1. *Lag Phase*

Immediately after inoculation of the cells into fresh medium:

a) the population remains temporarily unchanged

b) there is no apparent cell division occurring

c) the cells may be growing in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity.

The length of the lag phase is apparently dependent on a wide variety of factors including:

a) the size of the inoculum.

b) time necessary to recover from physical damage or shock in the transfer.

c) time required for synthesis of essential coenzymes or division factors.

d) time required for synthesis of new (inducible) enzymes that are necessary to metabolize the substrates present in the medium.

2. *Exponential (log) Phase*

The exponential phase of growth is a pattern of balanced growth where in:

a) all the cells are dividing regularly by binary fission, and are growing by geometric progression.

b) The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation.

c) The rate of exponential growth of a bacterial culture is expressed as **generation time**, also the **doubling time** of the bacterial population.

((Generation time (G) is defined as the time (t) per generation (n = number of generations). Hence, G=t/n is the equation from which calculations of generation time derive))

3. *Stationary Phase*

— Exponential growth cannot be continued forever in a **batch culture** (e.g. a closed system such as a test tube or flask). Population growth is limited by one of three factors:

1. exhaustion of available nutrients.

2. accumulation of inhibitory metabolites or end products.

3. exhaustion of space, in this case called a lack of "biological space".

- During the stationary phase, if viable cells are being counted, it cannot be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing.
- The stationary phase, like the lag phase, is not necessarily a period of ***quiescence***.
- Bacteria that produce **secondary metabolites**, such as antibiotics, do so during the stationary phase of the growth cycle ***(Secondary metabolites are defined as metabolites produced after the active stage of growth).***
- It is during the stationary phase that spore-forming bacteria have to induce or unmask the activity of dozens of genes that may be involved in sporulation process.

4. ***Death (decline) Phase***

- If incubation continues after the population reaches stationary phase a death phase follows, in which the viable cell population declines. (Note, if counting by turbidimetric measurements or microscopic counts, the death phase cannot be observed.).

- During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase.

- In most cases the rate of cell death is much slower than that of exponential growth.

- Frequently, after the majority of cells have died, the death rate decreases drastically, so that a small number of survivors may persist for months or even years. This persistence may in some cases reflect cell turnover, a few cells growing at the expense of nutrients released from cells that die and lyses.

Calculation of Generation Time

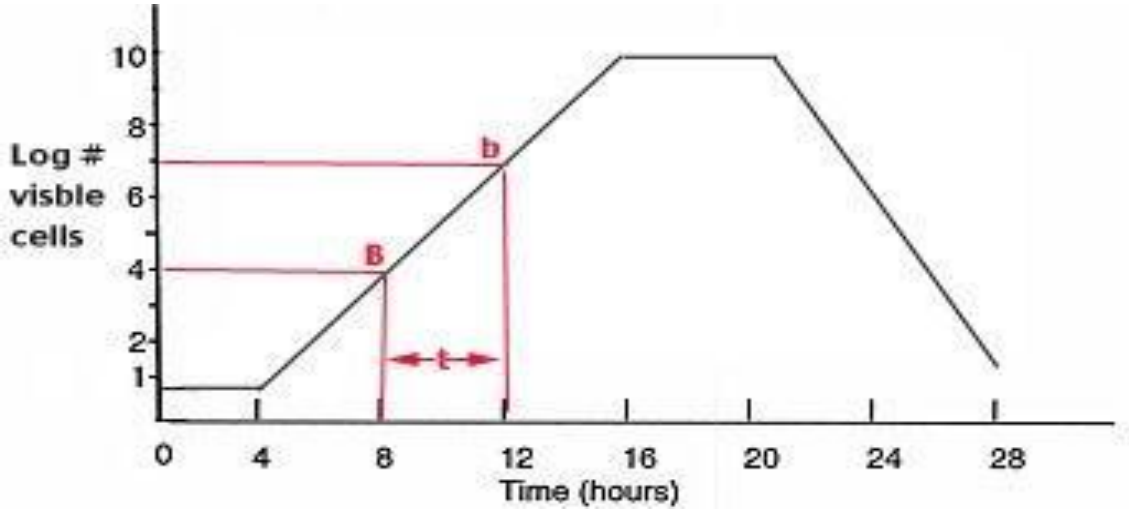
- When growing exponentially by binary fission, the increase in a bacterial population is by geometric progression. If we start with one cell, when it divides, there are 2 cells in the first generation, 4 cells in the second generation, 8 cells in the third generation, and so on. The **generation time** is the time interval required for the cells (or population) to divide.

- G (generation time) = (time, in minutes or hours)/n(number of generations)

- G = t/n

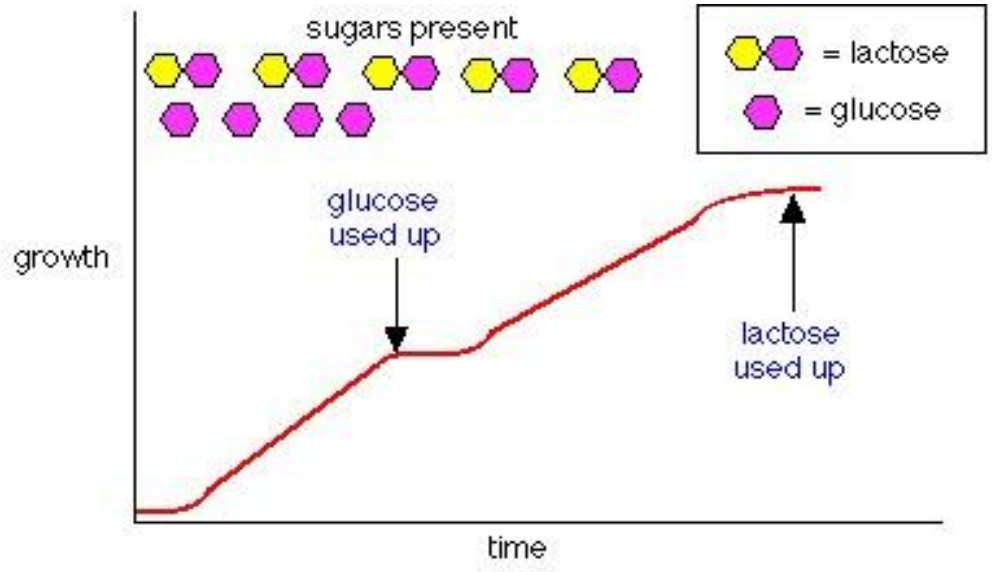
- t = time interval in hours or minutes
- B = number of bacteria at the beginning of a time interval
- b = number of bacteria at the end of the time interval
- n = number of generations (number of times the cell population doubles during the time interval)
- $b = B \times 2^n$ (This equation is an expression of growth by binary fission)
- G= t/3.3logb/B

- **Example: What is the generation time of a bacterial population that increases from 10,000 cells to 10,000,000 cells in four hours of growth?**



Diauxic Growth of Microorganisms

In a medium containing two carbon sources, bacteria such display a growth curve which is called diauxic



Diauxic growth is a phenomenon in which the microorganism grows successively on two different substrates. In the presence of multiple carbon sources, the microorganism will preferentially consume one carbon substrate for growth, and only after that carbon substrate is (substantially) depleted, will the microorganism consume the second substrate for growth. In between, a short lag period is seen.

Microbes have a choice of carbon source in the medium: a) In most cases the substrate which permits the highest growth rate will be used up first.

b) They will utilise first the one which is closest to the one they were using before inoculation.

c) They will use the simplest structure first (usually glucose)

Key features of diauxic growth:

a) Initially the growth curve is the same as a standard growth curve.

b) The *stationary phase* is in fact another *lag phase.*

c) In this second *lag phase* new enzymes are being synthesised by the bacteria to utilise the secondary carbon source.

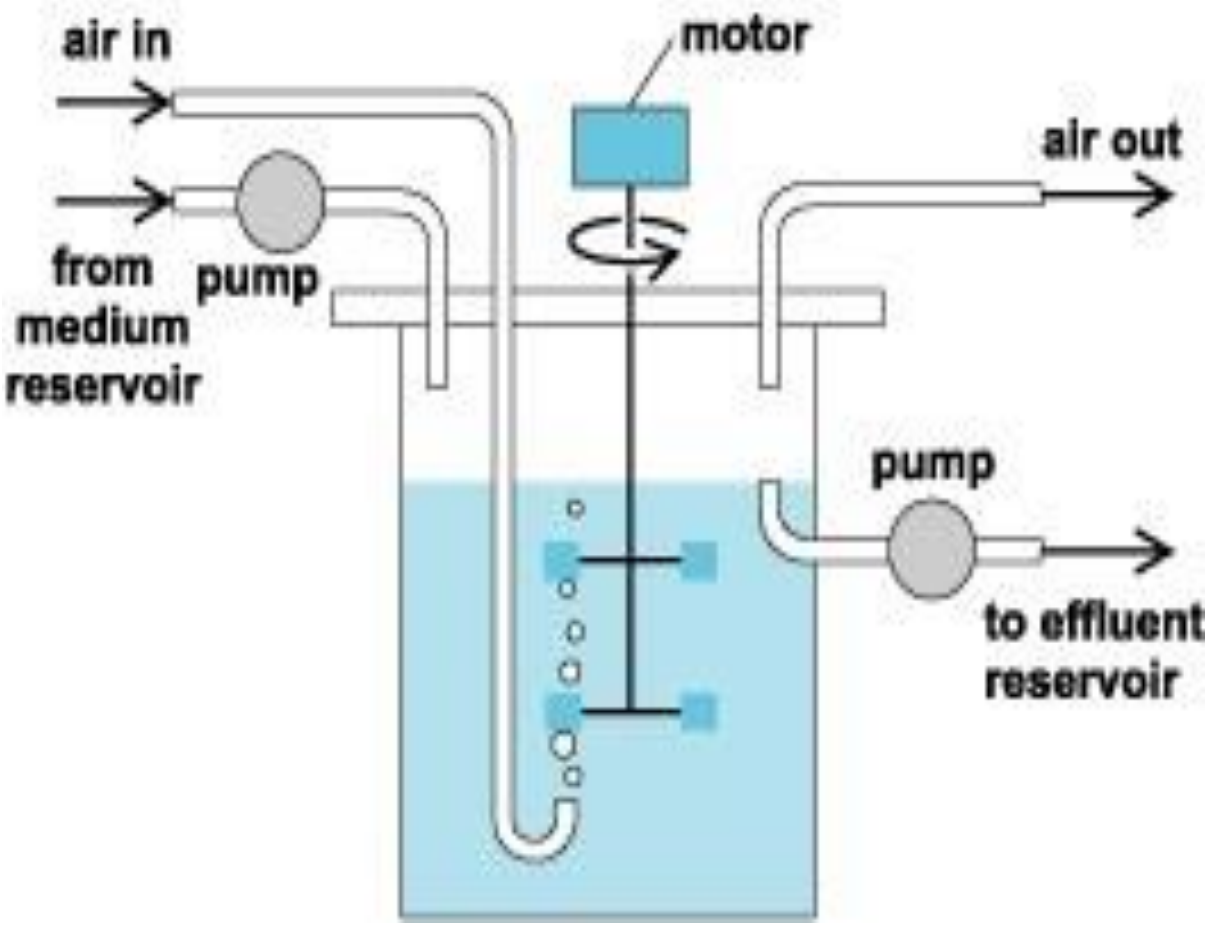
d) The second *lag phase* is then followed by a second *exponential phase* followed by the *stationary phase* and *death phase*

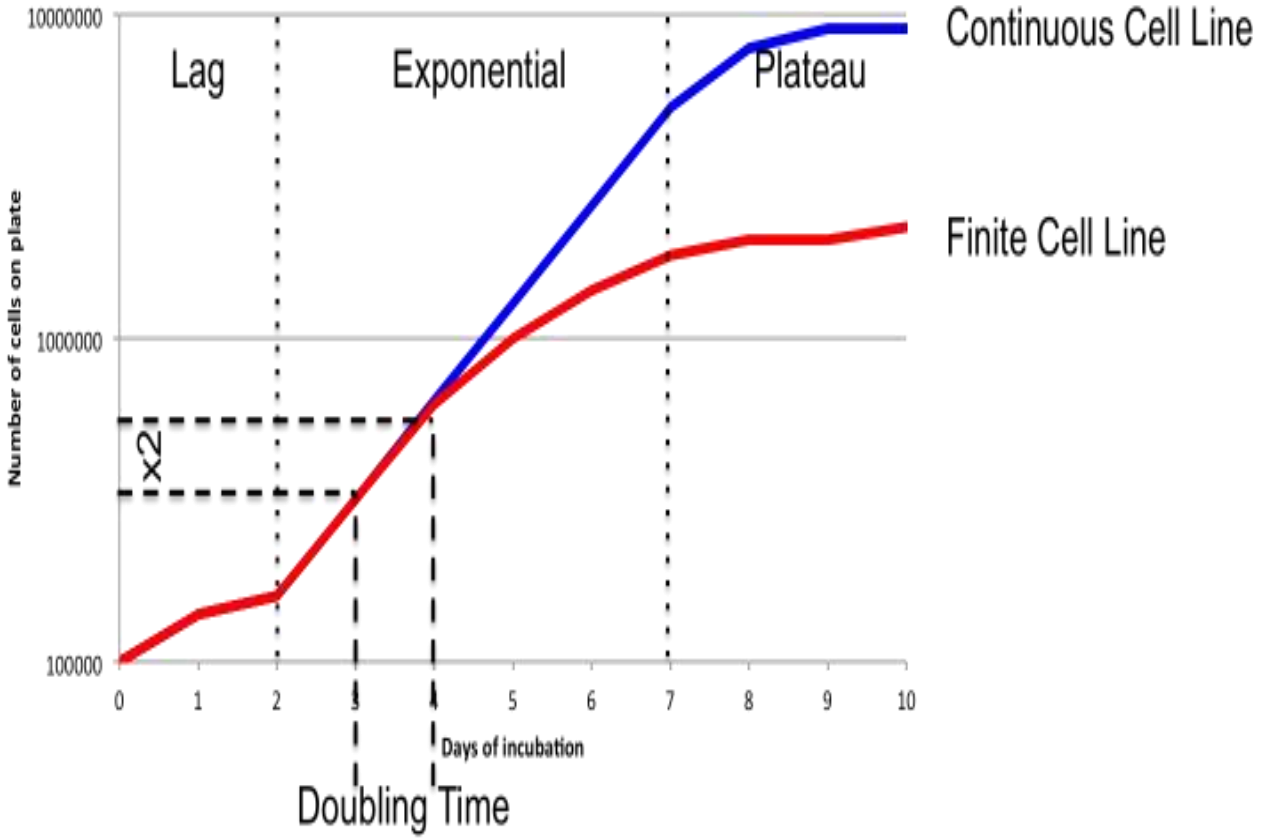
Lec. 2

Continuous Culture of Bacteria

The cultures so far discussed for growth of bacterial populations are called batch cultures. Since the nutrients are not renewed, exponential growth is limited to a few generations. Bacterial cultures can be maintained in a state of exponential growth over long periods of time using a system of continuous culture, designed to relieve the conditions that stop exponential growth in batch cultures.

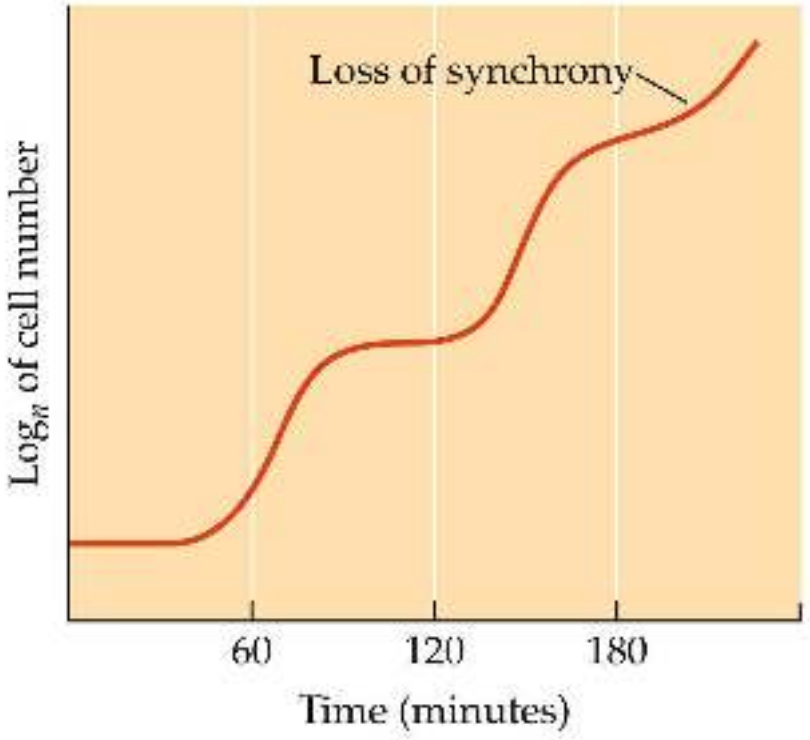
Continuous culture, in a device called a ***chemostat***, can be used to maintain a bacterial population at a constant density.


air in
motor
air out
from
medium
reservoir
pump
pump
to effluent
reservoir


10000000
1000000
100000
Number of cells on plate
Lag
Exponential
Plateau
x2
0
1
2
3
4
5
6
7
8
9
10
Days of incubation
Doubling Time
Continuous Cell Line
Finite Cell Line

Synchronous Growth

A growing microbial culture contains cells dividing asynchronously and the properties of the population are the average properties of the individual cells. While studying cell cycle events we want to measure changes in the biochemical features of individual cells and we therefore need to amplify the physiological events by producing a synchronously dividing culture in which all the cells divide at roughly the same time, consequently, a "stair-step" shape of curve results



Microbial Life 2e, Figure 6.9

Synchronous growth: A hypothetical situation in which the number of cells in a culture would increase in a stair-step pattern, dividing together at the same rate

Nonsynchronous growth: A natural situation in which an actual culture has cell dividing at one rate and other cells dividing at a slightly slower rate

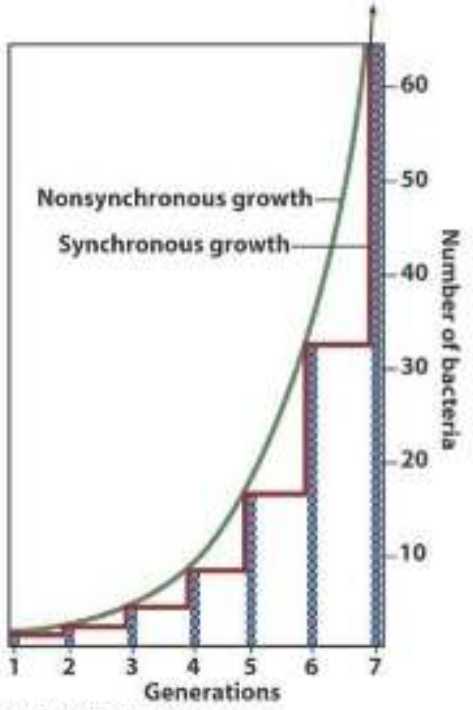


Figure 6-4 Microbiology, 6/e
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There are two methods available for producing the synchronous cultures:

1. Induction methods - They rely on synchronising an exponential phase culture by appropriate and usually sudden changes in the environment, such as:

1) alteration in temperature
2) concentration of nutrients
3) illumination for photoautotrophs

- **2. Selection methods -** The cells are physically separated from an exponential-phase culture at a particular point in the growth cycle. The methods include.

- **(I)** Centrifugation on a density gradient.
- **(II)** Filtration of cells through a cellulose nitrate filter and inverting it and passing medium through the filter from above. When cells divide they fall off the filter giving a continuous supply of newly born cells.

- ***Synchronous cultures can be obtained in several ways:***

ØExternal conditions can be changed, so as to arrest growth of all cells in the culture, and then changed again to resume growth. The newly growing cells are now all starting to grow at the same stage, and they are synchronized. For example:

1-***for photosynthetic cells light can be eliminated for several hours and then re-introduced.*** 2-***Another method is to eliminate an essential nutrient from the growth medium and later to re-introduce it.***

ØCell growth can also be arrested using chemical growth inhibitors. After growth has completely stopped for all cells, the inhibitor can be easily removed from the culture and the cells then begin to grow synchronously.Nocodazole, for example, is often used in biological research for this purpose.

ØCells in different growth stages have different physical properties. Cells in a culture can thus be physically separated based on their density or size, for instance. This can be achieved using centrifugation (for density) or filtration(for size).

ØIn the Helmstetter-Cummings technique, a bacterial culture is filtered through a membrane. Most bacteria pass through, but some remain bound

to the membrane. Fresh medium is then applied to the membrane and the bound bacteria start to grow. Newborn bacteria that detach from the membrane are now all at the same stage of growth; they are collected in a flask that now harbors a synchronous culture.

Lec. 3

Biofilm

• A biofilm is any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). is a polymeric conglomeration generally composed of :

1) extracellular DNA

2) proteins

3) polysaccharides

- Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings

- The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are singlecells that may float or swim in a liquid medium

- Microbes form a biofilm in response to many factors, which may include :

qcellular recognition of specific or non-specific attachment sites on a surface

qnutritional cues

qexposure of planktonic cells to sub-inhibitory concentrations of antibiotics

- When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behavior in which large suites of genes are differentially regulated

5. *Formation*

- Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first colonists adhere to the surface initially through weak, reversible adhesion via van der Waals forces.

- If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili.

- Hydrophobicity also plays an important role in determining the ability of bacteria to form biofilms, as those with increased hydrophobicity have reduced repulsion between the extracellular matrix and the bacterium.

- Some species are not able to attach to a surface on their own but are sometimes able to anchor themselves to the matrix or directly to earlier colonists.

- It is during this colonization that the cells are able to communicate via quorum sensing using products such as AHL.

- Some bacteria are unable to form biofilms as successfully due to their limited motility. Nonmotile bacteria cannot recognize the surface or aggregate together as easily as motile bacteria.

- Once colonization has begun, the biofilm grows through a combination of cell division and recruitment.

- Polysaccharide matrices typically enclose bacterial biofilms. In addition to the polysaccharides, these matrices may also contain material from the surrounding environment, including but not limited to minerals, soil particles, and blood components, such as erythrocytes and fibrin.

- The final stage of biofilm formation is known as dispersion, and is the stage in which the biofilm is established and may only change in shape and size.

- The development of a biofilm may allow for an aggregate cell colony (or colonies) to be
  increasingly antibiotic resistant. Cell-cell communication or quorum sensing (QS) has been shown to be involved in the formation of biofilm in several bacterial species.

- Acinetobacter baumannii is infamous for its ability to form biofilms both on inanimate objects as well as biotic surfaces.

- A. baumannii has been reported to commence secretion of exopolysacchrides once it has successfully adhered to a surface, be it hydrophilic or hydrophobic like glass and plastic, respectively, or surfaces of living cells.

- Previous findings indicate that within the protective environment of the biofilm, the pathogen remains protected from:

1) starvation
2) desiccation
3) action of antibiotics

- As such, the ability to form biofilms alone may be linked to the increased virulence in some of the strains.

- reports have shown that multidrug resistant strains are efficient biofilm producers, indicating a direct relationship between biofilm formation and antibiotic resistance.

- reports have also shown that the biofilm-associated protein (BAP) in A. baumannii, involved in biofilm formation, is capable of stimulating humoral response in mice, which suggests that it may have a role in virulence.

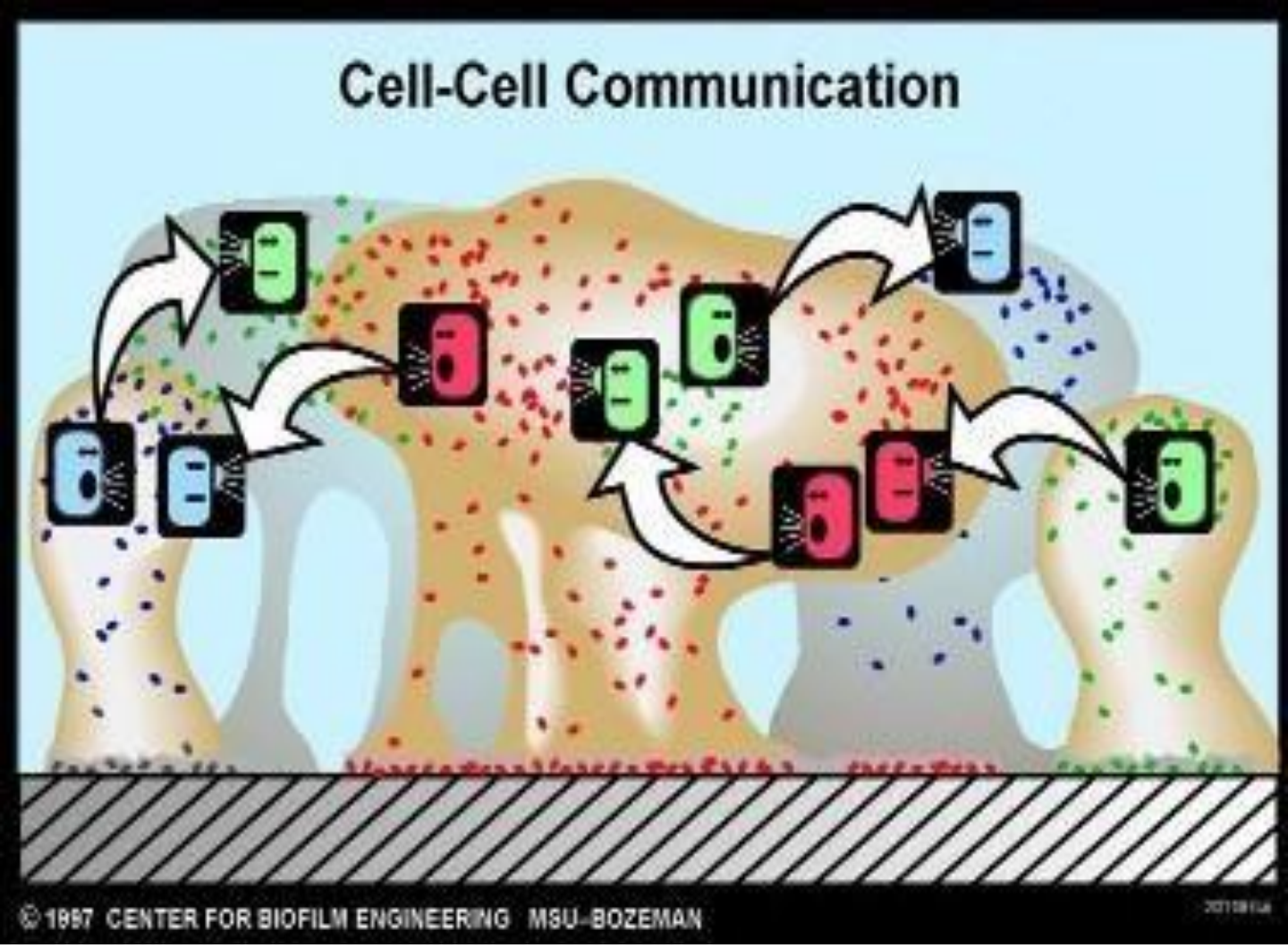
- The ability of A. baumannii to form biofilms has been shown to be related to certain outer membrane surface associated proteins like OmpA and BAP as well as certain pili-associated adhesins.

- The presence of metal cations has also been reported to be required for biofilm formation as indicated by the reduced ability of A. baumannii to produce biofilms in presence of chelators like ethylenediaminetetraacetic acid.

- The formation of CsuA/BABCDE-dependent pili appears to be essential for the adherence and biofilm formation on abiotic surfaces and the assembly of these pili seems to involve chaperone and usher-like proteins. However, this system does not seem to be involved in the adherence of A. baumannii to living cells

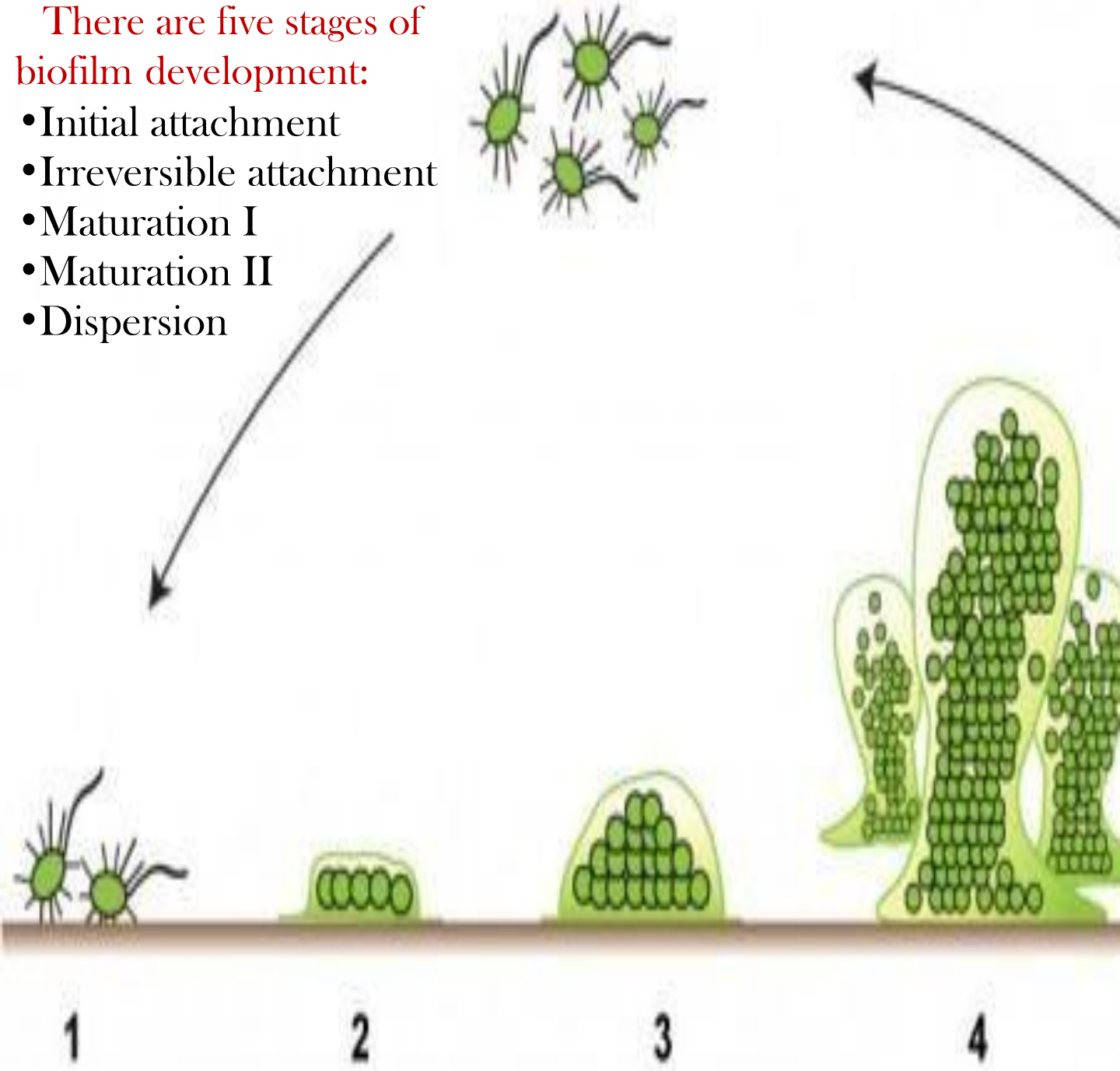
- Quorum sensing has also been implicated in the regulation of biofilm formation. A. baumannii has been shown to be capable of producing quorum sensing molecules namely N-acylhomoserine lactones of various chain length with -(3-hydroxydodecanoyl)-L-HSL reported as the primary signal molecule. However, only a single autoinducer synthase gene named abaI has been identified till date.

- Quorum sensing is the main method of communication between the bacterial cells within the biofilm and may also serve as a mechanism to coordinate and regulate the multiple virulence factors in A. baumannii. There are reports indicating that quorum sensing might possibly be involved in host–pathogen interactions as well. Thus, biofilm formation and quorum sensing are important components in the wide arsenal of virulence determinants produced by A. baumannii.


Cell-Cell Communication
© 1997 CENTER FOR BIOFILM ENGINEERING MSU–BOZEMAN

There are five stages of biofilm development:

- Initial attachment
- Irreversible attachment
- Maturation I
- Maturation II
- Dispersion



Lec. 4

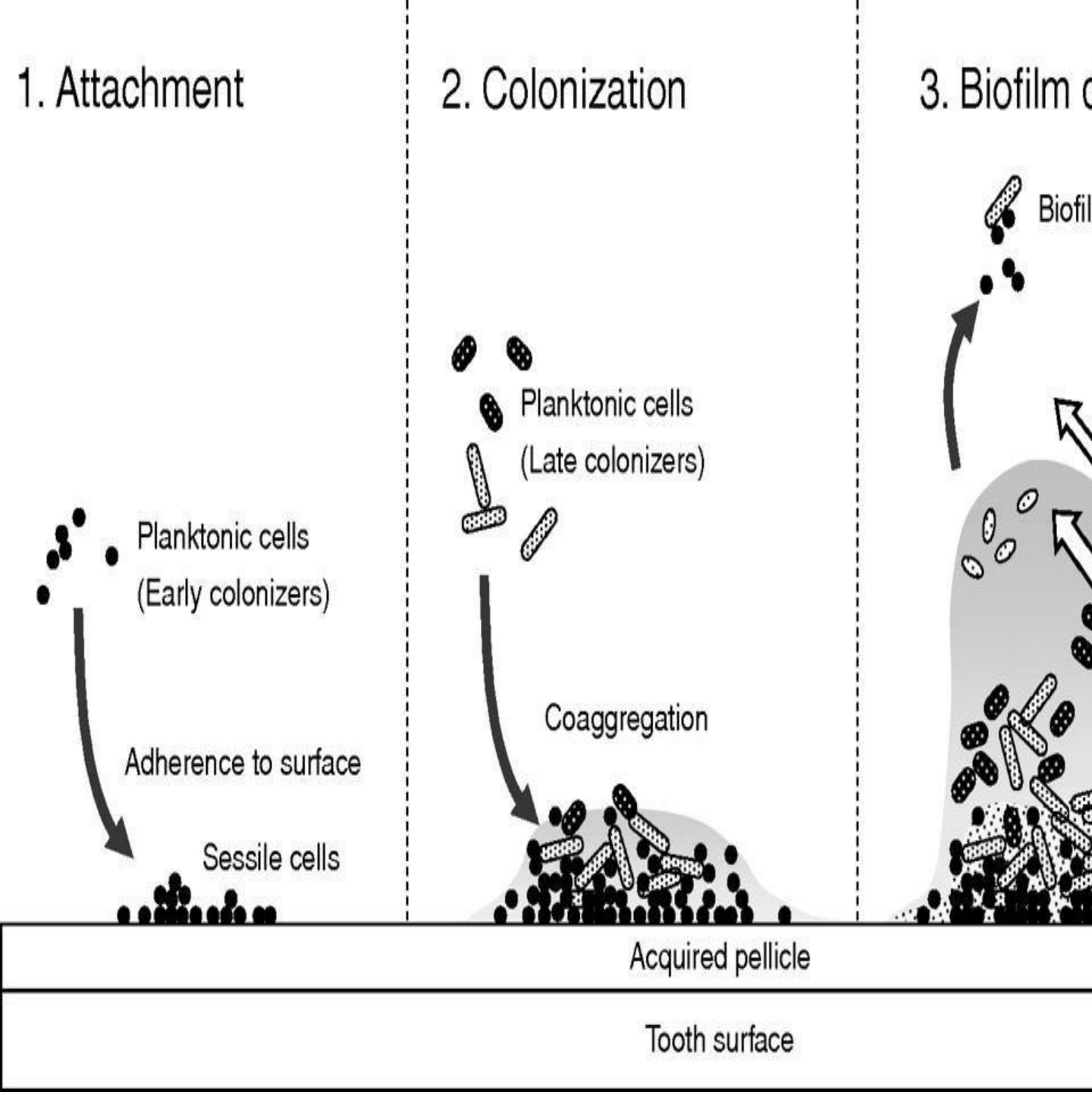
Biofilm: Adhesion and Microcolony Formation

- The attachment of a small number of bacterial cells is all that is needed to initiate biofilm formation anywhere along the system.

- Within a few seconds, the progression of phenotypic changes in the bacteria remarkably alters protein expression to further produce species-specific adhesions that irreversibly anchor the cell to the surface. Type IV pili are involved in a type of surface-associated motility called twitching, and this twitching might be required for the aggregation of cells into microcolonies.

- Within as few as 12 minutes, the adherent cells upregulate genes that direct production of accumulation proteins and polysaccharides, which firmly attach the cells to the substratum and to each other as they undergo exponential binary division.

- After initial attachment, the cells begin to grow and spread as a monolayer on the surface. As the cells continue to divide, the daughter cells spread outward and upward from the attachment point to form cell clusters. The production of exopolysaccharides (EPS) or "slime" embeds the aggregating cells to form microcolonies. Typically, the microcolonies are composed of 10% to 25% cells and 75% to 90% EPS matrix, with a consistency similar to a viscous polymer hydrogel.


1. Attachment
2. Colonization
3. Biofilm
Biofil
Planktonic cells
(Early colonizers)
Adherence to surface
Sessile cells
Planktonic cells
(Late colonizers)
Coaggregation
Acquired pellicle
Tooth surface

- The continued formation of the biofilm community evolves according to:

1) the biochemical and hydrodynamic conditions
2) the availability of nutrients in the immediate environment.

The structural organization is mainly influenced by hormone-like regulatory signals produced by the biofilm cells themselves in reaction to growth conditions.

Ø This interactive network of signals allows for communication among the cells, not only controlling colony formation but also regulating:

1) growth rate
2) species interactions
3) toxin production
4) invasive properties

The cell clusters are structurally and metabolically heterogeneous, and both aerobic and anaerobic processes occur simultaneously in different parts of the multicellular community.

- Cellular density typically increases to a steady state within 1-2 weeks, depending on the species and local environmental conditions. Expanded growth evolves into complex 3-D structures of tower- and mushroom-shaped cell clusters.

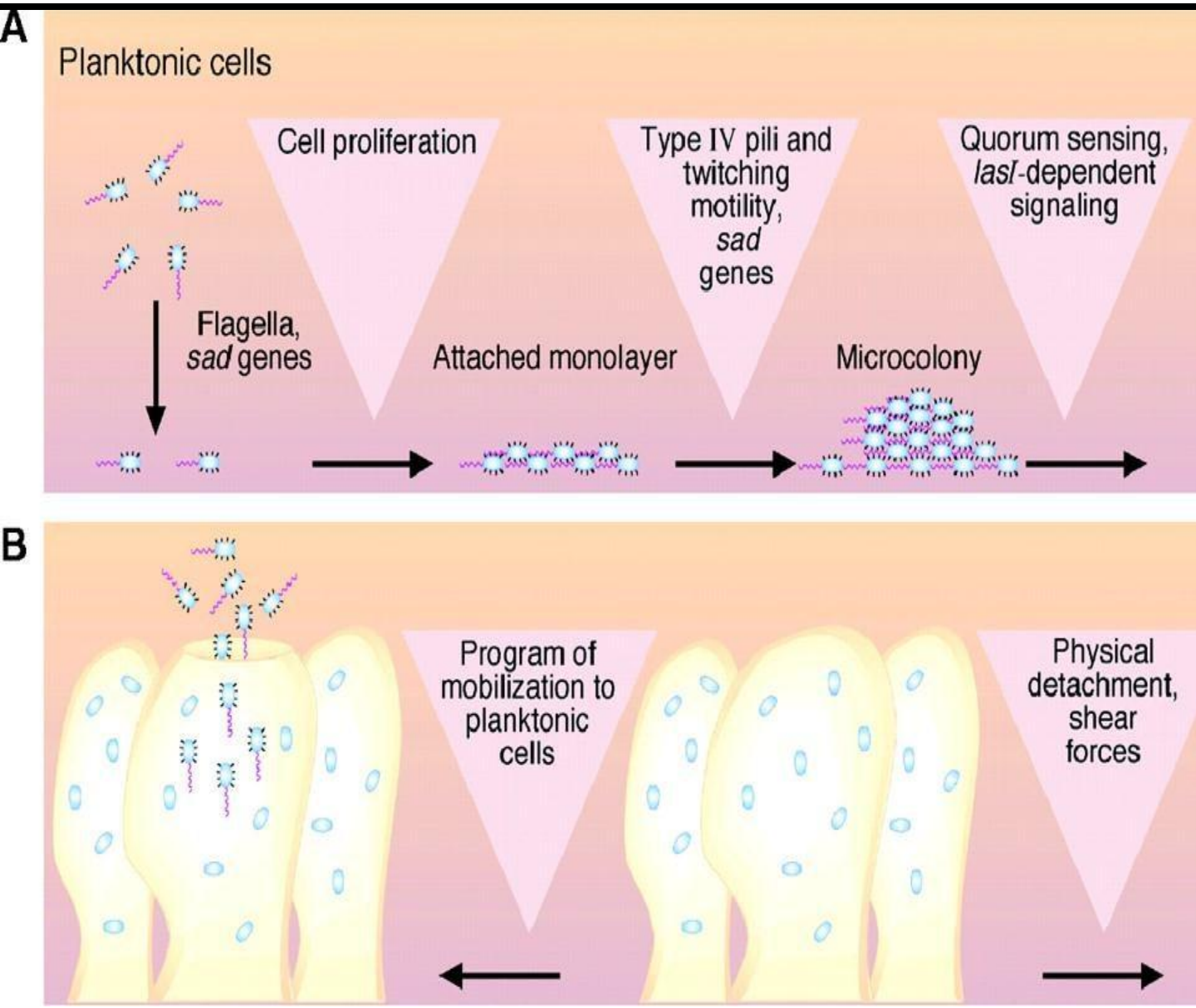
- Joining microcolonies are connected by water channels that serve as a primitive circulatory system for delivery of nutrients and removal of wastes.


Microbial cells
Water channel
2338 X
ASM MicrobeLibrary.org©Vic

- The thickness of the biofilm is variable (13-60 µm) and uneven, as determined by the balance between growth of the biofilm and detachment of cells.

- Depending on the initial number of attached organisms, the multilayered cell clusters develop as patchy networks or form a contiguous layer over the surface of the catheter.

- The dimension of biofilms in vivo is only on the order of tens of micrometers, but they contain thousands of bacteria in a very compact space.



Dispersal and Dissemination of Biofilm

Lec. 5

Biofilm formation

The formation of biofilm is a universal strategy for microbial survival.

- In order to colonize new surfaces and to prevent density-mediated starvation within the mature biofilm, the cells must detach and disseminate. However, those released in clumps retain antibiotic resistance and may embolize at a distant anatomic site to develop metastatic infections such as endocarditis or osteomyelitis. Dispersal is accomplished by:

1) shedding
2) Detachment
3) shearing

- Shedding:

- occurs when daughter cells from actively growing bacteria in the upper regions of the microcolonies are released from the cell clusters.

- Due to increased cell density a programmed set of events (cell-cell signaling) within the biofilm leading to a local hydrolysis of the extracellular polysaccharide matrix (alginase in the case of P. aeruginosa), and conversion of a subpopulation of cells into motile planktonic cells, which leave the biofilm.

- Shearing:

- biofilms are exposed to variable flow rates and shear forces. When the shear force of the infusion exceeds the tensile strength of the viscous biofilm, fragments break away.

- Detachment:

- Clumps or fragments of detached biofilm may contain thousands of cells, but they leave behind an adherent layer of cells on the surface to regenerate the biofilm. Intriguingly it has been observed that biofilms

with thick extrapolysaccharides have less chance of detachment which tends to reduce the spread of infections

- Dispersal of cells from the biofilm colony is an essential stage of the biofilm life cycle. Dispersal enables biofilms to spread and colonize new surfaces.

❖ Enzymes that degrade the biofilm extracellular matrix, such as dispersin B and deoxyribonuclease, may play a role in biofilm dispersal. Biofilm matrix degrading enzymes may be useful as antibiofilm agents.

❖ Recent evidence has shown that a fatty acid messenger, cis-2decenoic acid, is capable of inducing dispersion and inhibiting growth of biofilm colonies. Secreted by Pseudomonas aeruginosa, this compound induces cyclo heteromorphic cells in several species of bacteria and the yeast Candida albicans.

❖ Nitric oxide has also been shown to trigger the dispersal of biofilms of several bacteria species at sub-toxic concentrations. Nitric oxide has the potential for the treatment of patients that suffer from chronic infections caused by biofilms.

- Properties

- Biofilms are usually found on solid substrates submerged in or exposed to an aqueous solution, although they can form as floating mats on liquid surfaces and also on the surface of leaves, particularly in high humidity climates.

- Given sufficient resources for growth, a biofilm will quickly grow to be macroscopic (visible to the naked eye). Biofilms can contain many different types of microorganism,
  e.g. bacteria, archaea, protozoa, fungi and algae; each group performs specialized metabolic functions. However, some organisms will form single-species films under certain conditions. The social structure (cooperation, competition) within a biofilm highly depends on the different species present.

- ***Extracellular matrix***

- The biofilm is held together and protected by a matrix of secreted polymeric compounds called EPS. EPS is an abbreviation for either extracellular polymeric substance or exopolysaccharide, although the latter one only

refers to the polysaccharide moiety of EPS. In fact, the EPS matrix consists not only of polysaccharides but also of proteins (which may be the major component in environmental and waste water biofilms) and nucleic acids.

- A large proportion of the EPS is more or less strongly hydrated, however, hydrophobic EPS also occur; one example is cellulose which is produced by a range of microorganisms. This matrix encases the cells within it and facilitates communication among them through biochemical signals as well as gene exchange.

- The EPS matrix is an important key to the evolutionary success of biofilms. One reason is that it traps extracellular enzymes and keeps them in close proximity to the cells. Thus, the matrix represents:

1) an external digestion system

2) allows for stable synergistic microconsortia of different species

ü Some biofilms have been found to contain water channels that help distribute nutrients and signalling molecules.

üThis matrix is strong enough that under certain conditions, biofilms can become fossilized (Stromatolites).

- Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways.

- One benefit of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community. In some cases antibiotic resistance can be increased a thousandfold.

- Lateral gene transfer is greatly facilitated in biofilms and leads to a more stable biofilm structure. Extracellular DNA is a major structural component of many different microbial biofilms. Enzymatic degradation of extracellular DNA can weaken the biofilm structure and release microbial cells from the surface.

- However, biofilms are not always less susceptible to antibiotics. For instance, the biofilm form of Pseudomonas aeruginosa has no greater resistance to antimicrobials than do stationaryphase planktonic cells, although when the biofilm is compared to logarithmic phase planktonic cells, the biofilm does have greater resistance to antimicrobials. This

resistance to antibiotics in both stationary phase cells and biofilms may be due to the presence of persister cells.

Habitat

- Biofilms can be found on rocks and pebbles at the bottom of most streams or rivers and often form on the surface of stagnant pools of water. In fact, biofilms are important components of food chains in rivers and streams and are grazed by the aquatic invertebrates upon which many fish feed.

- Biofilms can grow in the most extreme environments: from, for example, the extremely hot, briny waters of hot springs ranging from very acidic to very alkaline, to frozen glaciers.

- In the human environment, biofilms can grow in showers very easily since they provide a moist and warm environment for the biofilm to thrive. Biofilms can form inside water and sewage pipes and cause clogging and corrosion. Biofilms on floors and counters can make sanitation difficult in food preparation areas.

- Biofilms in cooling- or heating-water systems are known to reduce heat transfer.

- Biofilms in marine engineering systems, such as pipelines of the offshore oil and gas industry, can lead to substantial corrosion problems. Corrosion is mainly due to abiotic factors; however, at least 20% of corrosion is caused by microorganisms that are attached to the metal subsurface (i.e., microbially influenced corrosion).

- Bacterial adhesion to boat hulls serves as the foundation for biofouling of seagoing vessels. Once a film of bacteria forms, it is easier for other marine organisms such as barnacles to attach. Such fouling can reduce maximum vessel speed by up to 20%, prolonging voyages and consuming fuel. Time in dry dock for refitting and repainting reduces the productivity of shipping assets, and the useful life of ships is also reduced due to corrosion and mechanical removal (scraping) of marine organisms from ships' hulls.

- Biofilms can also be harnessed for constructive purposes. For example, many sewage treatment plants include a treatment stage in which waste water passes over biofilms grown on filters, which extract and digest organic compounds. In such biofilms, bacteria are mainly responsible for removal of organic matter, while protozoa and rotifers are mainly responsible for removal of suspended solids, including pathogens and other microorganisms.

- Slow sand filters rely on biofilm development in the same way to filter surface water from lake, spring or river sources for drinking purposes. What we regard as clean water is effectively a waste material to these microcellular organisms.

- Biofilms can help eliminate petroleum oil from contaminated oceans or marine systems. The oil is eliminated by the hydrocarbon-degrading activities of microbial communities, in particular by a remarkable recently discovered group of specialists, the socalled hydrocarbonoclastic bacteria (HCB).

- Stromatolites are layered accretionary structures formed in shallow water by the trapping, binding and cementation of sedimentary grains by microbial biofilms, especially ofcyanobacteria. Stromatolites include some of the most ancient records of life on Earth, and are still forming today.

- Biofilms are present on the teeth of most animals as dental plaque, where they may cause tooth decay and gum disease.

- Biofilms are found on the surface of and inside plants. They can either contribute to crop disease or, as in the case of nitrogen-fixing Rhizobium on roots, exist symbiotically with the plant. Examples of crop diseases related to biofilms include Citrus Canker, Pierce's Disease of grapes, and Bacterial Spot of plants such as peppers and tomatoes.

- Biofilms are used in microbial fuel cells (MFCs) to generate electricity from a variety of starting materials, including complex organic waste and renewable biomass.

- Recent studies in 2003 discovered that the immune system supports bio-film development in the large intestine. This was supported mainly with the fact that the two most abundantly produced molecules by the immune system also support bio-film production and are associated with the biofilms developed in the gut. This is especially important because the appendix holds a mass amount of these bacterial bio-films. This discovery helps to distinguish the possible function of the appendix and the idea that the appendix can help reinoculate the gut with good gut flora.

Lac. 6

Biofilms and infectious diseases

- Dental plaque

- Dental plaque is an oral biofilm that adheres to the teeth and consists of:

1) many species of both fungal and bacterial cells (such as Streptococcus mutans and Candida albicans)
2) salivary polymers
3) microbial extracellular products.

- The accumulation of microorganisms subjects the teeth and gingival tissues to high concentrations of bacterial metabolites which results in dental disease.

- The biofilm on the surface of teeth is frequently subject to oxidative stress and acid stress. Dietary carbohydrates can cause a dramatic decrease in pH in oral biofilms to values of 4 and below (acid stress). A pH of 4 at body temperature of 37 °C causes depurination of DNA, leaving apurinic (AP) sites in DNA, especially loss of guanine.

- A peptide pheromone quorum sensing signaling system in S. mutans includes the Competence Stimulating Peptide (CSP) that
  controls genetic competence.

- Genetic competence is the ability of a cell to take up DNA released by another cell.

- Competence can lead to genetic transformation, a form of sexual interaction, favored under conditions of high cell density and/or stress where there is maximal opportunity for interaction between the competent cell and the DNA released from nearby donor cells.

- This system is optimally expressed when S. mutans cells reside in an actively growing biofilm. Biofilm grown S. mutans cells are genetically transformed at a rate 10- to 600-fold higher than S. mutans growing as free-floating planktonic cells suspended in
  liquid.

- When the biofilm, containing S. mutans and related oral streptococci, is subjected to acid stress, the competence regulon is induced, leading to resistance to being killed by acid.

- transformation in bacterial pathogens likely provides for effective and efficient recombinational repair of DNA damages.

- It appears that S. mutans can survive the frequent acid stress in oral biofilms, in part, through the recombinational repair provided by competence and transformation.

Streptococcus pneumoniae

- S. pneumoniae is the main cause of community -acquired pneumonia and meningitis in children and the elderly, and of septicemia in HIV-infected persons.

- When S. pneumonia grows in biofilms, genes are specifically expressed that respond to oxidative stress and induce competence.

- Formation of a biofilm depends on competence stimulating peptide (CSP). CSP also functions as a quorum-sensing peptide. It not only induces biofilm formation, but also increases virulence in pneumonia and meningitis.

- It has been proposed that competence development and biofilm formation is an adaptation of S. pneumoniae to survive the defenses of the host. In particular, the host's polymorphonuclear leukocytes produce an oxidative burst to defend against the invading bacteria, and this response can kill bacteria by damaging their DNA.

- Competent S. pneumoniae in a biofilm have the survival advantage that they can more easily take up transforming DNA from nearby cells in the biofilm to use for recombinational repair of oxidative damages in their DNA.

- Competent S. pneumoniae can also secrete an enzyme (murein hydrolase) that destroys non-competent cells (fratricide) causing DNA to be released into the surrounding medium for potential use by the competent cells.

- Legionellosis

- Legionella bacteria are known to grow under certain conditions in biofilms, in which they are protected against disinfectants.

- Workers in cooling towers, persons working in air conditioned rooms and people taking a shower are exposed to Legionella by inhalation when the systems are not well designed, constructed, or maintained.

- Biofilms in medicine

- The rapidly expanding worldwide industry for biomedical devices and tissue engineering related products is already at $180 billion per year, yet this industry continues to suffer from microbial colonization.

- No matter the sophistication, biofilms are known to develop on all medical devices and tissue engineering constructs.

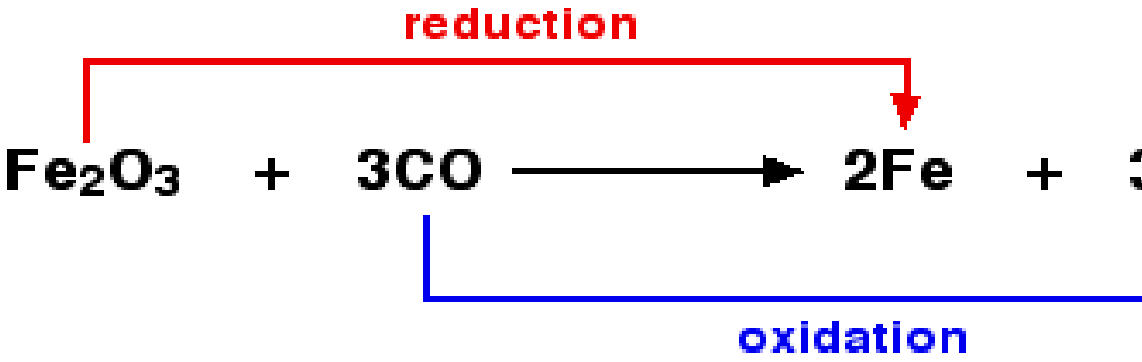
- Biofilms also account for more than 65% of nosocomial infections. This leads to 2 million cases annually in the U.S., costing the healthcare system over $5 billion in additional healthcare expenses.

Lec. 7

Definitions of Oxidation and Reduction with respect to Oxygen Transfer

Oxidation is the **gain** of oxygen.

Reduction is the **loss** of oxygen.



- Because both reduction and oxidation are occurring simultaneously, this is known as a ***redox reaction***.

6. • *Oxidizing and reducing agents*

- An oxidizing agent is substance which oxidizes something else. In the example, the iron(III) oxide is the oxidizing agent*(give oxygen to another substance).*

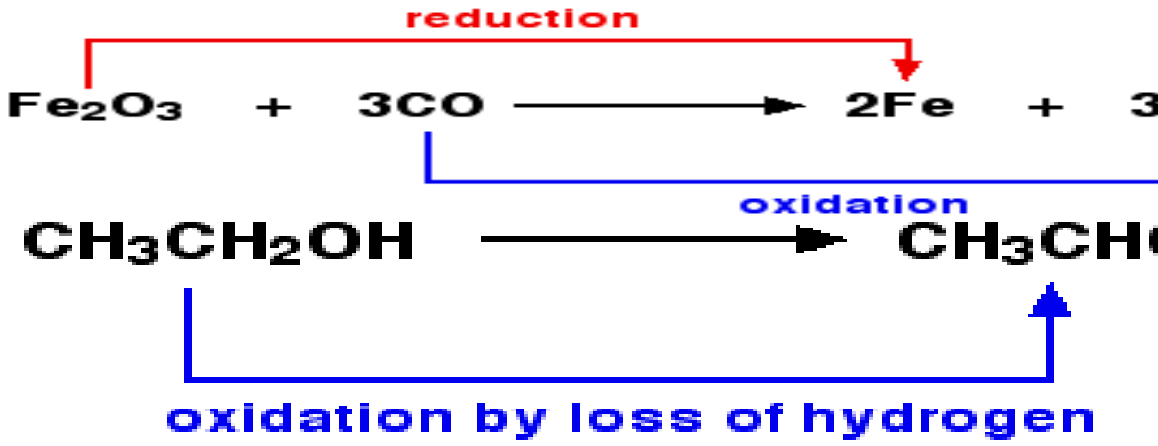
- A reducing agent reduces something else. In the equation, the carbon monoxide is the reducing agent *(remove oxygen from another substance).*

***Definition 2*: Oxidation and Reduction with respect to Hydrogen Transfer** Oxidation is the **loss** of hydrogen.

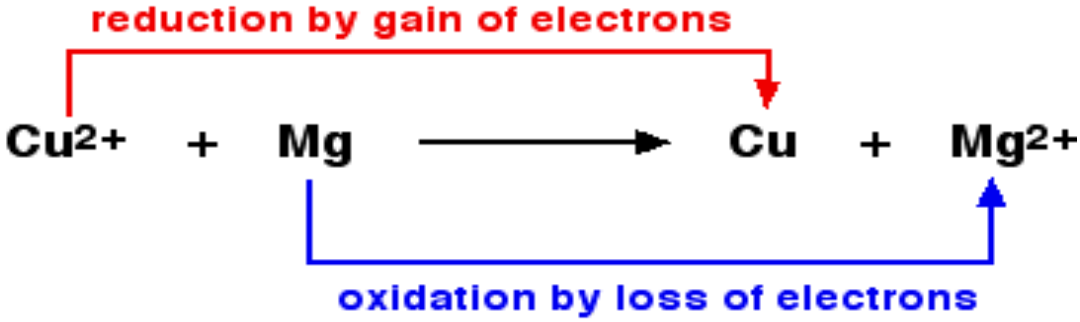
Reduction is the **gain** of hydrogen.

Notice that these are exactly the opposite of the oxygen definitions.

For example, ethanol can be oxidized to ethanal:



- ***Definition 3*: Oxidation and Reduction with respect to Electron Transfer**
- Oxidation is **loss** of electrons
- Reduction is **gain** of electrons



7. *The Effect of Oxygen*

- ***Obligate aerobes*** require O_2 for growth; they use O_2 as a final electron acceptor in aerobic respiration and cannot grow in its absence.

- ***Obligate anaerobes*** (occasionally called **aerophobes**) do not need or use O_2 as a nutrient. In fact, O_2 is a toxic substance, which either kills or inhibits their growth; hence, they must depend on other substances as electron acceptors. Obligate anaerobic procaryotes may live by:

1) fermentation or anaerobic respiration.
2) bacterial photosynthesis.
3) novel process of methanogenesis.

- ***Facultative anaerobes*** (or **facultative aerobes**) are organisms that can switch between aerobic and anaerobic types of metabolism. however, they grow better in the presence of molecular oxygen.

- ***Aerotolerant anaerobes*** are bacteria with an exclusively anaerobic (fermentative) type of metabolism but they are insensitive to the presence of O_2. They live by fermentation alone whether or not O_2 is present in

their environment, since they lack the ability to use oxygen as a terminal electorn acceptor.

- ***Microaerophiles*** are organisms that use oxygen, but only low concentrations (low micromolar range); their growth is inhibited by normal oxygen concentrations (approximately 200 micromolar).

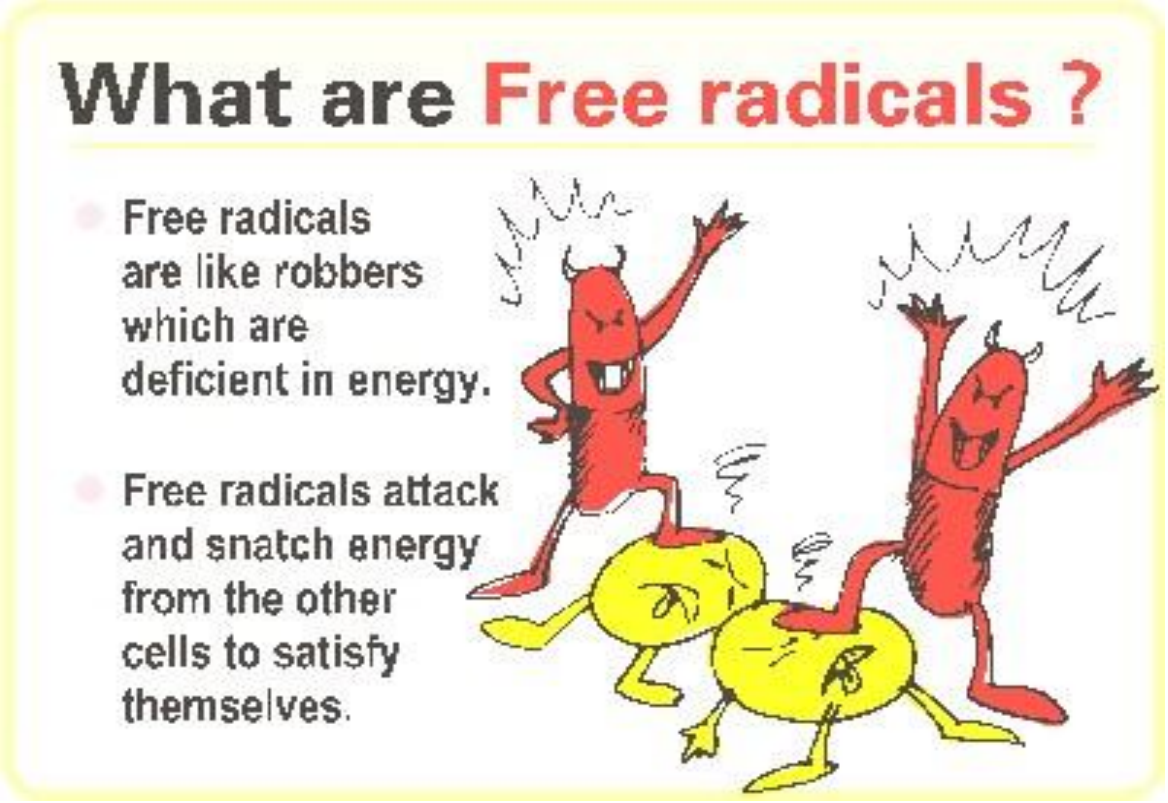
- ***Nanaerobes*** are organisms that cannot grow in the presence of micromolar concentrations of oxygen, but can grow with and benefit from lower (nanomolar) concentrations of oxygen (e.g. *Bacteroides fragilis*).

- The response of an organism to O_2 in its environment depends upon the occurrence and distribution of various enzymes which react with O_2 and various oxygen radicals that are invariably generated by cells in the presence of O_2.

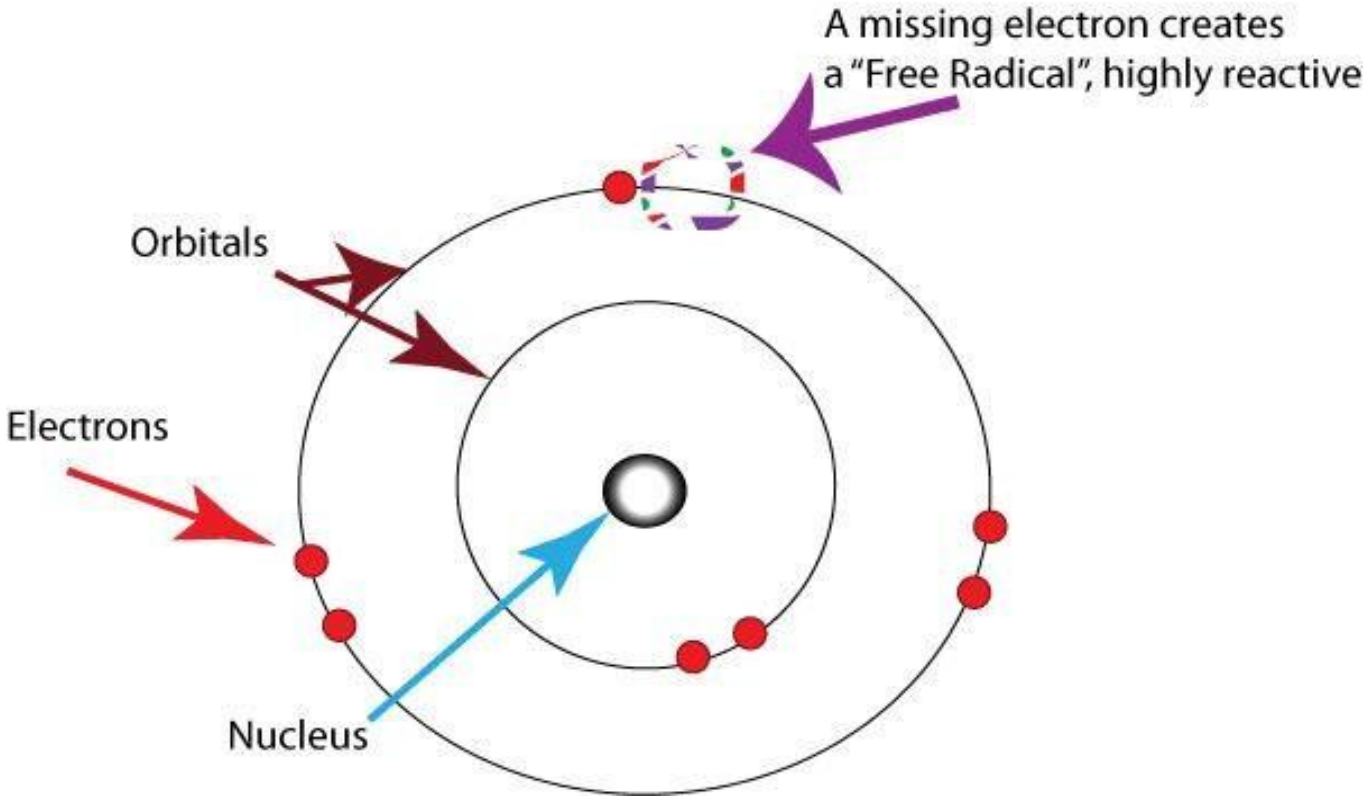
- ***All cells contain enzymes capable of reacting with O_2. For example:***

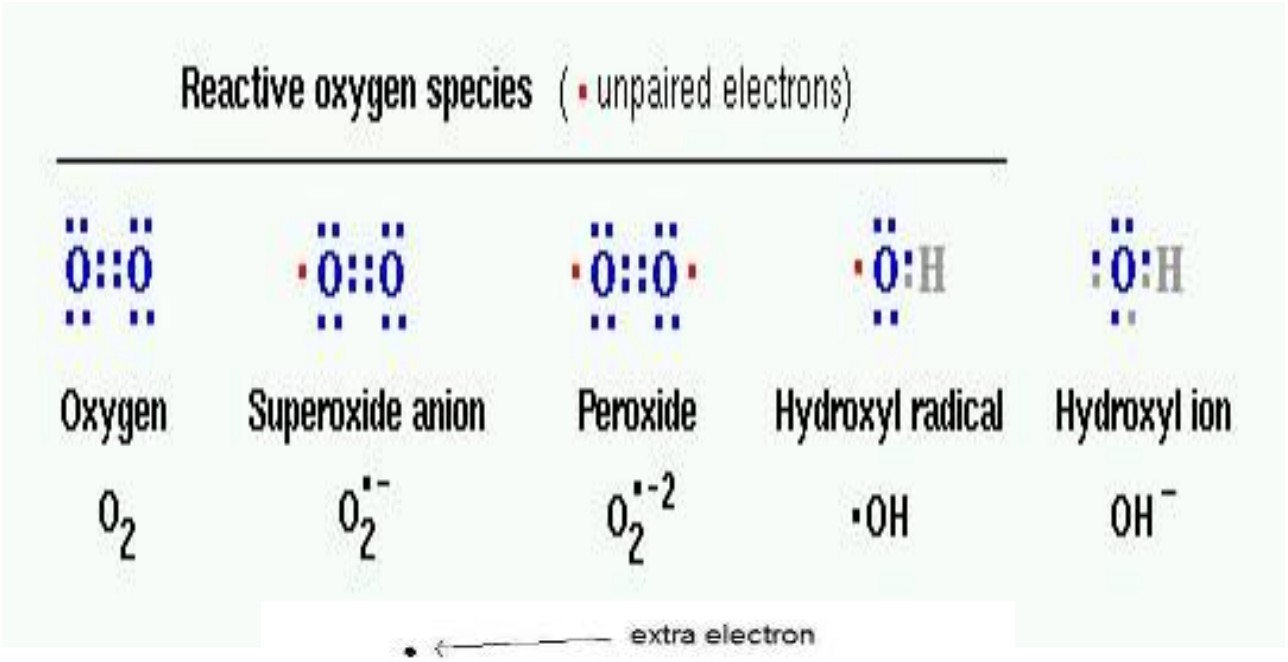
- oxidations of flavoproteins by O_2 invariably result in the formation of **H_2O_2** (peroxide) as one major product and small quantities of an even more toxic free radical, superoxide or **$^{\bullet}O_2^-$**.

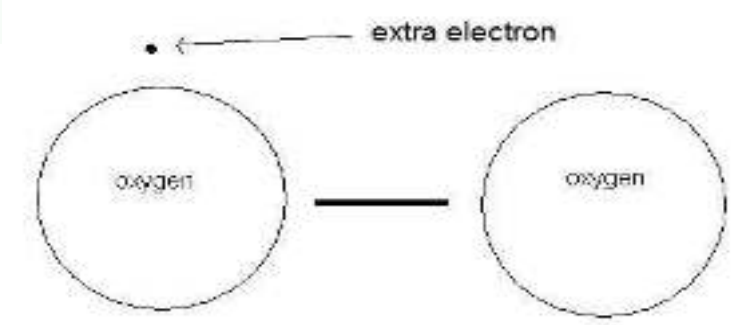
- Also, chlorophyll and other pigments in cells can react with O_2 in the presence of light and generate singlet oxygen **$^1O2$**, another radical form of oxygen which is a potent oxidizing agent in biological systems.



A free radical is an atom or group of atoms that has an unpaired electron and is therefore unstable and highly reactive.


A missing electron creates
a "Free Radical", highly reactive
Orbitals
Electrons
Nucleus


Reactive oxygen species (• unpaired electrons)
Oxygen
Superoxide anion
Peroxide
Hydroxyl radical
Hydroxyl ion
O_2
$O_2^{\bullet -}$
$O_2^{\bullet -2}$
•OH
OH^-


extra electron
oxygen
oxygen

- In aerobes and aerotolerant anaerobes the potential for lethal accumulation of superoxide is prevented by the enzyme superoxide dismutase.
- All organisms which can live in the presence of O_2 (whether or not they utilize it in their metabolism) contain superoxide dismutase.
- Nearly all organisms contain the enzyme catalase, which decomposes H_2O_2. Even though certain aerotolerant bacteria such as the lactic acid bacteria lack catalase, they decompose H_2O_2 by means of peroxidase enzymes which derive electrons from $NADH_2$ to reduce peroxide to H_2O.
- **Obligate anaerobes lack superoxide dismutase and catalase and/or peroxidase, and therefore undergo lethal oxidations by various oxygen radicals when they are exposed to O_2.**

Group	*Superoxide dismutase* $\bullet O_2^-$	*Catalase* H2O2	*Pero* H2O

Obligate aerobes and most facultative anaerobes (e.g. Enterics)	+	+	-
Most aerotolerant anaerobes (e.g. Streptococci)	+	-	+
Obligate anaerobes (e.g. Clostridia, Methanogens, Bacteroides)	-	-	-

- All photosynthetic (and some nonphotosynthetic) organisms are protected from lethal oxidations of singlet oxygen by their ***possession of carotenoid pigments which physically react with the singlet oxygen radical*** and lower it to its nontoxic "ground" (triplet) state. Carotenoids are said to "quench" singlet oxygen radicals.

- Much more usable energy, in the form of highenergy phosphate, is obtained when a molecule of glucose is completely catabolized to carbon dioxide and water in the presence of oxygen ***(38 molecules of ATP)*** than when it is only partially catabolized by a fermentative process in the absence of oxygen ***(2 molecules of ATP)***. The ability to utilize oxygen as a terminal electron acceptor provides organisms with an extremely efficient mechanism for generating energy.

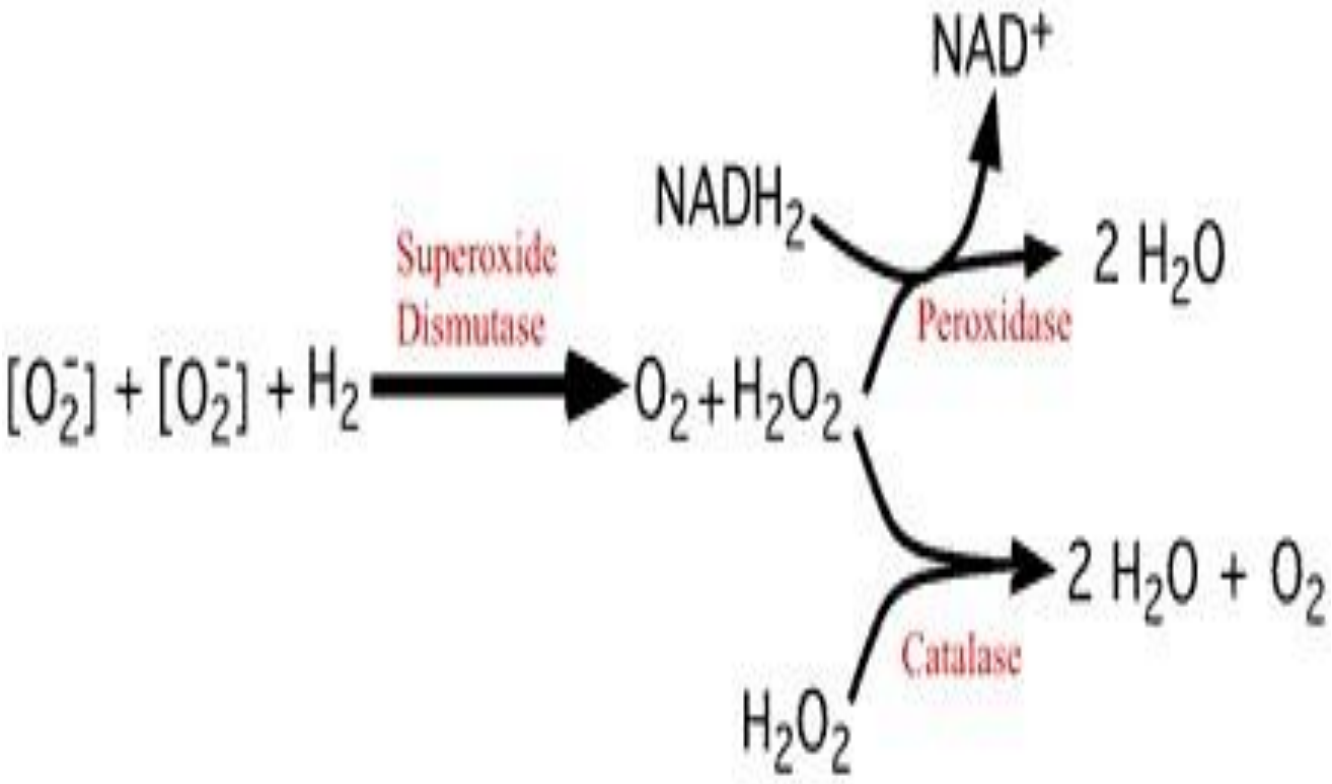
- Most facultative and aerobic organisms contain a high concentration of an enzyme called superoxide dismutase. This enzyme converts the superoxide anion into ground-state oxygen and hydrogen peroxide, thus ridding the cell of destructive superoxide anions:

- $2O_2^- + 2H^+ \xrightarrow{\text{Superoxide Dismutase}} O_2 + H_2O_2$

  - The hydrogen peroxide generated in this reaction is an oxidizing agent, but it does not damage the cell as much as the superoxide anion and tends to diffuse out of the cell. Many organisms possess catalase or peroxidase or both to eliminate the H_2O_2. Catalase uses H_2O_2 as an oxidant (electron acceptor) and a reductant (electron donor) to convert peroxide into water and ground-state oxygen:

  - $H2O2 + H2O2 \xrightarrow{Cat} 2H_2O + O_2$ Peroxidase uses a reductant other than H_2O_2:

  - $H_2O_2 + H_2R \xrightarrow{\text{Peroxidase}}$


NAD+
NADH2
2 H2O
Superoxide
Dismutase
Peroxidase
[O2-] + [O2-] + H2
O2+H2O2
2 H2O + O2
Catalase
H2O2

ØOne study showed that facultative anaerobes and aerobic organisms lacking superoxide dismutase possess high levels of catalase or peroxidase. High concentrations of these enzymes may alleviate the need for superoxide dismutase, because they effectively scavenge H_2O_2 before it can react with the superoxide anion to form the more active hydroxyl radical.

ØHowever, most organisms show a positive correlation between the activity of superoxide dismutase and resistance to the toxic effects of oxygen.

ØIn another study, facultative and aerobic organisms demonstrated high levels of superoxide dismutase.

- The enzyme was present, generally at lower levels, in some of the anaerobes studied, but was totally absent in others. The most oxygen-sensitive anaerobes as a rule contained little or no superoxide dismutase.

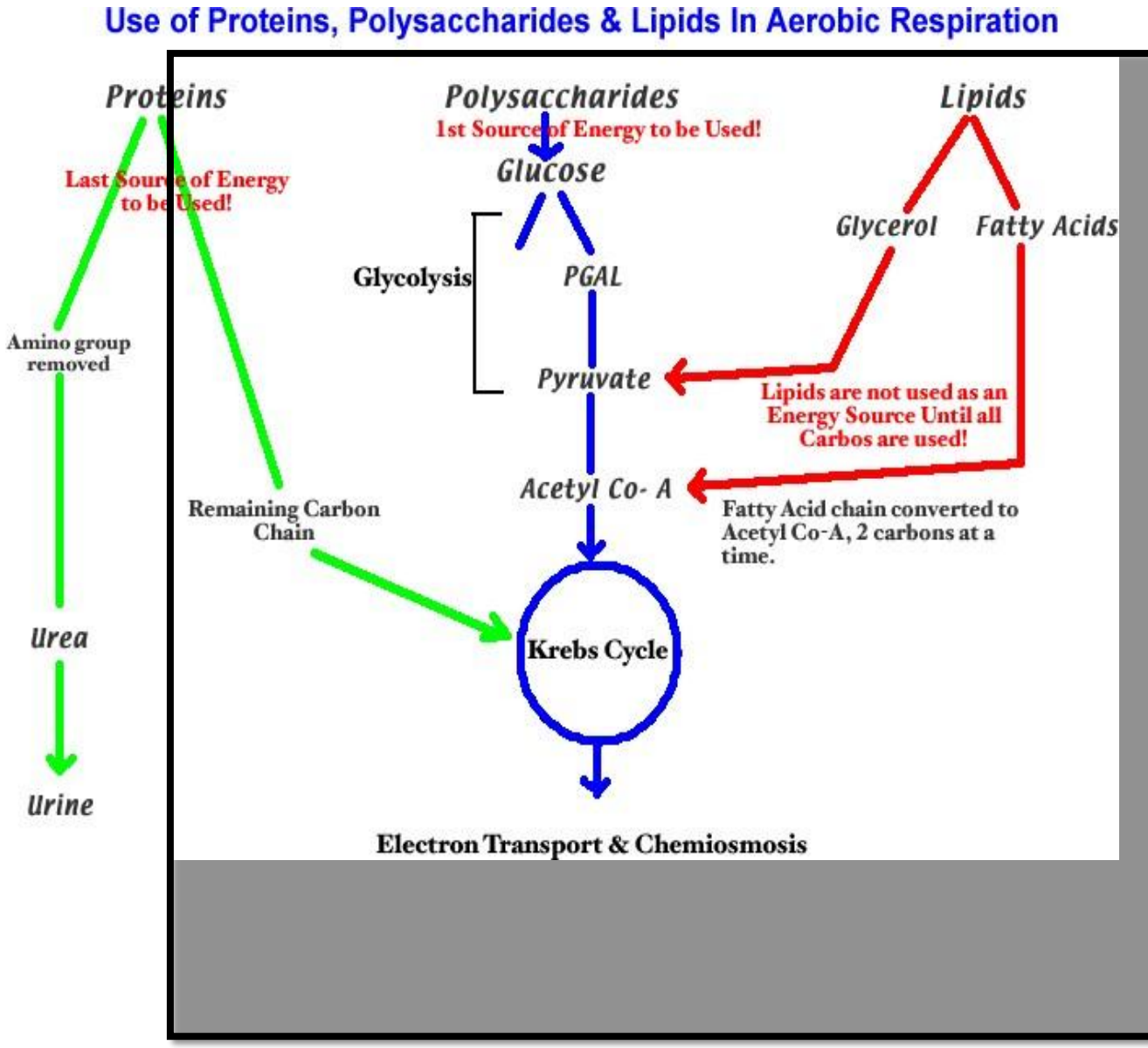
- In addition to the activity of superoxide dismutase, the rate at which an organism takes up and reduces oxygen was determined to be a factor in oxygen tolerance.

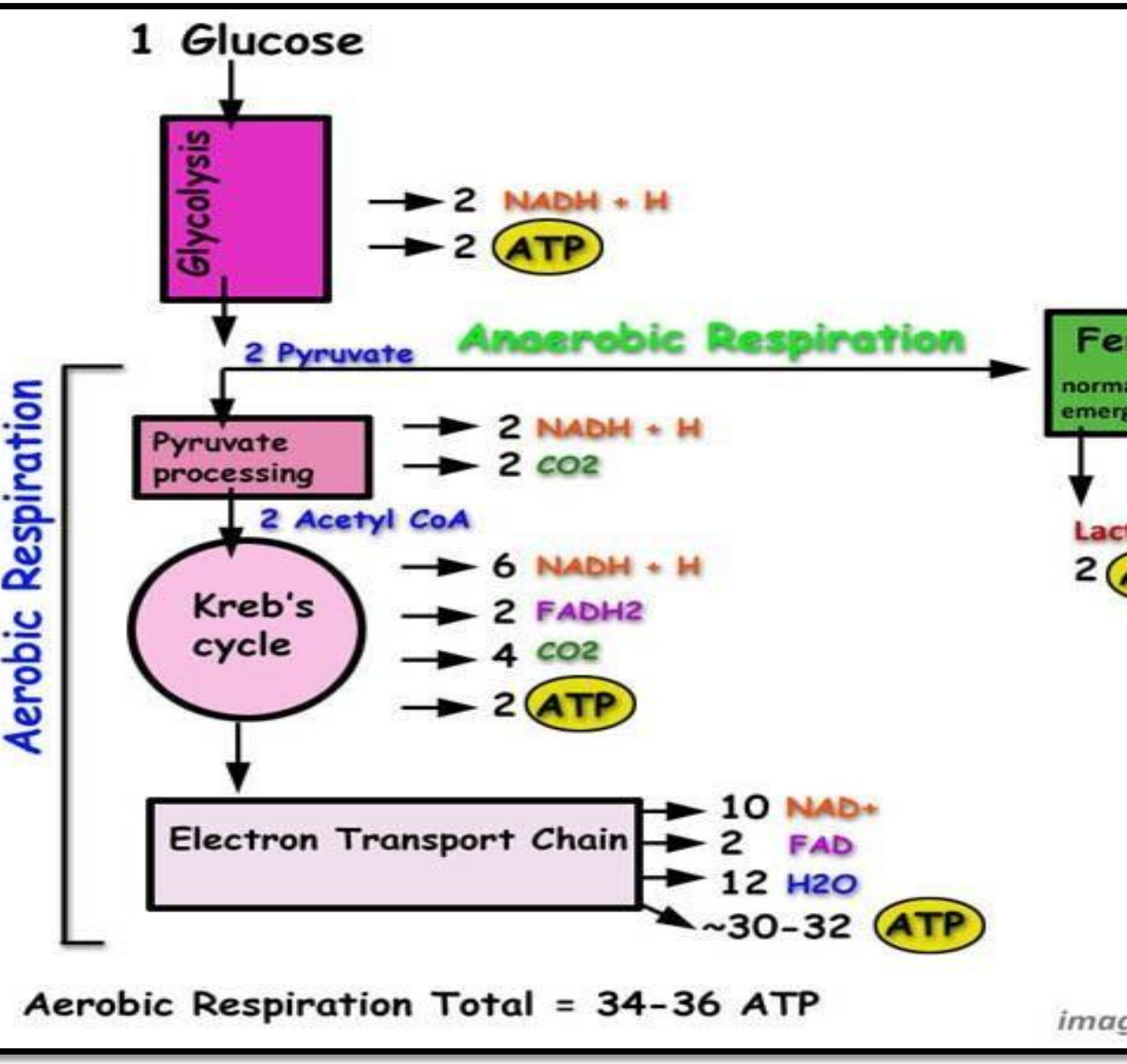
- Very sensitive anaerobes, which reduced relatively large quantities of oxygen and exhibited no superoxide dismutase activity, were killed after short exposure to oxygen.

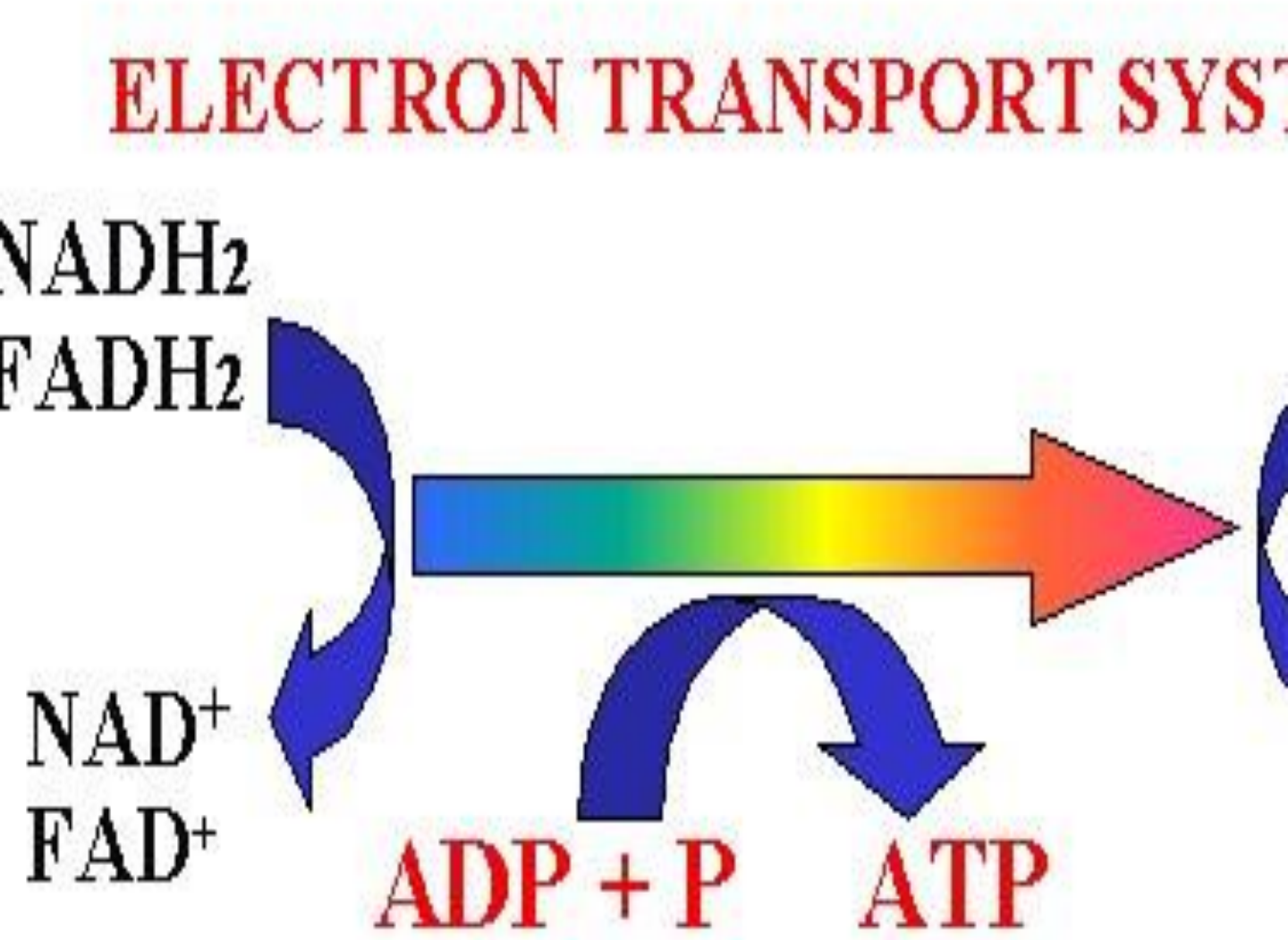
- More tolerant organisms reduced very little oxygen or else demonstrated high levels of superoxide dismutase activity.

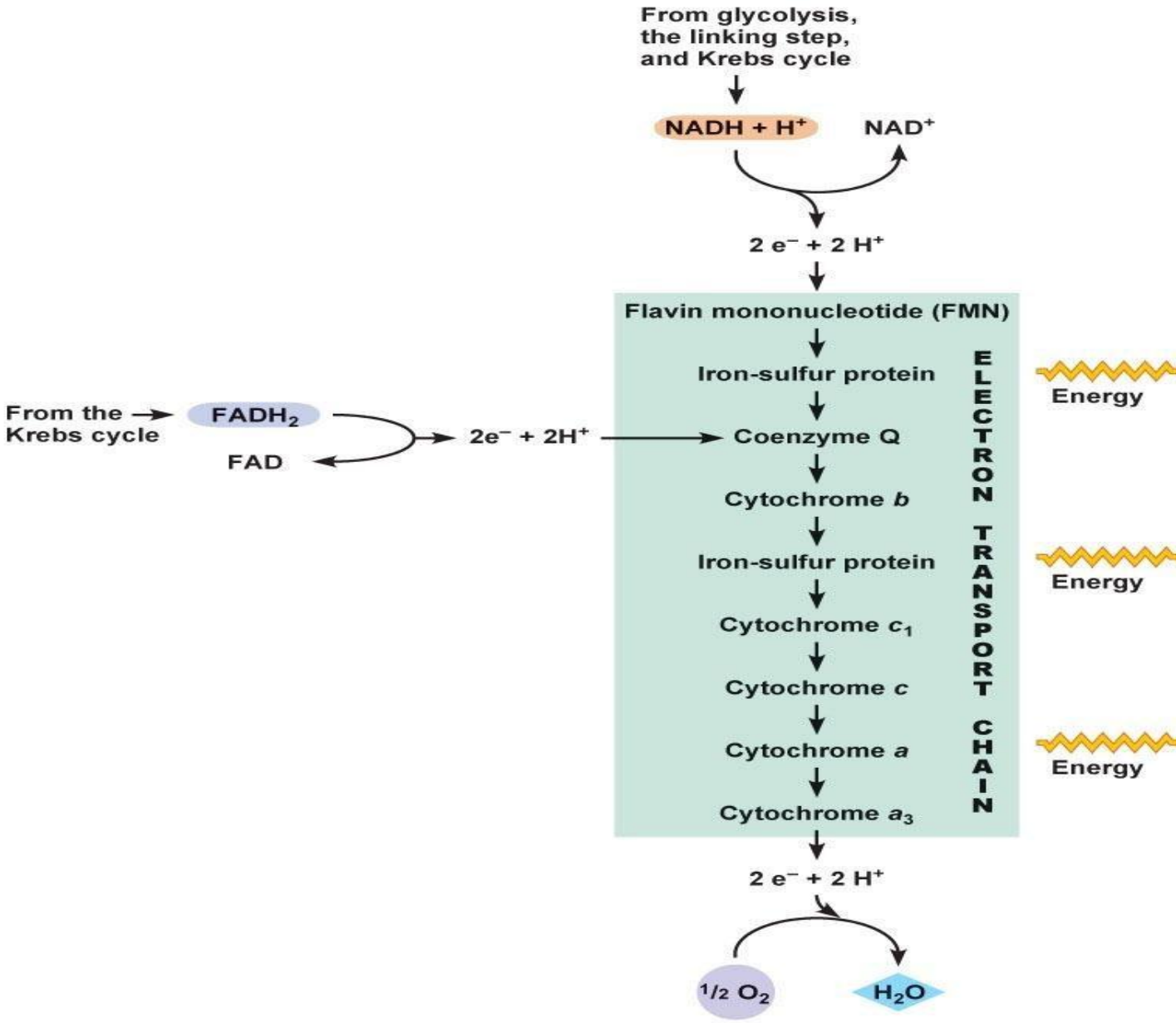
- ***The continuous spectrum of oxygen tolerance among bacteria appears to be due to:***

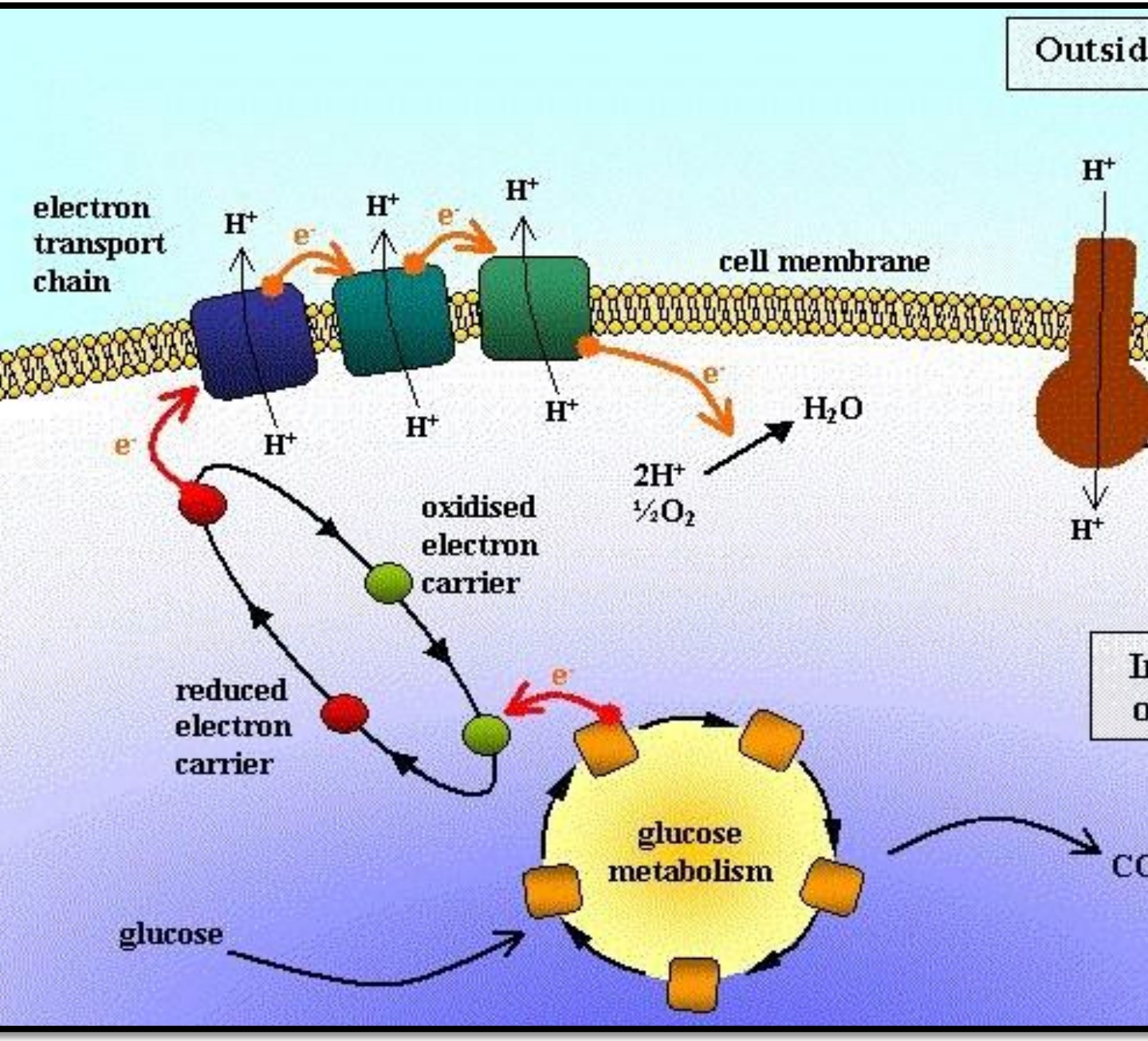
1. The activities of superoxide dismutase, catalase, and peroxidase in the cell.
2. Partly, to the rate at which the cell takes up oxygen.
3. The location of protective enzymes in the cell (surface versus cytoplasm).
4. The rate at which cells form toxic oxygen products (e.g., the hydroxyl radical or singlet oxygen).
5. The sensitivities of key cellular components to the toxic oxygen products.


Use of Proteins, Polysaccharides & Lipids In Aerobic Respiration
Proteins
Polysaccharides
1st Source of Energy to be Used!
Lipids
Glucose
Last Source of Energy to be Used!
Glycerol
Fatty Acids
Glycolysis
PGAL
Amino group removed
Pyruvate
Lipids are not used as an Energy Source Until all Carbos are used!
Acetyl Co- A
Remaining Carbon Chain
Fatty Acid chain converted to Acetyl Co-A, 2 carbons at a time.
Urea
Krebs Cycle
Urine
Electron Transport & Chemiosmosis


1 Glucose
Glycolysis
2 NADH + H
2 ATP
2 Pyruvate
Anaerobic Respiration
Aerobic Respiration
Pyruvate processing
2 NADH + H
2 CO2
2 Acetyl CoA
Kreb's cycle
6 NADH + H
2 FADH2
4 CO2
2 ATP
Electron Transport Chain
10 NAD+
2 FAD
12 H2O
~30-32 ATP
Aerobic Respiration Total = 34-36 ATP


ELECTRON TRANSPORT SYST
NADH2
FADH2
NAD+
FAD+
ADP + P
ATP




Outsid
electron transport chain
H+
e-
cell membrane
H2O
2H+
½O2
oxidised electron carrier
reduced electron carrier
glucose metabolism
glucose

Lec. 8

Anaerobic Respiration

- Unlike eukaryotes, some prokaryotes can respire using inorganic molecule other than oxygen as a terminal electron acceptor. They all use an electron transport system (ETS) in a membrane and synthesis of ATP via ATP synthase.

- Anaerobic respiration is a less efficient form of energy transformation than is aerobic respiration. This is partly due to the lesser amount of energy released in reactions that involve the reduction of inorganic compounds other than molecular oxygen.

- ***Nitrate reduction***

- Some microbes are capable of using nitrate as their terminal electron accepter. The energy transfer system (ETS) used is somewhat similar to aerobic respiration, but the terminal electron transport protein donates its electrons to nitrate instead of oxygen. Nitrate reduction in some species (the

best studied being *E. coli*) is a two electron transfer where nitrate is reduced to nitrite. Electrons flow through the quinone pool and the cytochrome b/c_1 complex and then nitrate reductase resulting in the transport of protons across the membrane.

- $NO_3^- + 2e^- + 2H^+ \longrightarrow NO_2^- + H_2O$

- This reaction is not particularly efficient. Nitrate does not as willingly accept electrons when compared to oxygen and the potential energy gain from reducing nitrate is less. If microbes have a choice, they will use oxygen instead of nitrate, but in environments where oxygen is limiting and nitrate is plentiful, nitrate reduction takes place.

• Denitrification

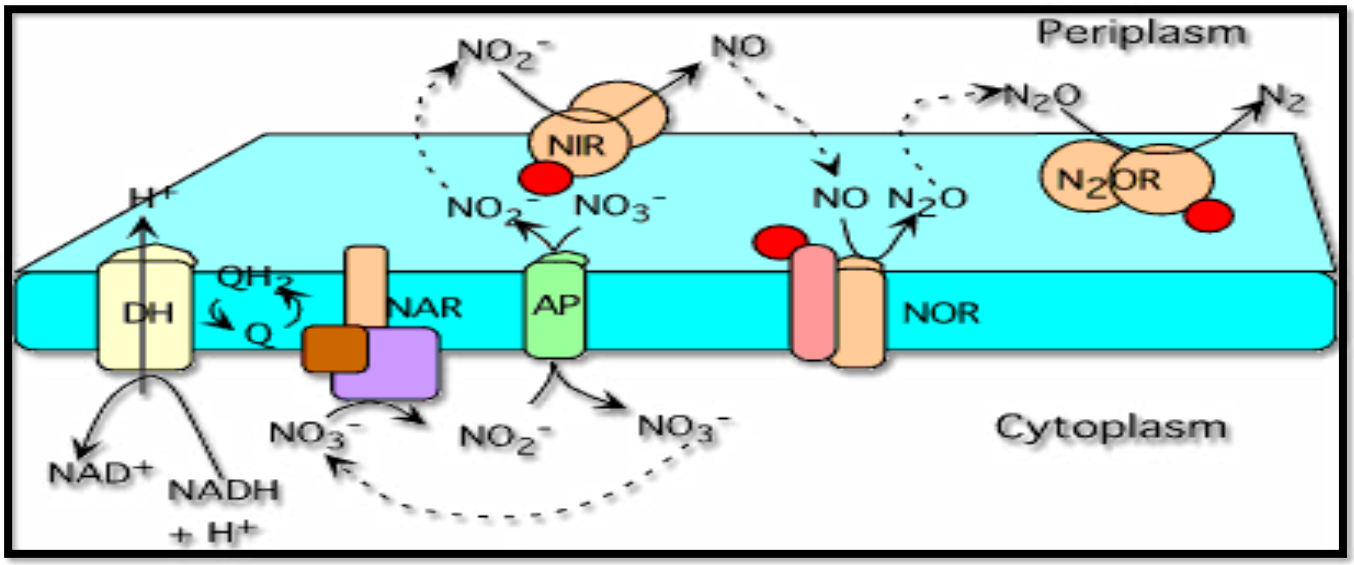
- Nitrite, the product of nitrate reduction, is still a highly oxidized molecule and can accept up to six more electrons before being fully reduced to nitrogen gas. Microbes exist (*Paracoccus denitrificans, Pseudomonas stutzeri, Pseudomonas aeruginosa*, and *Rhodobacter sphaeroides* are a few examples) that are able to reduce nitrate all the way to nitrogen gas. The process is carefully regulated by the microbe since some of the products of the reduction of nitrate to nitrogen gas are toxic to metabolism. This may explain the large number of genes involved in the process and the limited number of bacteria that are capable of denitrification. Below is the chemical equation for the reduction of nitrate to N_2.

- NO3- → NO2- → NO → N2O → N2
- Nitrate Nitrite Nitric oxide Nitrous oxide Nitrogen

- The advantage for the cell of carrying out a complete reduction of nitrate is two fold:

1) The nitrate ETS serves as a place to oxidize NADH and free it to be used in catabolism of more substrate.

2) Denitrification takes eight electrons from metabolism and adds them to nitrate to form N_2 versus just two for nitrate reduction alone. Also, donation of electrons from NADH through the cytochrome b/c_1 complex and eventually to nitrous oxide (N_2O) reductase provides another opportunity to pump protons across the membrane.



Denitrification in the membrane: ***NADH dehydrogenase complex (DH)***
nitrate reductase (NAR)
nitrite reductase (NIR) NO reductase (NOR) N_2O reductase (N_2OR) Nitrate proton (AP)

- Nitrate reduction has been extensively studied in bacteria due to its significance in the global nitrogen cycle. Denitrification removes nitrate, an accessible nitrogen source for plants, from the soil and converts it to N_2 a much less tractable source of nitrogen that most plants cannot use. This decreases soil fertility making farming more expensive.

- Intermediates of denitrification, nitrous oxide and nitric oxide, are gases and will sometimes escape the cell before being completely reduced. These compounds, when in the atmosphere, contribute to the greenhouse effect and exacerbate global warming. The use of high nitrate fertilizers in modern agriculture makes matters worse.

- ***Sulfate reduction***

- The dissimilatory reduction of sulfate seems to be a strictly anaerobic process as all the microbes capable of carrying it out only grow in environments devoid of oxygen. Sulfate (SO_4^2 is reduced to sulfide (S^{-2}), typically in the form of hydrogen sulfide (H_2S). Eight electrons are added to sulfate to make sulfide

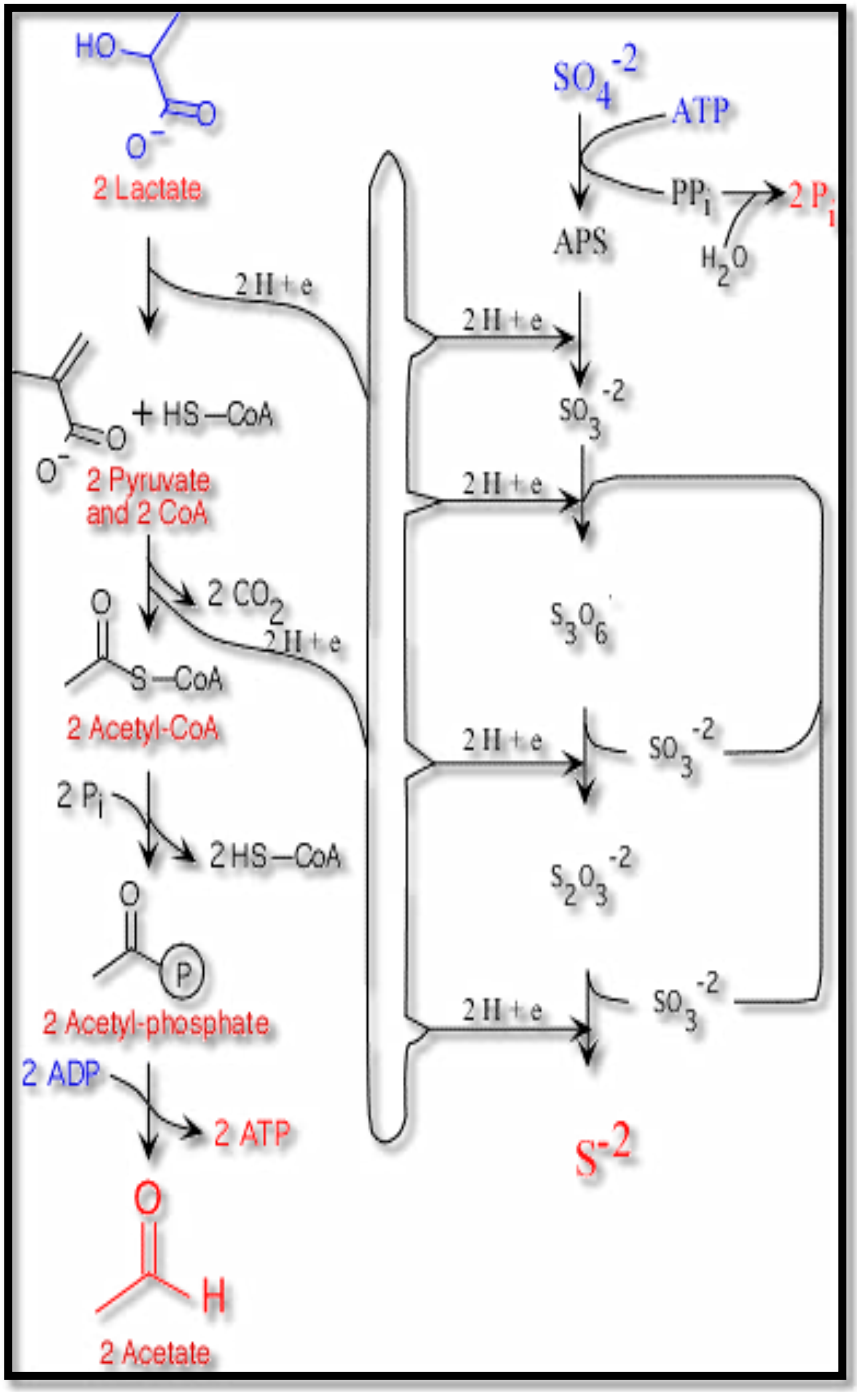
- $Acetate + SO_4^{-2} + 3H^+ \quad 2 \quad CO_2 + H_2S + 2H_2O$

- The electron potential and energy yield for sulfate reduction is much lower than for nitrate or oxygen. However, there is still enough energy to allow the synthesis of ATP when the catabolic substrate used results in the formation of NADH or FADH. ***Substrates for sulfate reducers range from hydrogen gas to aromatic compounds such as benzoate. The most commonly utilized are acetate, lactate and other small organic acids (lactate, malate, pyruvate and ethanol are some examples).*** These compounds are prevalent in anaerobic environments where anaerobic catabolism of complex organic polymers such as cellulose and starch is taking place.

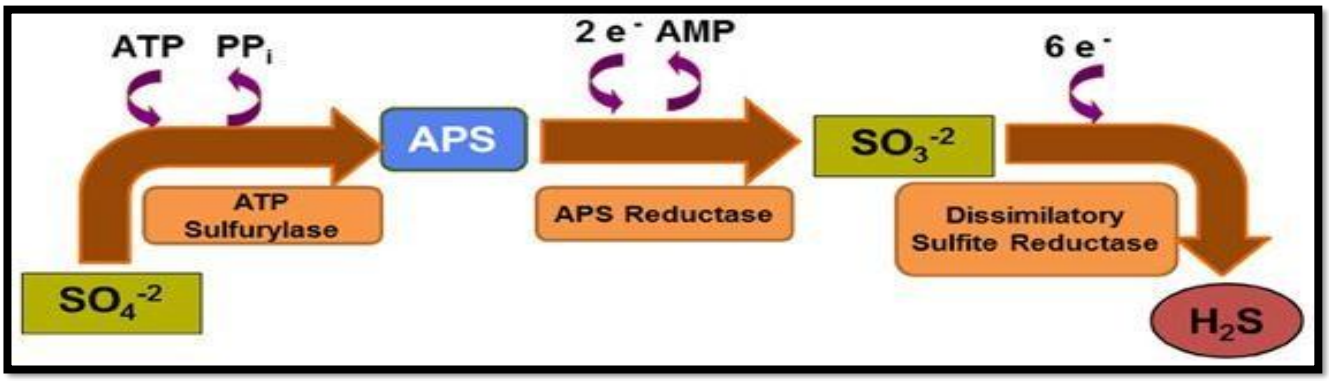
• Biochemistry of sulfate reducers

• Sulfate reducers take these growth substrates and metabolize them to acetate. The reducing power generated travels down an electron transport chain eventually reducing sulfate to hydrogen sulfide and generating energy using ATP synthase.

Pathway of sulfate reduction when grown on lactate. Lactate (in blue) is oxidized to acetate (in red) and the electrons remove eventually end up reducing sulfate (in blue) to sulfide (in red). Note that the energy gained in the process by substrate level phosphorylation (SLP) - converting acetyl phosphate to acetate - is used up to activate sulfate in the first step of sulfate reduction. Energy for metabolism is only generated via an electron transport chain.



APS=adenosylphosphosulphate



Dissimilatory sulfate reduction pathway

Microbial sulfate reduction relies on sequential catalytic reactions in which reduction of sulfate is coupled with oxidation of H_2 or simple organic molecules. This anaerobic respiration pathway is less favorable thermodynamically than aerobic respiration.

• Carbonate reduction

- Several groups of microbes are capable of using carbonate (CO_2) as a terminal electron accepter.

- ***Carbonate is a poor choice to leave the electrons with due to:***

1) its low reduction potential

2) energy yields from CO_2 reduction are low

- However, carbonate is one of the most common anions in nature and its ready availability makes it a tempting target.

- Several groups of microbes have evolved mechanisms to take advantage of carbonate. The most important group among these is the methanogens.

- **$HCO_3^- + 4H_2 + H^+$** → **$CH_4 + 3H_2O$**

- ***Methanogens*** use compounds that contain very high energy electrons as their electron donors and in the process convert CO_2 to methane (CH_4). Above is shown the use of hydrogen as the source of electrons. They are the only group of microbes that produce a hydrocarbon as major end product of their metabolism.

- Another group of carbonate reducing microbes is the ***homoacetogens***. They utilize hydrogen as the electron source and use it to reduce CO_2 to acetic acid.

- $HCO_3^- + 4H_2 + H^+$ → $CH_3\text{-}COO^- + 4H_2O$

- ***Fermentation***

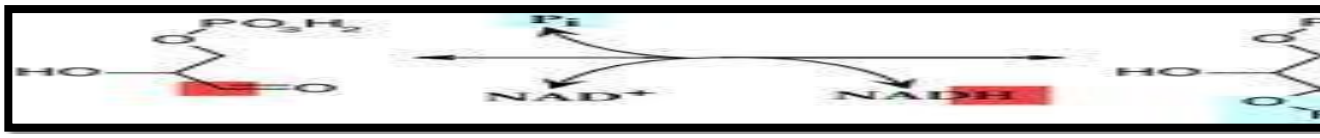
- Fermentation is defined as an energy yielding process whereby organic molecules serve as both electron donors and electron accepters. The molecule being metabolized does not have all its potential energy extracted from it. In other words, it is not completely oxidized.

- ***General Concepts***

- Despite the many methods bacteria employ to ferment organic compounds, there are some unifying concepts that are true of all fermentations:

- **1- NAD^+ is almost always reduced to NADH:** Remember that metabolism involves the oxidation of the substrate. These electrons are removed from the organic molecule and most often given to NAD.

(This is true both in fermentation and respiration). Below is shown an example of NAD reduction.



- **2- Fermentation results in an excess of NADH:** Accumulation of NADH causes a problem for anaerobes. They have too much of it and it prevents further oxidation of substrate due to a lack of an NAD^+ pool to accept electrons. In many fermentation pathways, the steps after energy generation are performed in part to get rid of the NADH.

- **3- Pyruvate is often an important intermediate:** Many of the reactions that we will look at eventually end up making pyruvate. Pyruvate is a valuable intermediate because it can be used for cell synthesis and many different enzymes can act on it. It gives the microbe flexibility.

- **4- Energy is derived from Substrate-Level Phosphorylation (SLP):** is a type of chemical reaction that results in the formation and creation of adenosine triphosphate (ATP) by the direct transfer and donation of a phosphate group to adenosine diphosphate (ADP) from a reactive intermediate. In cells, it occurs primarily and firstly in the cytoplasm (in glycolysis) under both aerobic and anaerobic conditions.Unlike oxidative phosphorylation, here the oxidation and phosphorylation are not coupled or joined, although both types of phosphorylation result in ATP.

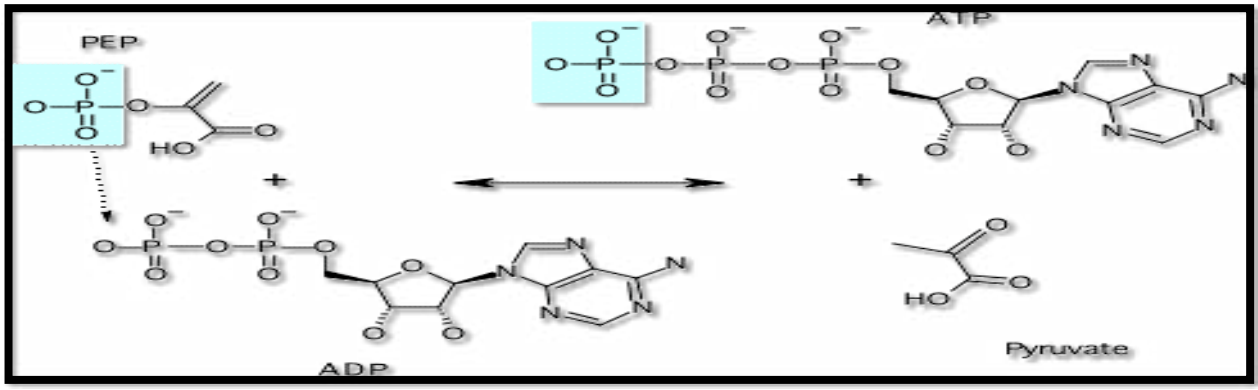
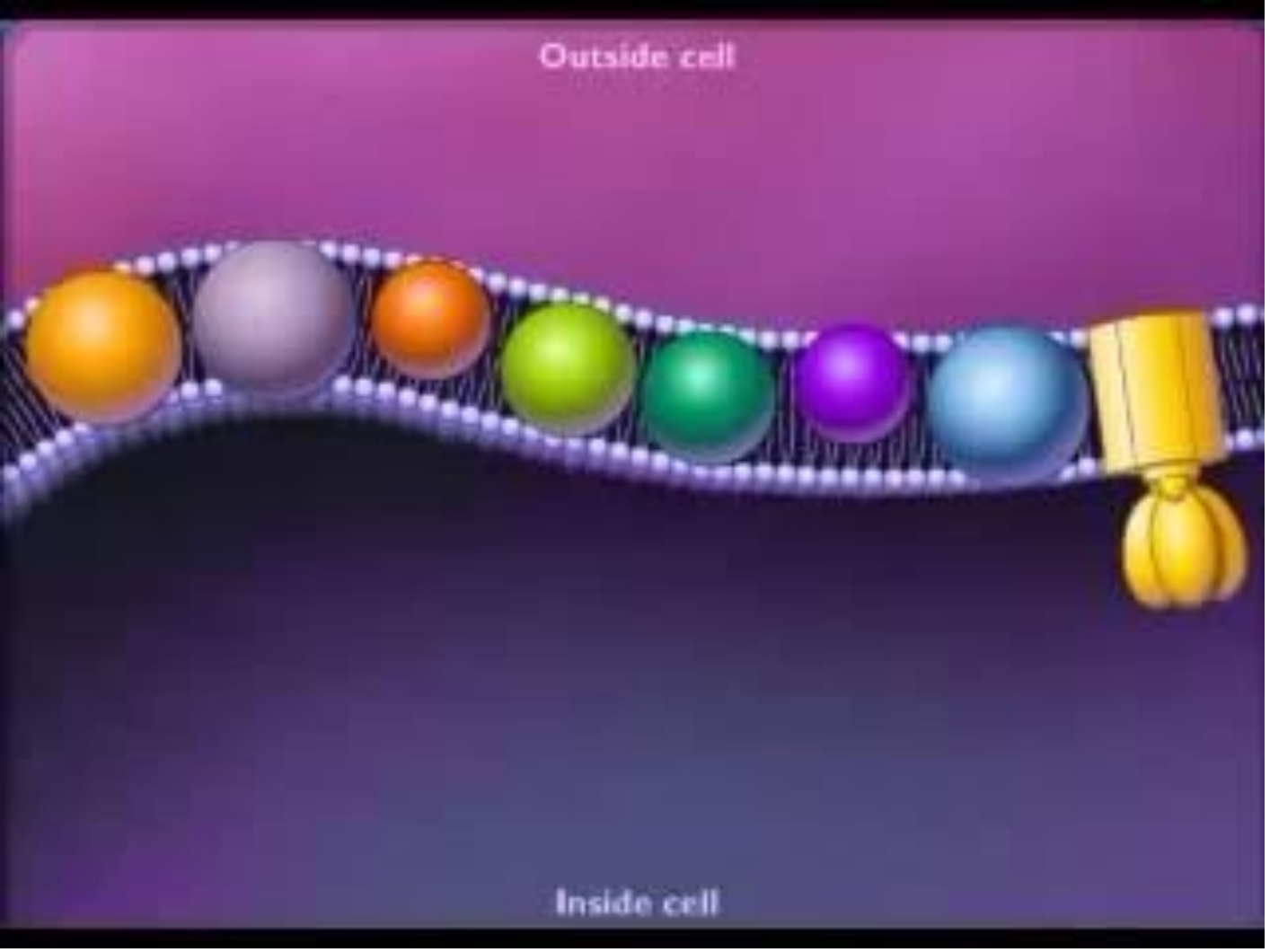


Figure 5: Phosphoenolpyruvate is converted to pyruvate with the formation of ATP. The phosphate highlighted in blue is transferred from PEP to ATP.

- **5- Energy yields are low:** SLP is an inefficient process and much of the energy of the electrons is lost. Typically energy yields are 1-4 ATP per substrate molecule fermented.

- **6- Oxygen is not involved:** Fermentation can involve any molecule that can undergo oxidation. Typical substrates include sugars (such as glucose) and amino acids. Typical products depend upon the substrate but can include organic acids (lactic acid, acetic acid), alcohols (ethanol, methanol, butanol), ketones (acetone) and gases (H_2 and CO_2).


Outside cell
Inside cell

Lec. 9

Quorum Sensing

Bacteria communicate with one another using chemical signaling molecules as words. Specifically, they release, detect, and respond to the accumulation of these molecules, which are called autoinducers.

—Detection of autoinducers allows bacteria to:

1) distinguish between low and high cell population density (accumulation of signaling molecules enable a single cell to sense the number of bacteria (cell density))

2) control gene expression in response to changes in cell number (allows a population of bacteria to coordinately control the gene expression of the entire community)

—Many bacterial behaviors are regulated by quorum sensing including:

1) symbiosis

2) bioluminescence

3) virulence

4) antibiotic production

5) sporulation

6) swarming

7) conjugation

8) biofilm formation

— This phenomenon was first described in the bioluminescent marine bacterium Vibrio fischeri

—V. fischeri lives in symbiotic associations with a number of marine animal hosts. In these partnerships, the host uses the light produced by V. fischeri for specific purposes such as:

1) attracting prey

2) avoiding predators

3) finding a mate

- In exchange for the light it provides, V. fischeri obtains a nutrient-rich environment in which to reside

- A luciferase enzyme complex is responsible for light production in V. fischeri. Bioluminescence only occurs when V. fischeri is at high cell number, and this process is controlled by quorum sensing.

- Specifically, the production and accumulation of, and the response to, a minimum threshold concentration of an acylated homoserine lactone (HSL) autoinducer will:

1) regulates density-dependent light production in

8. *fischeri*

2) enables V. fischeri to emit light only inside the specialized light organ of the host but not when free-living in the ocean.

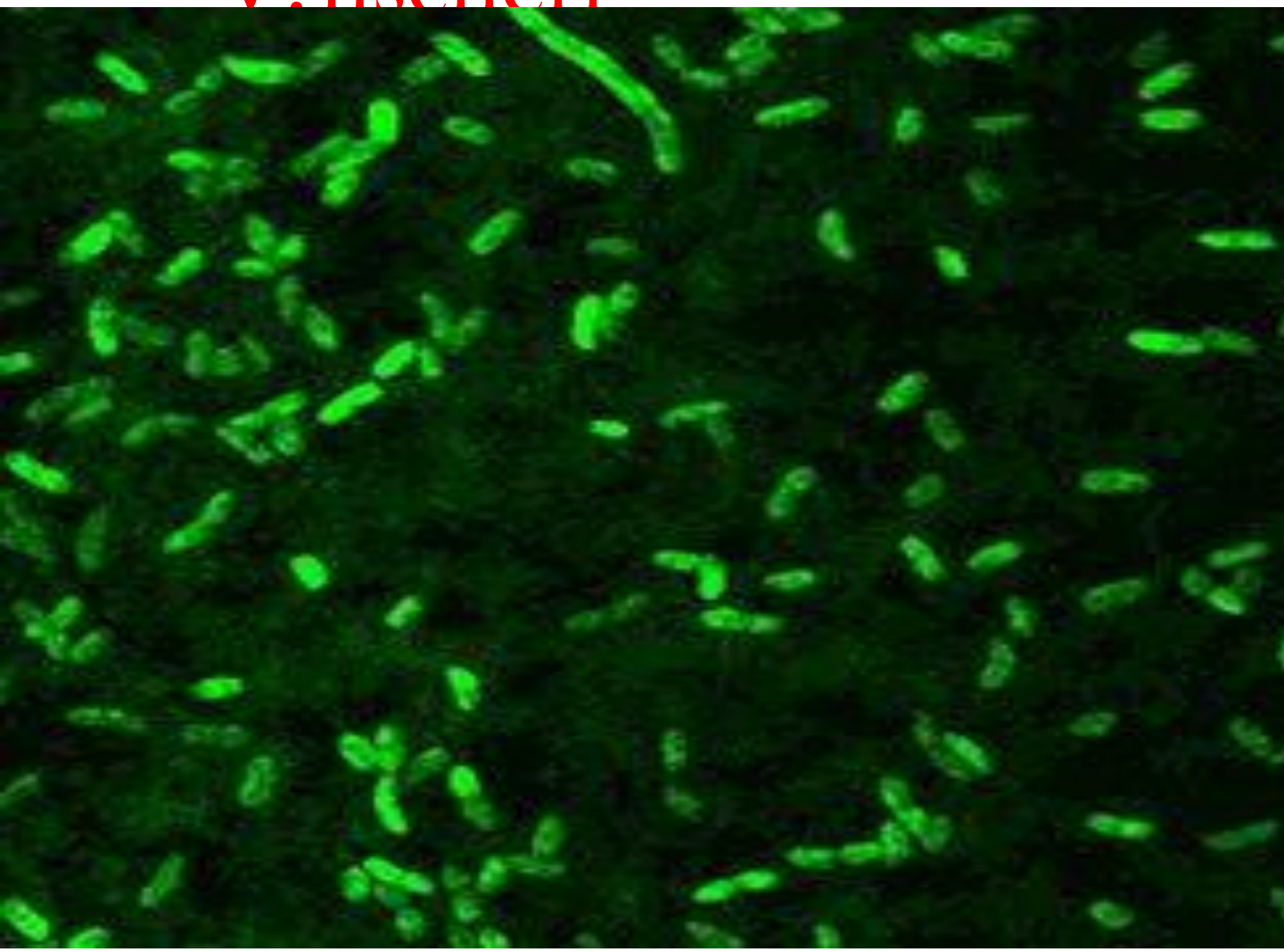
—There are two reasons for this:

- First, only under the nutrient-rich conditions of the light organ can V. fischeri grow to high population densities

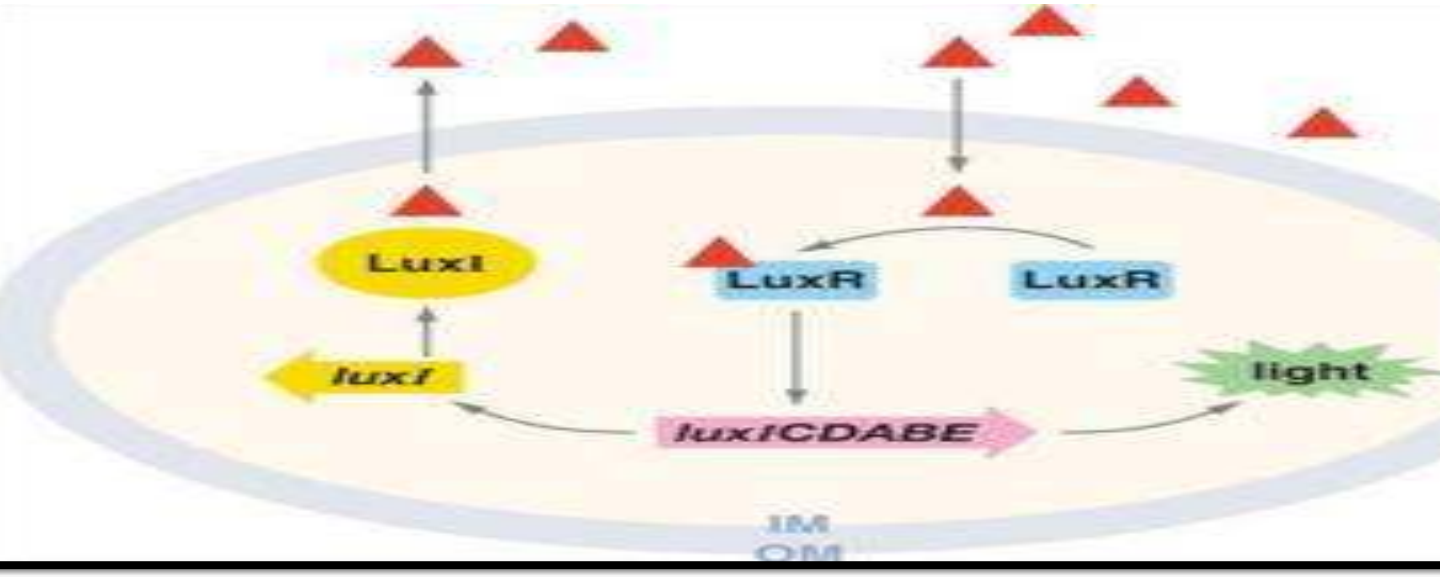
- second, trapping of the diffusible autoinducer molecule in the light organ with the bacterial cells allows it to accumulate to a sufficient concentration that V. fischeri can detect it

Gram negative bacteria homoserinelactones as w

- V. fischeri



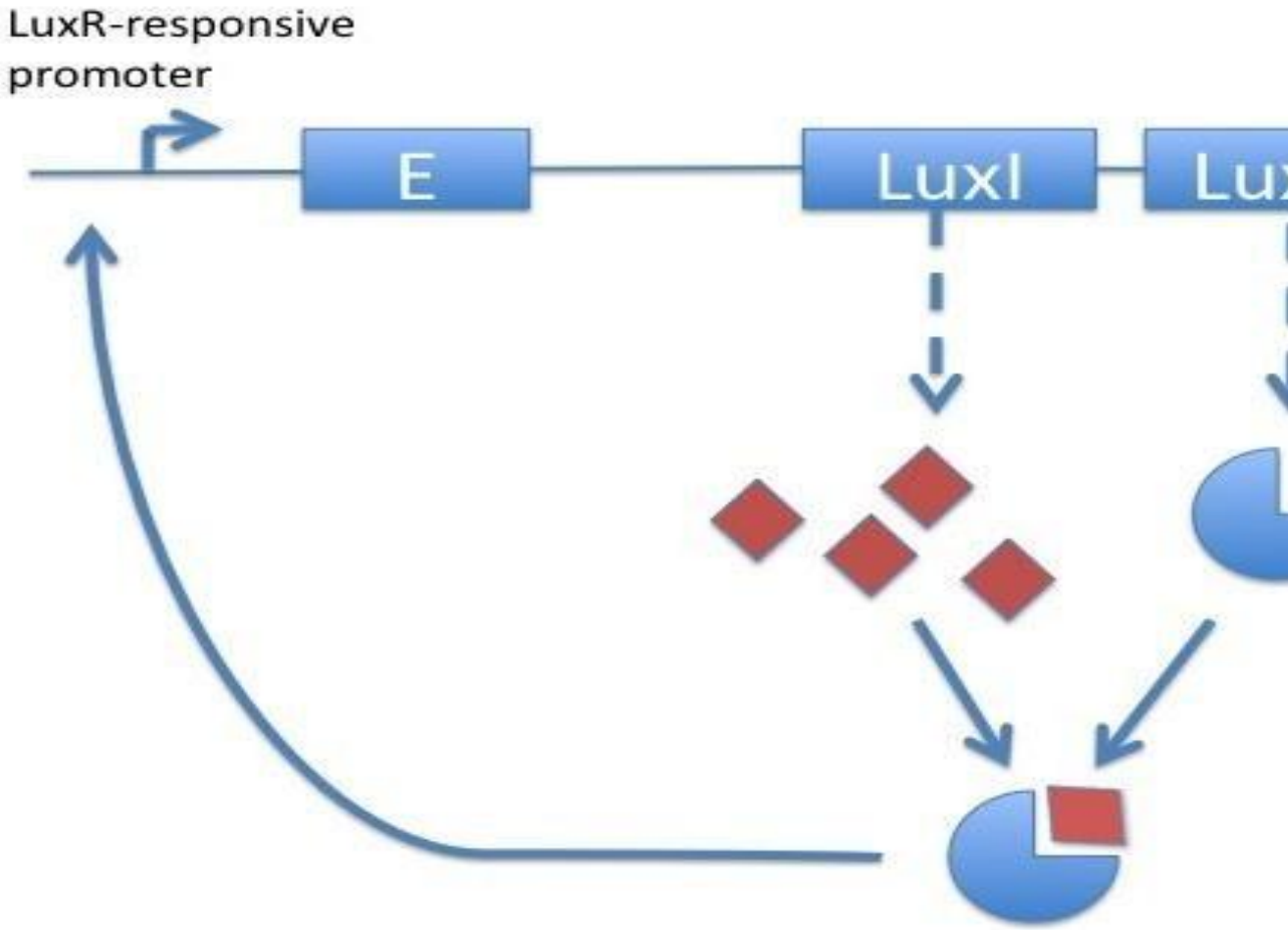
- The LuxI protein is responsible for production of the HSL autoinducer

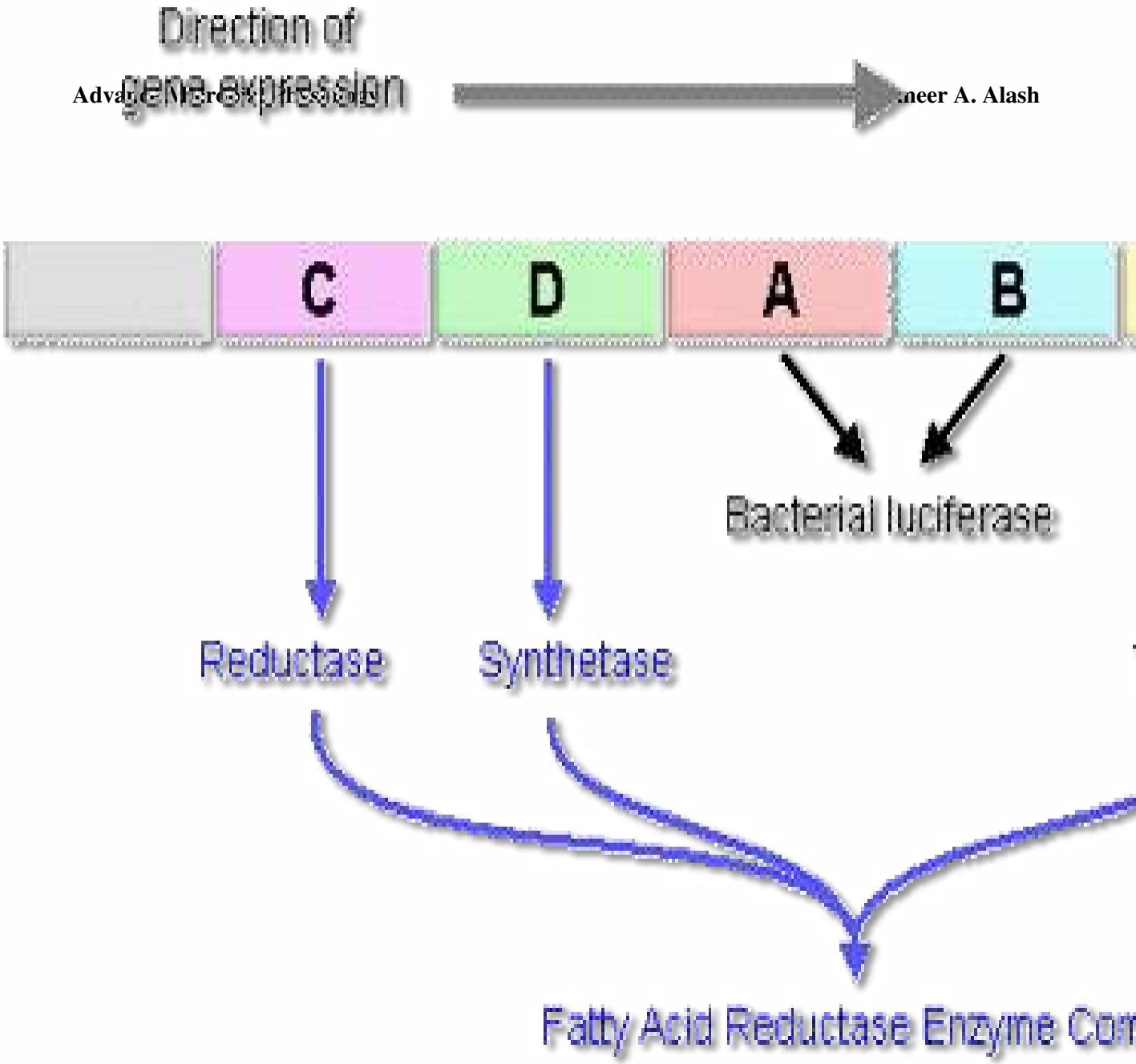


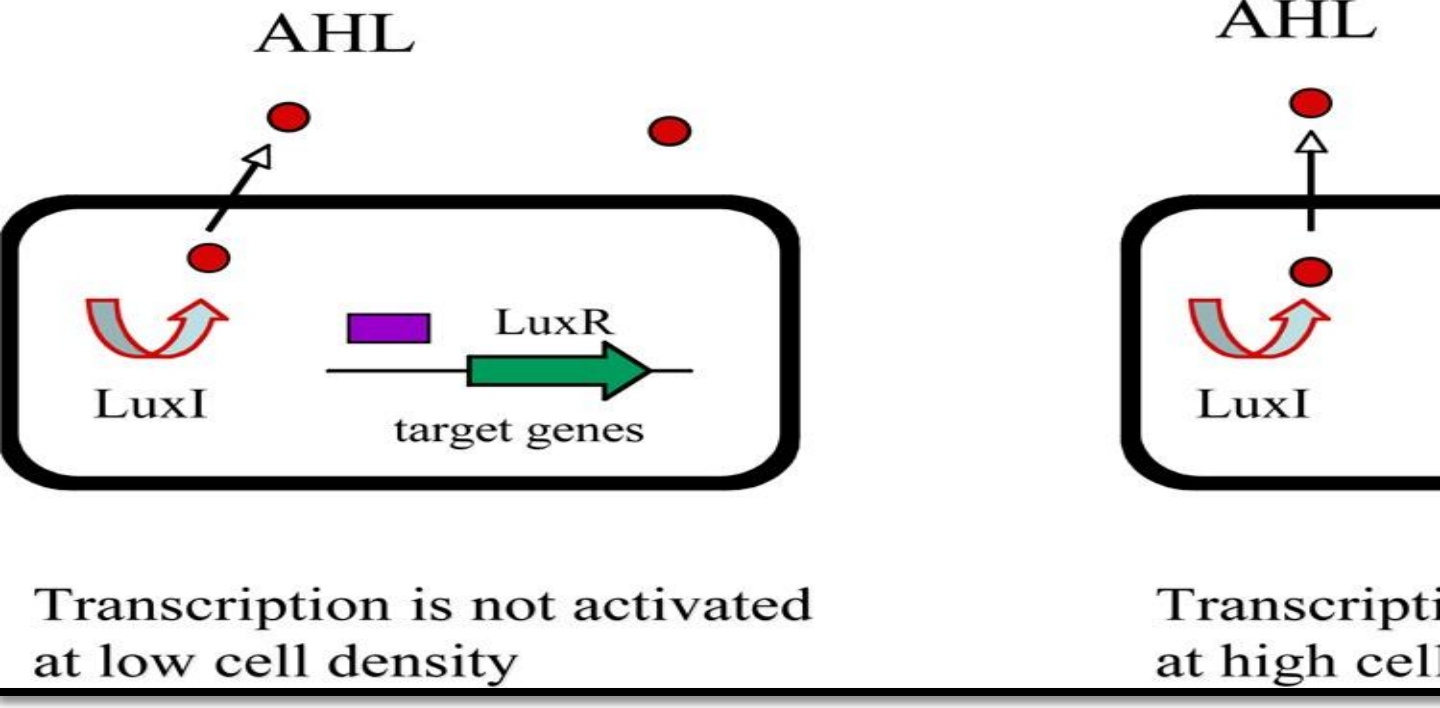
- the LuxR protein is responsible for binding the HSL autoinducer and activating transcription of the luciferase structural operon at high cell density

- ***(The receptor/activator proteins that mediate the cell's response to them constitute evolutionarily conserved families of regulatory proteins known as the LuxI and LuxR families)***

Acyl-HSL autoinducers freely diffuse through the bacterial membrane, allowing them to increase in concentration in the external environment in conjunction with cell population growth.


LuxR-responsive
promoter
E
LuxI
Lux


Direction of gene expression
C
D
A
B
Bacterial luciferase
Reductase
Synthetase
Fatty Acid Reductase Enzyme Com



As an autoinducer-producing population of V. fischeri cells grows, the concentration of autoinducer increases as a function of increasing cell-population density. When the autoinducer concentration reaches the micromolar range, it can interact with the LuxR protein, and the LuxR-autoinducer complex binds the luciferase promoter to activate transcription. Therefore, this quorum sensing circuit allows light production to be tightly correlated with the cell population density.

- Communication via LuxI/LuxR (HSL/transcriptional activator) signaling circuits appears to be the standard mechanism by which Gram-negative bacteria talk to each other, as quorum sensing systems resembling the V. fischeri circuit have been shown to control gene expression in over 25 species of Gram-negative bacteria.

- In every case, an acylated HSL is the signal molecule whose synthesis is dependent on a LuxI-like protein.

- A cognate LuxR-like protein is responsible for recognition of the HSL autoinducer and subsequent transcriptional activation of downstream target genes.

Escherichia coli

- A new communication factor have been discovered, that is produced by the intestinal bacteria Escherichia coli. The new factor is secreted by the bacteria and serves as a communication signal between single bacterial cells.

- The communication factor formed by E. coli enables the activation of a built-in "suicide module" which is located on the bacterial chromosome and is responsible for bacterial cell death under stressful conditions.

  Therefore, the new factor has been designated EDF (Extra-cellular Death Factor).

- EDF is a symmetric, linear pentapeptide whose amino acid sequence is Asn-Asn-TrpAsn-Asn.

- While suicidal cell death is counterproductive for the individual bacterial cell, it becomes effective for the bacterial community as a whole by the simultaneous action of a group of cells that are signaled by EDF.

- Under stressful conditions in which the EDF is activated, a major sub-population within the bacterial culture dies, allowing the survival of the population as a whole.

- EDF functions may provide a lead for a new and more efficient class of antibiotics that specifically trigger bacterial cell death in the intestine bacteria Escherichia coli and probably in many other bacteria, including
  those pathogens that also carry the "suicide module"

Lec. 10

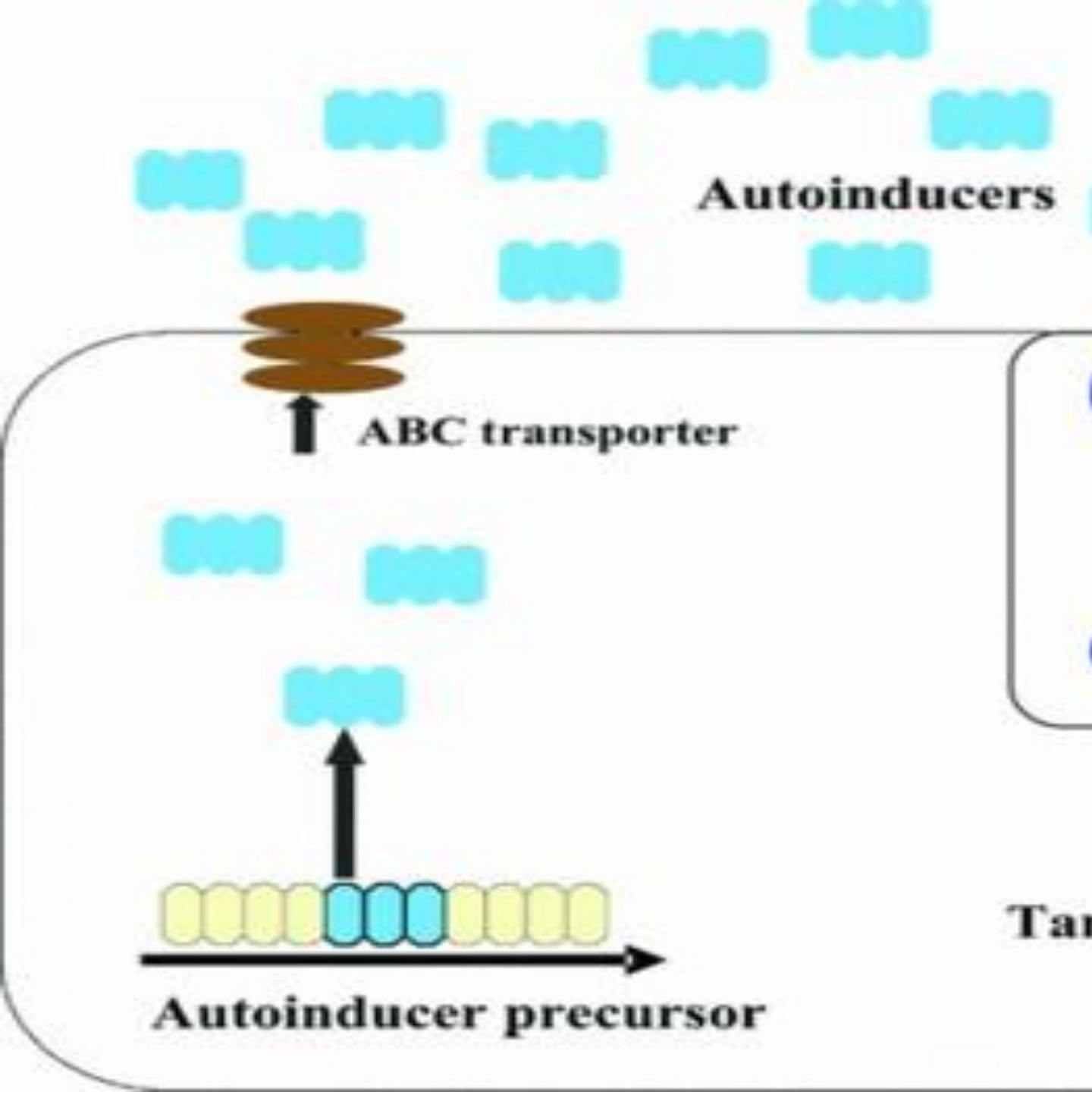
Quorum Sensing

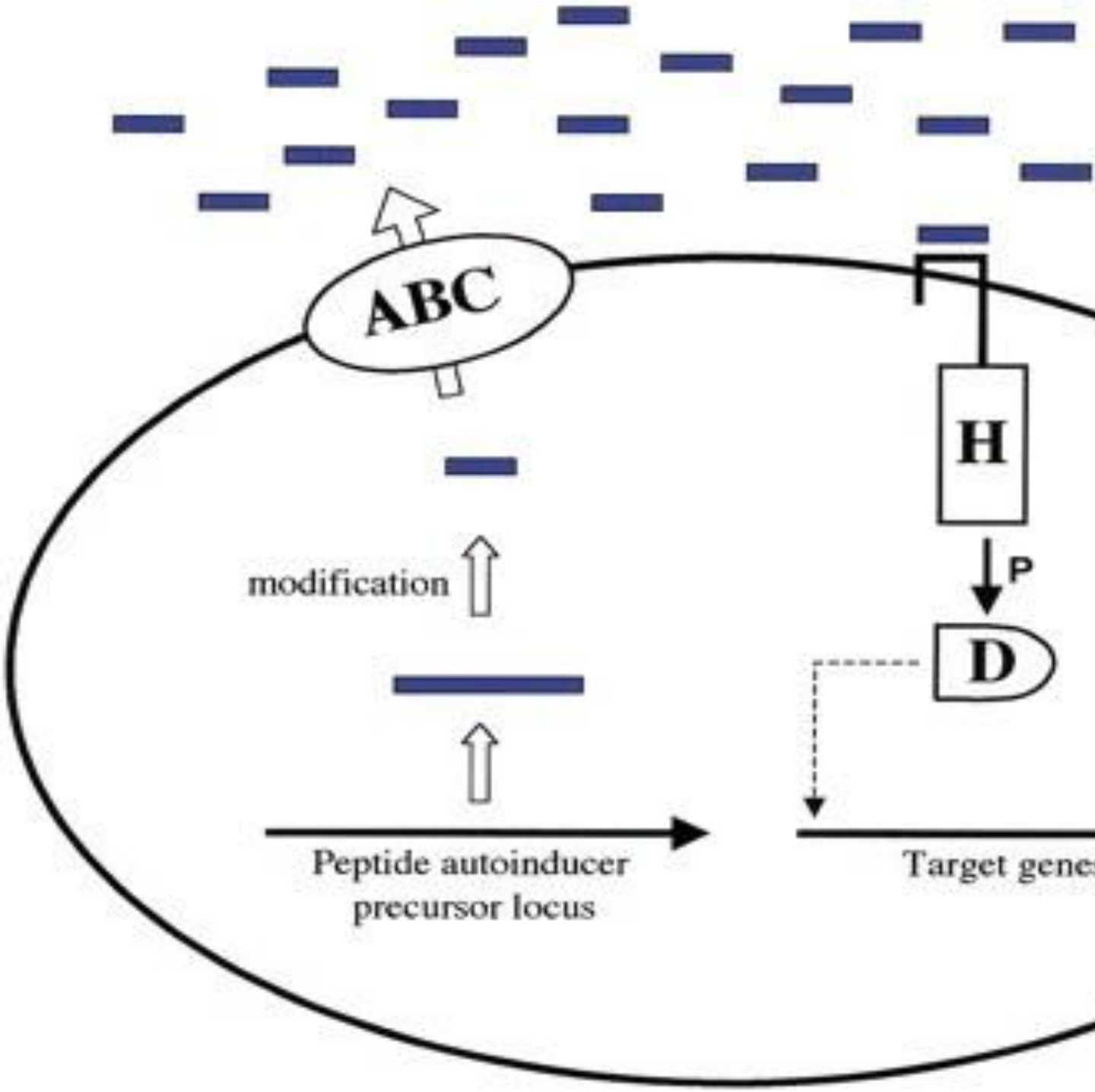
Gram-positive bacteria speak with oligopeptides

- Gram-positive bacteria have evolved a basic communication mechanism that is different from that used by Gram-negative bacteria.

- In this case, the signals are modified oligopeptides that are secreted into the medium and accumulate at high cell density.

- The detectors for the oligopeptide signals are two-component adaptive response proteins.
- Bacteria use two-component proteins to detect fluctuations in environmental stimuli and relay the information regarding these changes into the cell. The mechanism of signal transduction is via a conserved phosphorylation/dephosphorylation mechanism.
- Analogous to Gram-negative quorum sensing bacteria, Gram-positive bacteria employ a conserved signal-response mechanism as the foundation of the quorum sensing process, and the addition of diverse regulatory components fine-tunes each circuit to the individualized needs of the species.


Autoinducers
ABC transporter
Autoinducer precursor


ABC
H
P
D
modification
Peptide autoinducer
precursor locus
Target gene

qA specific precursor peptide is produced.

qThe precursor peptide is modified, processed, and an ATP-binding cassette (ABC) exporter complex secretes the mature oligopeptide autoinducer

qThe oligopeptide autoinducer accumulates as the cells grow.

qAt high cell density, the autoinducer is detected by a two-component signal transduction system.

- Specifically, the sensor kinase protein recognizes the autoinducer and subsequently autophosphorylates at a conserved histidine residue (H).
- The phosphoryl group is transferred to a cognate response regulator protein, and this protein is phosphorylated on a conserved aspartate residue (D).
- The phosphorylated response regulator binds to specific target promoters to modulate the expression of quorum sensing regulated genes.
- P denotes that the mechanism of signal transduction is by phosphate transfer between the regulatory elements.

Multilingual bacteria

- recent studies suggest that bacteria may have evolved multiple languages that serve different purposes.

- It appears that many bacteria possess a species-specific language as well as a species-nonspecific language.

—These findings imply that bacteria:

1) can assess their own population numbers and also the population density of other species of bacteria in the vicinity.

2) Furthermore, distinct responses to the intraspecies and interspecies signals allow a particular species of bacteria to properly modulate its behavior depending on whether it makes up a majority or a minority of any given consortium.

— In Gram-negative bacteria, two types of AIs have been observed (AI-1 and AI-2):

1) AI-1 molecules are N-acyl-homoserine lactones (AHL)

2) AI-2 is a unique furanosyl borate diester

—The AI-1 regulatory system consists of two structural genes :

1) luxI that encodes the AI-1 synthase
2) luxR that encodes the AI-1 response regulator

—luxI and luxR homologues are present in a wide variety of gram-negative bacteria and control numerous processes ranging from virulence genes to biofilm formation.

- The gene responsible for AI-2 production (luxS) is highly conserved across many species and the ability of AI-2 from a diverse group of species to regulate gene expression in other bacterial species indicates that it may have a role in inter-species communication as opposed to intra-species communication typical of AI-1 autoinducers.

- The AI-2 system is particular interesting because it has been correlated with pathogenicity of several organisms.

- With respect to symbiosis multiple signaling systems may be important in a complex community structure such as the rhizosphere where bacterial species need to coordinate their activities with bacteria of the same species as well as a host of other bacterial species.

- the cognate sensors LuxN and LuxPQ recognize AI-1 and AI-2, respectively.

- LuxP is a soluble periplasmic protein, which is proposed to be the primary receptor for AI-2. LuxP, in complex with AI-2, interacts with LuxQ to send the AI2 signal.

- LuxN and LuxQ are two component hybrid sensor kinase/response regulator proteins that funnel their phosphorylation signals to a shared integrator protein called LuxU which is in turn channels the signal to a final response regulator protein called LuxO that induces the expression of a repressor of the luciferase structural operon (luxCDABE).

- This putative repressor is called X.

- A transcriptional activator called LuxR is also required for expression of luxCDABE. However, the V. harveyi LuxR is not similar to the LuxR from V. fischeri and other Gram-negative quorum sensing bacteria.

- The V. harveyi quorum sensing circuit is proposed to function as follows:

1) At low cell density, in the absence of AI-1 and AI-2, LuxN and LuxQ are autophosphorylating kinases that transfer phosphate to LuxU.

2) LuxU subsequently transfers the phosphate to LuxO.

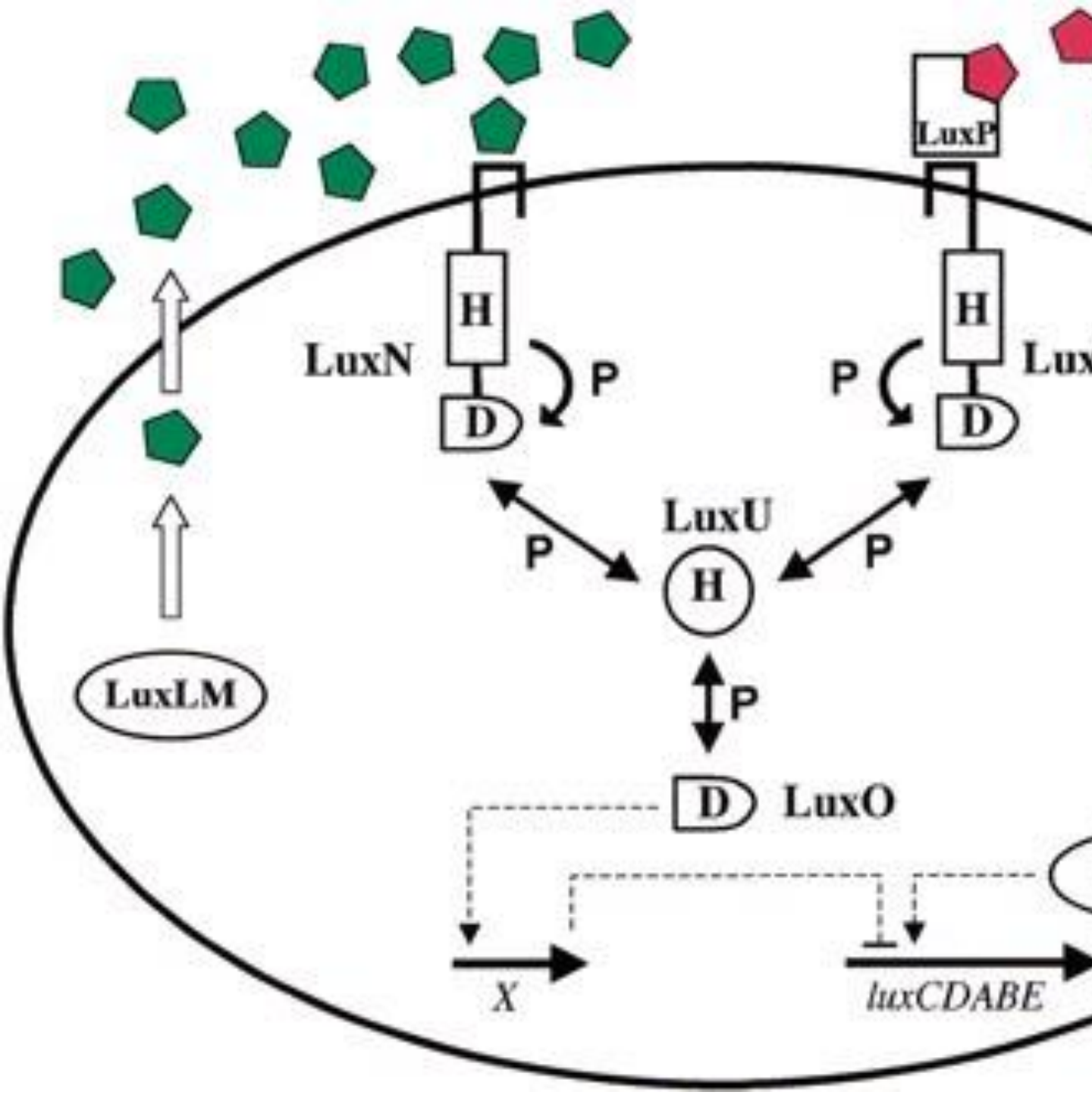
3) Phospho-LuxO is active.

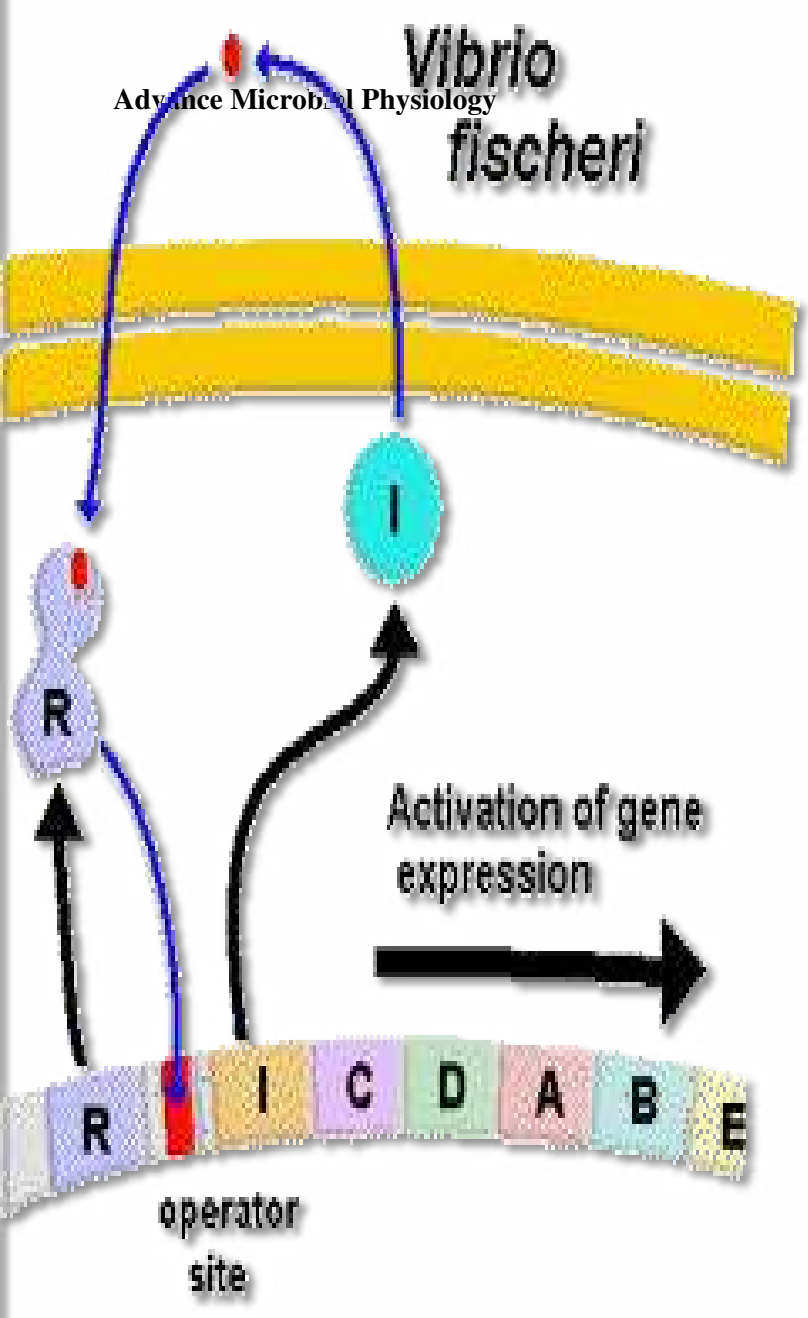
4) In conjunction with the alternative sigma factor ^{54}, phospho-LuxO activates the transcription of X, and X, in turn, represses the expression of luxCDABE. Therefore, under the low cell density condition V. harveyi makes no light.

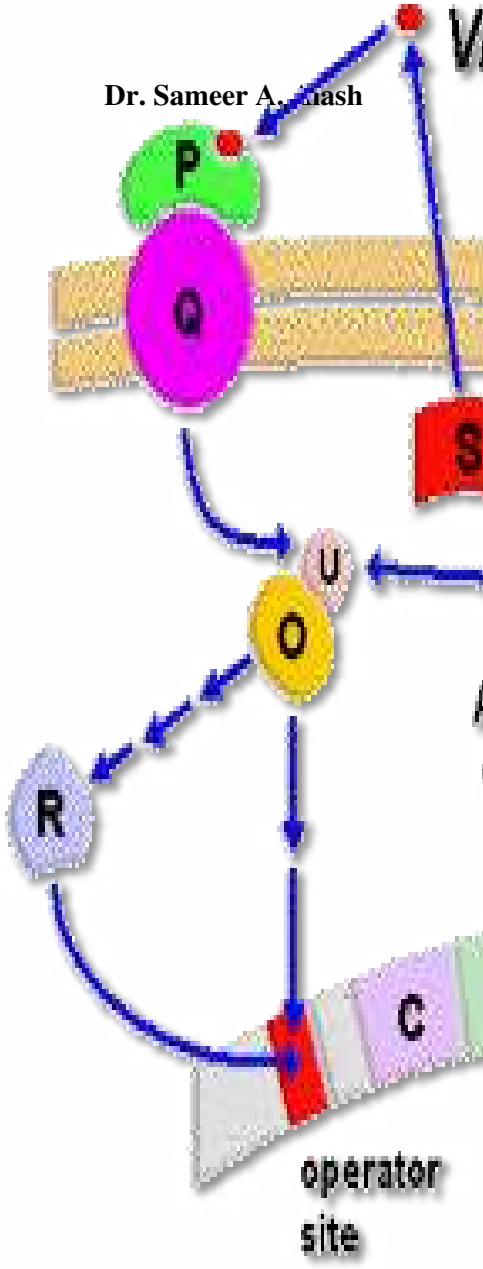
- At high cell density, when the autoinducers have accumulated, interaction of AI-1 and AI-2 with LuxN and LuxPQ induces LuxN and LuxQ to switch from kinase mode to phosphatase mode.

- As phosphatases, LuxN and LuxQ drain phosphate from LuxO via LuxU. DephosphoLuxO is inactive. Thus, the repressor X is not transcribed, and the LuxR protein activates transcription of luxCDABE. Therefore, under the high cell density condition V. harveyi emits light.

- Many experiments led to the hypothesis that V. harveyi uses AI-1 for intra-species communication and AI-2 for interspecies cell-cell signaling


LuxP
H
LuxN
P
D
P
H
P
D
Lux
LuxU
P
P
H
LuxLM
P
D
LuxO
X
luxCDABE


Vibrio
fischeri
I
R
Activation of gene
expression
R
I
C
D
A
B
E
operator
site


P
Q
U
O
R
C
operator
site

—P. aeruginosa biofilms exist in the lungs of cystic fibrosis (CF) sufferers. An intact quorum sensing circuit is required for proper biofilm formation by P. aeruginosa.

—B. cepacia is an emerging pathogen in CF infections. Usually CF individuals infected with B. cepacia are coinfected with P. aeruginosa.

— Addition of P. aeruginosa autoinducers to

B. cepacia induces the expression of B. cepacia virulence factors. It is hypothesized that

P. aeruginosa is the primary colonizer in the CF lung, and interspecies communication allows

B. cepacia to establish itself in the host