

## Microbial Nutrition and Growth

Some of the information relating to microbial nutrition and growth has been presented in previous lectures or in laboratory sessions. This information will be included here as a review.

### Nutritional Categories:

Microorganisms (and macroscopic forms also) can be divided into four nutritional categories based on the sources they use for **energy** and **carbon**. The four categories are:

- 1) **Chemoheterotrophs** = Organisms requiring preformed organic compounds for both their energy and carbon requirements. These organisms may also be referred to as **chemoorganotrophs**.
- 2) **Chemoautotrophs** = Organisms that use chemicals for energy, but are capable of using inorganic compounds (e.g.,  $\text{CO}_2$ ,  $\text{HCO}_3^-$ , etc.) for carbon. If these organisms use only inorganic chemicals to meet their nutritional needs, they may be called **chemolithotrophs**.
- 3) **Photoheterotrophs** = Organisms that use light energy and require preformed organic compounds as their source of carbon.
- 4) **Photoautotrophs** = Organisms that use light energy and are capable of using inorganic compounds (e.g.,  $\text{CO}_2$ ,  $\text{HCO}_3^-$ , etc.) for carbon.

Animals (including humans), fungi, protozoa and many types of prokaryotic organisms are chemoheterotrophs. Of these, some are **saprotrophs**, some **parasites**, some **hypotrophs** and many are free-living organisms consuming other life forms as food materials. Free-living protozoa may be considered predators (**carnivores**) or **herbivores**, depending on the prey they consume, but many of these are **omnivorous**.

Green plants, algae, cyanobacteria and some other types of prokaryotic organisms are photoautotrophs. These are ecologically considered as **producers** because they provide much of the organic material consumed by various different types of chemoheterotrophs. Most photoheterotrophs are prokaryotic, but because some single-celled eukaryotic forms are capable of switching between nutritional categories, they might function as photoheterotrophs for short periods of time. All chemoautotrophs are prokaryotic.

### Culture media:

Most of the microorganisms grown under laboratory conditions are chemoheterotrophs. This is particularly true in clinical laboratories where human pathogens are of primary interest. Many chemoheterotrophs can be grown on or in mixtures of materials designed to support their metabolic processes. Mixtures of materials providing all the nutrients needed to grow microorganisms **in vitro**, i.e., in artificial containers, are called **culture media** (singular = **medium**). When considering the nutrients necessary for growth, it is useful to consider the composition of protoplasm and the elements incorporated into various compounds. Some common nutrients are listed below.

- 1) **Carbon** – Carbon is essential for the synthesis of all organic compounds, carbohydrates, proteins, lipids and nucleic acids. Though autotrophs can obtain carbon (carbon dioxide) from the air, heterotrophs cannot, and must have this element provided to them in the culture medium. Carbon is often provided in carbohydrate form (glucose, lactose, mannitol, etc.) or as protein or protein breakdown products (peptones, peptides or proteoses). Media without carbon can be used to grow cyanobacteria.
- 2) **Nitrogen** – Nitrogen is essential for the synthesis of proteins, nucleic acids and some carbohydrates, e.g., glucosamine. Since proteins and protein breakdown products provide both carbon and nitrogen, these are common ingredients in culture media. Some prokaryotic organisms can obtain nitrogen from the air (molecular nitrogen =  $N_2$ ), and are called nitrogen-fixing organisms. Bacteria with this capability can grow readily on nitrogen-free media.
- 3) **Minerals** – Many of the elements incorporated into organic compounds are minerals such as sulfur, phosphorous, iron, calcium, magnesium, iodine, manganese and copper. Sulfur is incorporated into certain amino acids, so is essential to protein formation. Phosphorous is used for the synthesis of phospholipids, nucleic acids and a variety of nucleotide derivatives such as **coenzymes** (NAD, FAD, NADP), and **high energy compounds** (ATP, GTP, etc.). Iron and copper often serve as **prosthetic groups** in enzymes with quaternary structure, and calcium, magnesium and manganese often function as **cofactors** essential to enzyme activity. Electrolytes such as NaCl, KCl and  $CaCl_2$ , dissociate into ions involved in maintaining membrane potentials, activating contractile elements (e.g., microfilaments) and other cellular functions. Iodine is incorporated into various organic compounds.
- 4) **Water** – Though not usually considered as a nutrient, water is essential to metabolism, and is incorporated into organic compounds (as  $H^+$  and  $OH^-$ ) during **hydrolysis reactions**. The amount of water present relative to solute materials also influences **tonicity**, making environments **isotonic**, **hypotonic** or **hypertonic**. Though bacteria are not damaged by osmosis when placed in hypotonic environments (because they are equipped with walls), the nutrient levels in hypotonic media tend to be low, and growth may be slow. Hypertonic environments tend to inhibit growth because they cause water to exit cells and metabolism to slow or stop. For this reason, salt and sugar are often used to preserve food materials. Since high salt environments tend to occur naturally in various places, e.g., along rocky ocean shores, in deserts, on skin surfaces and inside nasal passages, many microorganisms have adapted to these environments. These salt-loving organisms are called **halophiles**.
- 5) **Buffers** – Some of the minerals listed above are also incorporated into buffers, i.e., substances that resist pH change when the acidic or alkaline waste products of various microorganisms are released into media.  $K_2HPO_4$ ,  $KH_2PO_4$  and  $CaCO_3$  are examples of materials often incorporated into buffer systems. Culture media without buffers often contain **pH indicators**, i.e., substances that change color in response to changes in pH (the acidity or alkalinity of the environment). Phenol red, bromothymol blue and bromocresol purple are pH indicators commonly used in culture media.
- 6) **Other** – Some organisms will only grow when provided with specific growth factors such as vitamins, amino acids, cells, tissue fragments or other materials. Such media are called **enriched media** and are used to grow **fastidious microorganisms** (picky eaters). Bacteria in the genera *Streptococcus* and *Neisseria* tend to be fastidious and are often grown on enriched media such as blood agar or chocolate agar.

As demonstrated in the laboratory, culture media may be categorized in a variety of ways. **Broth media** are liquid in form and when incubated on a moving surface such as an orbital shaker, allow all organisms present to maintain contact with the nutrients provided. **Solid media** contain some type of solidifying agent in addition to the nutrients present. Though a variety of solidifying agents could be used, **agar** (a polysaccharide) is the material most commonly used to change broth media into solid media. Agar is an ideal solidifying agent for culture media because most microorganisms do not catabolize it (break it down). Unlike those containing gelatin or starch, solidifying agents catabolized by many different types of microorganisms, media made with agar will remain solid as microbial cultures grow. Another advantage of agar is its response to temperature changes. Media made with agar will remain solid at relatively high incubation temperatures (considerably above 37° C) while media made with gelatin will liquefy. Agar is made from algae in the phylum Rhodophyta.

Culture media containing agar are prepared as liquids and poured into various containers where they are allowed to solidify. Agar deeps, slants/slopes and plates are commonly used in our laboratory. Agar plates provide broad surfaces where cultures can be streaked and colonies isolated to check for purity. Agar slants and deeps are often used for enzymatic testing, but can also be used for long-term storage if the culture tubes used are equipped with screw-on caps.

### **Factors influencing microbial growth:**

Many factors influencing microbial growth were described in association with criteria used in microbial classification. These same factors can also be associated with microbial control, and will be described in that context later. Some factors known to influence microbial growth include:

- 1) **Gas requirements** – Microorganisms can be categorized as **obligate aerobes**, **facultative anaerobes**, **obligate anaerobes** or **microaerophiles** based on their gas requirements. Obligately aerobic organisms have **respiratory/oxidative** metabolic processes and use molecular oxygen (O<sub>2</sub>) as a final electron acceptor. Aerobic bacteria often grow near the surface in broth media and sometimes form **pellicles** or skin-like coverings at the medium surface. Facultatively anaerobic organisms can often use either respiratory or fermentative metabolic processes and switch back and forth between the two as their environments permit. Facultative organisms will grow throughout broth media if they are motile, or if agitated during incubation; but non-motile forms will often form a mass of sediment at the bottom of a stationary tube. Obligately anaerobic forms may also form sediments on tube bottoms, but typically require special containment or anaerobic media for growth. Many of the organisms inhabiting the Winogradsky columns and window are obligately anaerobic.
- 2) **Temperature** – Organisms can be categorized as **psychrophiles**, **mesophiles**, **thermophiles** or **hyperthermophiles** based on the temperatures they prefer for growth. Bacteria as a group can inhabit environments with extreme temperature differences, e.g., the freezing temperatures of polar ice Vs the near boiling temperatures of hot springs or deep-sea thermal vents; but individual species typically have relatively narrow temperature ranges. Enzyme activity and therefore metabolism is dependent on temperature, and enzymes vary considerably with respect to their temperature optimums. Though most of the bacteria grown in our laboratory are mesophiles, placing cultures in a 37° C incubator will not necessarily increase their growth rate. Many organisms associated with air plates prefer temperatures below this.

- 3) **pH requirements** – The amount of acidic or alkaline material present in culture media can significantly influence microbial growth. Most media used in our laboratory are initially pH-adjusted to be near **neutral** (pH = 6.8 – 7.2), particularly if they contain pH indicators. Many bacteria can tolerate slightly acidic environments (shifts toward the low end of the pH scale) more readily than they can alkaline environments (shifts toward the high end of the pH scale). Some organisms, e.g., *Ferroplasma*, grow best in acidic environments and can be considered as **acidophiles**. Although bacteria can form both acidic and alkaline end products through their metabolic processes, acidic products tend to stay in the media and have a greater influence on pH (alkaline end products such as amines and ammonia are volatile and escape into the air). Organic acids (e.g., lactic acid and acetic acid) formed by fermentative microorganisms are used extensively in food preservation (cheese, yogurt, pickles, sauerkraut and silage), because high levels of acid inhibit the growth of organisms likely to cause spoilage.
- 4) **Osmotic pressure requirements** – The effective osmotic pressure or **tonicity** of an environment can influence growth by causing water to either enter or exit cells through osmosis. Hypotonic environments do not damage organisms equipped with **cell walls** (e.g., fungi, algae and bacteria), because walls prevent these cells from taking on excess water and blowing up. Hypertonic environments cause water to exit cells, and will often inhibit metabolism and growth. Since high salt environments occur naturally in various places, many microorganisms have adapted to these. Some salt-loving organisms or **halophiles** can only grow in hypertonic environments containing high levels of salt.
- 5) **Symbiosis** – In natural environments, the growth of microorganisms is significantly influenced by interactions with other organisms. Relationships are often subtle and many have yet to be investigated or are not thoroughly understood. A few examples have been described earlier, and are included here as reminders. **Symbiotic relationships** between bacteria in the genera *Rhizobium* and *Photobacterium* and plants or fish/squid, respectively, are mutually beneficial to all organisms concerned, i.e., are **mutualistic**. Bacteria, protozoa and fungi living inside the gastrointestinal tracts of animals (including humans) gain nutrients provided to them by their hosts, but can also provide animals with nutrients they could otherwise not obtain. Maintaining balance between the various populations present is essential to good health. **Parasites** such as flatworms, roundworms and various arthropods take nutrients from animal hosts, but generally give nothing in return. Fungi form **haustoria** or **mycorrhizae** when interacting with plant hosts, form **antibiotics** that inhibit bacterial growth and live symbiotically with algae or cyanobacteria within lichens. Ecologically speaking, all symbiotic relationships are probably mutualistic, because even parasites and pathogens benefit their hosts through population control, a concept foreign to many humans.

When microorganisms are grown in vitro, symbiotic relationships are limited, but some can still be observed. On agar plates exposed to air or soil, mixed populations of fungi and bacteria will sometimes interact in ways visible to the naked eye. Clear areas or zones of inhibition are often formed by colonies producing chemicals (antibiotics) inhibiting the growth of others. When the effects of antibiotics are synergistic, i.e., greater when combined, this is also visible. Fungi will often grow on nitrogen-free media if nitrogen-fixing bacteria are already present. Though fungi cannot fix nitrogen, they can and do use by-products formed by bacteria. On blood agar plates, hemolysis reactions can be enhanced by the combined action of different bacteria, as

demonstrated by the CAMP-reaction. Organisms with this ability can also cause more severe damage when interacting together inside a host.

### **Microbial Growth:**

When applied to microorganisms, the term **growth** refers to an increase in cell number rather than to an increase in the size of an individual organism. When bacteria are placed on the surface of a solid medium such as nutrient agar within a Petri plate, each living cell in contact with the nutrients available has the potential to reproduce itself and grow into a mass visible to the naked eye. The visible mass is called a **colony**, and though variable in morphology, will often appear as a circular form with an entire margin, convex elevation, smooth-shiny surface texture, opaque optical character and pigment varying from white to brightly-colored (yellow, pink, purple, brown, etc.).

Like many single-celled eukaryotic organisms, bacteria typically reproduce themselves by means of an asexual process called **binary fission** during which one cell divides into two. Unlike eukaryotes, bacteria do not undergo mitosis. Prokaryotic cells do not form microtubules, centrioles or a spindle apparatus, so the separation of chromosomes requires an alternative mechanism. Though fission typically proceeds as a more-or-less continuous process, it can be broken into a number of steps or phases as described below.

- 1) **Replication of DNA** (and other essential cellular components) – Before it can divide itself into two parts, a cell must replicate its genetic information (DNA). This insures that each new cell formed will contain the information necessary to function as a separate organism. The details of DNA replication will be presented later; but replication basically involves the separation of the two, nucleotide strands present in each DNA molecule, and the formation of new complimentary strands in association with each one. Additional cellular components such as ribosomes and enzymes are also made.
- 2) **Elongation** – Though bacteria do not grow extensively as individuals, each cell does undergo some degree of elongation before dividing into two parts. Since peptidoglycan is not elastic, i.e., does not stretch, elongation requires a partial decomposition of this rigid wall material and the deposition of new peptidoglycan. (The process is somewhat like the growth of long bones in association with epipheseal plates.) Decomposition typically occurs in several places along the long axes of rod-shaped cells (bacilli), or in one centrally located (equatorial) region of each spherical cell.

During elongation, the chromosomes are separated. Regions of each chromosome are attached to the cell membrane or cytoplasmic membrane (recall membranous folds called **mesosomes**), and as the cell elongates, the chromosomes are pulled or pushed apart.

- 3) **Septum formation** – Following elongation, the cell membrane folds inward across the long axis of the cell, until it forms a two-layered, membranous septum separating the cytoplasm into two parts. As in eukaryotic cells, this inward folding or pinching of the cell membrane involves microfilaments.

- 4) **Deposition of new wall within the septum** – Once the septum has been formed; new layers of peptidoglycan are deposited between the membrane layers or within the septum. This effectively separates the cell into two parts. Many of the diplobacilli observed in bacterial smears are cells at this stage of the fission process.
- 5) **Physical separation** – When sufficient wall material has been deposited, the two cells can break away from one another and exist as separate individuals; however, physical separation does not always occur. Cells with a characteristic arrangement, e.g., diplococci, streptococci, streptobacilli, etc. are examples of those maintaining connections following fission. If cells do not physically separate, their **arrangement is influenced by the orientation of the fission plane**. Chains of cells (streptococci or streptobacilli) will be formed if the fission plane always has the same orientation. Tetrads, sarcinae and staphylococci will form if the orientation of the fission plane changes between fission cycles.

### Population Dynamics in a Batch Culture:

Bacteria grown in a broth medium within a closed container such as a tube, flask or bottle (i.e., grown in vitro), form a **batch culture**. Under batch culture conditions, the population can receive no additional nutrients, and most metabolic wastes will stay within the medium.

A population of bacteria grown in a batch culture will typically undergo changes in density (number of cells per volume) that when plotted against time will form a predictable growth curve. The phases of this curve can change somewhat with respect to time and cell density, but the **overall pattern is similar for all populations**. The growth curve and the phases represented are described below. Note that ordinate values are represented on a logarithmic scale.

1. **Lag phase** – The lag phase is represented by a straight, horizontal line of variable length. During this phase of growth, the cells present are **increasing in DNA content, metabolic activity, size and dry weight**, but the population is experiencing **no increase in cell number**. The cells are "gearing up" for growth and completing some of the steps involved in fission, but physical separation has not yet occurred. The number of cells introduced and the batch culture volume will influence cell density (line height), and the time required to manufacture enzymes needed to complete various metabolic processes will influence the time involved (line and phase length).
2. **Exponential growth or Logarithmic growth phase** – During the exponential or logarithmic growth phase, the cells are dividing at a rapid rate and population numbers are represented by a diagonal line extending upward from the lag phase level. The **population is metabolically very active and its numbers are increasing exponentially** (1 cell becomes 2, 2 become 4, 4 become 8, 8 become 16, 16 become 32, 32 become 64, 64 become 128, 128 become 256, etc.).

The time required for one cell to divide into two cells is called the **generation time** or **doubling time** and for rapidly growing organisms such as *E. coli*, *Staphylococcus* or *Salmonella* is typically around 20 minutes. Since an *E. coli* cell growing under optimum

conditions generally requires about 60 minutes to replicate its chromosome, this short generation time seems impossible; but the bacteria accomplish this amazing feat by overlapping their DNA replication cycles in time, i.e., before the first replication cycle is complete, a 2<sup>nd</sup> and 3<sup>rd</sup> cycle have begun.

It has been estimated that if bacteria growing in a batch culture could be maintained in their exponential growth phase for 45 hours, they would form a mass the size of the earth. Needless to say, this is not possible.

3. **Stationary phase** - The stationary phase is represented by a horizontal line again indicating no overall increase in cell number. During this phase the **number of cells dying is equal to the number of new cells being formed** and the population has reached its maximum density, referred to as the **maximum concentration** or **m-concentration**. For most bacteria grown in a batch culture, the m-concentration is  $10^9$  (one or more billion) cells per ml of culture medium.

During the stationary phase, some of the cells present in the population are **dying due to a lack of nutrients and a buildup of toxic metabolic waste** products. Since conditions within cells and within the environment are not uniform, the death of some cells can provide nutrients for others not yet overcome by the toxins present, but only for a limited time. During this phase of growth, population density is influenced by an environmental feature called **carrying capacity**. Carrying capacity refers to the number of organisms an environment can support, and is influenced by **limiting factors** such as nutrient availability, pH, redox potential and temperature. The numerical value of the carrying capacity is identical to that of the m-concentration, because population density cannot exceed the environment's capacity to support it. **All populations are limited by the carrying capacity of their environment.**

4. **Exponential death phase** – During the death phase, represented by a diagonal line extending downward from the stationary level, the population is experiencing an exponential decrease in number. Many cells are dying due to a lack of nutrients and are being poisoned by their own metabolic waste products. The density of live cells decreases rapidly and typically drops below the number of organisms initially introduced into the medium. Conditions within the container are generally nasty, but a few hardy individuals will often manage to survive; especially if metabolism is slowed by a drop in temperature (e.g., if some conscientious individual places the culture into a refrigerator).

If the growth curve described above were drawn without using a logarithmic scale for the ordinate, it would form a J-shaped curve extending steeply upward until the carrying capacity was reached, and then dropping in an equally steep downward sweep. The growth curve representing earth's human population shows a similar J-shape. Although the carrying capacity of this planet is not uniform and cannot be accurately predicted, it is a feature of our environment, and cannot be indefinitely ignored. Environmental factors such as nutrient availability, toxic waste buildup and temperature will eventually limit our population just as they do those of microorganisms.

As a supplement to this week's lecture information, you may explore the population Web sites listed below. Students interested can obtain 5 points extra credit by visiting two or more of these sites and writing a brief report summarizing their content.

United Nations Population Fund - <http://www.unfpa.org/index.htm>

Population Connection - <http://www.populationconnection.org/>

The Population Institute - <http://www.populationinstitute.org/>

Though humans have considerable potential for intelligent achievement, our population growth curve strongly resembles that of *Escherichia coli* growing in a batch culture. Somehow it seems we should know better.

### **Growth in a Continuous Culture:**

Microorganisms such as bacteria and yeast can be maintained in **continuous cultures** if wastes (including dead cells) are removed and a steady supply of nutrients is maintained. Continuous cultivation is often used when microorganisms are being maintained for the synthesis of organic compounds such as enzymes, solvents, etc., or when biochemical processes are being investigated. Population density within a continuous culture is much more stable, and metabolic processes tend to be more consistent than are those occurring in batch cultures.

### **Growth on solid media:**

Bacteria grown on solid media typically form colonies of a characteristic size and shape. Although colonies are often surrounded by what appears to be available nutrient, colony growth is limited by the same factors limiting growth in a batch culture. Bacteria typically release enzymes that break down nutrients beyond the colony border, and open agar surfaces are often depleted of nutrients. Although the youngest, most viable organisms are often found at the colony margin, cell reproduction and expansion of the colony is essentially impossible.

Note – Motile organisms and filamentous forms can temporarily overcome nutrient limitations by **swarming** (swimming over the agar surface) or by extending their filaments into regions with available nutrients. Eventually however, the plate edge is reached, and all available nutrients are depleted.

Students seeking to maintain pure cultures of various microorganisms must transfer samples to new media every 2-3 weeks. Nutrient depletion and water loss will eventually cause most of the cells on an agar plate to die, even though the colonies may appear unchanged.