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Review Article

A Composite Review on: Microbial Culture and Growth Curve of Bacteria

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Abstract

Many researchers have studied the population dynamics of microbe of microbes as a typical example of population dynamics. The Monod equation, which mainly focuses on the growth and stationary phases, is used when plotting a growth curve. The microbes were divided into two populations: one grew by consuming the limiting substrate and the other degraded the products by metabolism. According to the numerical analysis of our model, microbes may choose one of two strategies: one consumes substrates and expands quickly, and the other grows slowly while cleaning up the environment in which they thrive. Determining the fitness of specific microbial genotypes has extensive application in microbial genetics, evolution, and biotechnology. While estimates from growth curves are simple and allow high throughput, they are inaccurate and do not account for interactions between costs and benefits accruing over different parts of a growth cycle. To plot a growth curve and determine a) Generation time and b) Specific growth rate of bacterial culture.

Keywords: Microbial Culture; Growth Curve; Nutrition Media; Incubation Condition

Introduction

Bacterial population growth studies require inoculation of viable cells into a sterile broth medium and incubation of the culture under optimum temperature, pH and gaseous conditions. Under these conditions, the cells will reproduce rapidly and the dynamics of the microbial growth can be charted by means of a population growth curve, which is constructed by plotting the increase in cell numbers versus time of incubation. The curve can be used to delineate stages of the growth cycles [1,2]. It also facilitates measurement of cell number and the rate of growth of a particular organism under standardized conditions as expressed by its generation time, the time required for a microbial population to double.

The stages of a typical growth curve are

- **Lag phase:** During this stage the cells are adjusting to their new environment. Cellular metabolism is accelerated, resulting in rapid biosynthesis of cellular macromolecules, primarily enzymes, in preparation for the next phase of cycle. Although the cells are increasing in size, there is no cell division and therefore no increase in numbers.
- **Logarithmic (log) phase:** Under optimum nutritional and physical conditions, the physiologically robust cells reproduce at a uniform and rapid rate by binary fission. Thus there is a rapid exponential increase in population, which doubles regularly until a maximum number of cells is reached. The time required for the population to double is the generation time. The length of the log phase varies, depending on the organism and the composition of the medium. The average may be estimated to last from 6 to 12 hours [3-6].
- **Stationary phase:** During this phase the number of cells undergoing division is equal to the number of cells that are dying. Therefore there is no further increase in cell number and the population is maintained at its maximum level for a period of

time. The primary factors responsible for this phase are the depletion of some essential metabolites and the accumulation of toxic acidic or alkaline end products in the medium.

- **Decline or death phase:** The decrease in population due to death closely parallels its increase during the log phase. Theoretically the entire population should die during a time interval equal to that of log phase. This does not occur, however, since a small number of highly resistant organisms persist for an indeterminate length of time.

Construction of a complete bacterial growth curve requires that aliquots of a 24-h shake flask culture be measured for population size at intervals during the incubation period. Spectrophotometric measurement of developing turbidity at regular intervals can be used as an index of increasing cellular mass. The generation time can be determined by simple extrapolation from the log phase. Instead of cell number, it is often more convenient to use dry cell weight per volume X as a measure of cell biomass concentration.

During the exponential phase in batch we can write:
dX/dt =X
Where is the specific growth rate of the cells.

Materials

- Culture: 12-18 h nutrient broth culture of E. coli DH5α
- Medium: Nutrient Broth Ingredients gl -1 Peptic digest of animal tissue 5.00 Beef extract 3.00
- Final pH (at 25°C) 6.9 ± 0.2
- The above constituents were dissolved in requisite amount of distilled water.
- The media was sterilized in an autoclave.

Equipment and accessories

Laminar hood, Orbital incubator shaker, 250 ml conical flasks, 15 ml test tubes, Glassware marker, 1.0 and 0.2 ml sterile disposable tips, Micropipettes.

Procedure [7,12]

- An over-night culture of E. coli DH5α is used to inoculate 100 ml of nutrient broth in a 250 ml conical flask at 1% level.
- The flask containing culture was incubated in an orbital shaker at 37°C, 180 rpm.
- Aliquots of the culture were taken aseptically at regular intervals and the turbidity was measured in a spectrophotometer at 600 nm using nutrient broth as blank.
- Optical density of the samples at 600 nm was recorded till 24 h of growth.
- The O.D600 values as a function of time were plotted in a semi-log paper to generate the growth curve.
- The generation time of the bacteria can be determined by extrapolation from the growth curve.
- Plot the growth curve and calculate the generation time from the curve.
- The biomass concentration in different samples is obtained by use of calibration curve obtained earlier.
- A graph is plotted between biomass concentrations vs. time.
- Linear part of the graph, which is exponential phase of growth, is taken for specific growth calculation.

Observations and Results

Incubation time (h) 0 1.0 2.0 3.0 4.0 5.0 6.0 8.0 12.0 18.0, Optical Density (600 nm)

Bacterial growth curve [13,18]

Bacteria 's growth can be take place by binary fission and during that so many phases¬ happen during that different events takes place. Five type of growth curves: 1) Growth cycle 2) Biphasic growth 3) Maintenance of cells in exponential phase 4) Synchronous growth 5) Bacterial growth *in vivo*.

Growth cycle or Growth Curve Bacterial growth is regulated by nutritional environment.

When suitable environment is there that time bacterium is incubated its growth leads to increase in number of cells which allow definite course.

The growth curve has got four phases:

- Lag phase
- Log phase (logarithmic) or exponential phase
- Stationary phase
- Decline phase

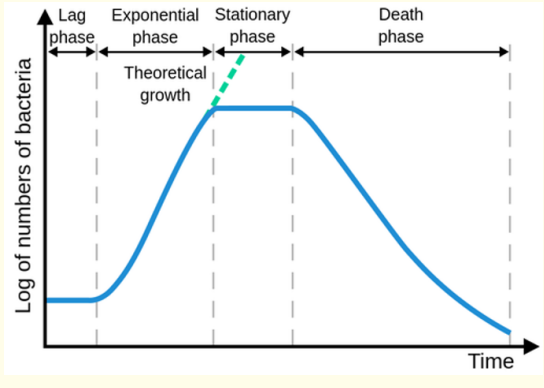

Figure 1: Bacterial Growth Curve.

Microbial nutrition and growth [19,23]

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:

- Chemoheterotrophs = Organisms requiring preformed organic compounds for both their energy and carbon requirements. These organisms may also be referred to as chemoorganotrophs.
- Chemoautotrophs = Organisms that use chemicals for energy, but are capable of using inorganic compounds (e.g., CO_2, HCO_3 -, etc.) for carbon. If these organisms use only inorganic chemicals to meet their nutritional needs, they may be called chemolithotrophs.
- Photoheterotrophs = Organisms that use light energy and require preformed organic compounds as their source of carbon.
- Photoautotrophs = Organisms that use light energy and are capable of using inorganic compounds (e.g., CO_2, HCO_3 -, etc.) for carbon. Animals (including humans), fungi, protozoa and many types of prokaryotic organisms are chemoheterotrophs.

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 [24,26]. 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.

- Carbon – Carbon is essential for the synthesis of all organic compounds, carbohydrates, proteins, lipids and nucleic acids.
- Nitrogen – Nitrogen is essential for the synthesis of proteins, nucleic acids and some carbohydrates, e.g., glucosamine.
- Minerals – Many of the elements incorporated into organic compounds are minerals such as sulfur, phosphorous, iron, calcium, magnesium, iodine, manganese and copper. 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.
- 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.
- Other – Some organisms will only grow when provided with specific growth factors such as vitamins, amino acids, cells, tissue fragments or other materials.

Factors influencing microbial growth [1,8]

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:

- Gas requirements
- Temperature
- pH requirements
- Osmotic pressure requirements
- Symbiosis

Conclusion

This is concluded as summary of this review that reveals, the Microbial growth curve relates the count of live cells inside a microbacterial population. The growth curve has four phases: lag, exponential phase (called log), stationary phase, and death phase. The lag phase occurs when bacteria are biologically active but just not dividing.

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