he following points highlight the four main phases of growth in bacteria. The phases are: 1. Lag Phase 2. Log or Exponential Growth Phase 3. Stationary Phase 4. Death or Decline Phase.

1. Lag Phase:

Lag phase represents a period of active growth during which bacteria prepare for reproduction, synthesizing DNA, various inducible enzymes, and other macromolecules needed for cell division. Therefore, during this phase, there may be increase in size (volume) but no increase in cell number. The lag phase may last for an hour or more, and near the end of this phase some cells may double or triple in size.

The lag phase is necessary before the initiation of cell division due to variety of reasons. If the cells are taken from an old culture or from a refrigerated culture, it might be possible that the cells may be old and depleted of ATP, essential cofactors and ribosomes.

If the medium is different from the one in which the microbial population was growing previously, new enzymes would be needed by the cells to use new nutrients in the medium.

However, these deficiencies are fulfilled by the cells during lag phase. It is, therefore, the lag phase is generally longer if the cells are taken from an old or refrigerated culture. In contrast, if the cells are taken from young, vigorously growing culture (microbial population) and inoculated to a fresh medium of the identical composition, the lag phase may be short or even absent.

2. Log or Exponential Growth Phase:

Bacterial cells prepared for cell division during lag phase now enter into the log phase or exponential growth phase during which the cells divide at a maximal rate and their generation time reaches a minimum and remains constant.

The growth in this phase is quite balanced (i.e. all cellular constituents are synthesized at constant rates relative to each other) hence, the most uniform in terms of chemical and physiological

properties, the log phase cultures are usually used in biochemical and physiological studies.

Since the generation time is constant, a logarithmic plot of growth during log phase produces an almost a straight line. This phase is called log phase because the logarithm of the bacterial mass increases linearly with time, and exponential growth phase because the number of cells increases as an exponential function of 2^n (i.e. 2^1 , 2^2 , 2^3, 2^4, 2^5 and so on).
The log phase also represents the time when bacterial cells are most active metabolically, and in industrial production, this is the period of peak activity and efficiency.

3. Stationary Phase:

Since the bacteria are growing in a constant volume of medium of batch culture, and no fresh nutrients are added, the growth of bacterial population eventually ceases and the growth curve becomes horizontal. Such a phase of growth in bacteria is attained at a population level of around 10^9 cells per ml.
The ceasation of growth may be because of the exhaustion of available nutrients or by the accumulation of inhibitory end products of metabolism. The ceasation of growth may also be due to O_2 availability particularly in case of aerobes. Oxygen is not very soluble and may be depleted so quickly that only cells on the surface of the culture may find necessary oxygen concentration for adequate growth.
Sooner or later, the bacterial cells start dying and the number of such cells balances the number of new born cells, and the bacterial population stabilizes. This state of growth, during which the total number of viable cells remains constant because of no further net-increase in cell number and the growth rate is exactly equal to the death rate, is called stationary phase.

The transition between the log and exponential and stationary phases involves a period of unbalanced growth during which the various cellular components are synthesized at unequal rates. Consequently, cells in the stationary phase have a different chemical composition from those in the exponential phase.

4. Death or Decline Phase:

After a while, the number of dying cells begins to exceed the number of new-born cells and thus the number of viable bacterial cells present in a batch culture starts declining.

This condition represents the death of decline phase which continues until the population is diminished to a tiny fraction of more resistant cells, or it may die out entirely. Like exponential growth, death is also exponential, but inverse, as the number of viable bacterial cells decreases exponentially.

he following points highlight the three main types of growth that take place in bacteria. The types are: 1. Diauxic Growth 2. Synchronous Growth 3. Continuous Growth.

Type # 1. Diauxic Growth (Diphasic Growth):

Diauxic growth is a diphasic growth represented by two growth curves intervened by a short lag phase produced by an organism utilizing two different substrates, one of which is glucose. When E. coli grows in a medium containing both glucose and lactose, it uses glucose preferentially until the glucose is exhausted.

Then after a short lag phase during which bacterium synthesizes the enzymes needed for lactose use, growth resumes with lactose as a carbon source. If this diphasic growth of E. coli is plotted in respect to bacterial density against time, two growth curves follow one after the other intervened by a short lag phase to produce a diauxic or diphasic growth curve (Fig. 19.3).

The enzyme needed for lactose use is β-galactosidase, which splits lactose into glucose and galactose, and the bacterium utilizes glucose for growth. Galactose can also be utilized, but only after it is converted to glucose. It has been demonstrated that E. coli growing in a medium containing both glucose and galactose produces a diauxic (diphasic) growth curve as in case of glucose and lactose.

Similar response has been found in case of other sugars such as arabinose, maltose, sorbitol, etc. when they are used in combination

with glucose by E. coli. Each of these sugars is utilized only after glucose has been used up in the growth medium.

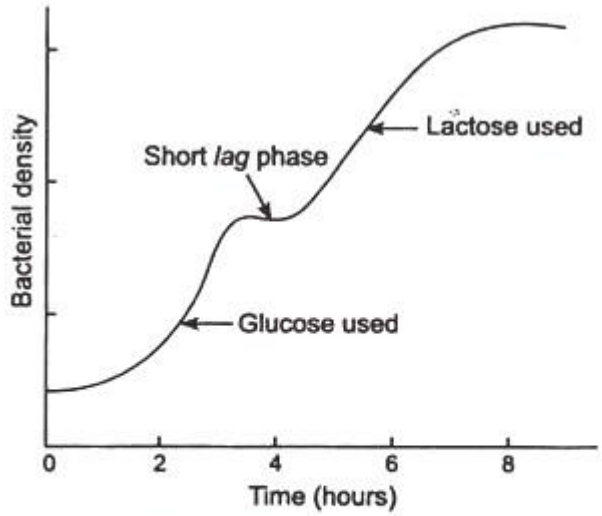

FIG. 19.3. Diauxic or diphasic growth curve of *E. coli* grown with a mixture of glucose and lactose. Glucose is first used, then lactose. A short *lag* phase in diauxic growth is present during which the bacterium synthesizes the enzymes needed for use of lactose.

The cause of diauxic (diphasic) growth is complex and not completely understood, it is considered that catabolite repression or the glucose effect probably plays a part in it. In catabolite repression of the lac-operon of E. coli, glucose exerts an inhibitory effect on the transcription of the lac genes.

As a result, lactose- utilization enzymes are not synthesized, even if lactose is present in the medium. When glucose is completely consumed by E. coli, the bacterium is now competent to transcribe the lac-operon genes resulting in production of necessary enzymes that help metabolise lactose.

Type # 2. Synchronous Growth:

Synchronous growth of a bacterial population is that during which all bacterial cells of the population are physiologically identical and in the same stage of cell division cycle at a given time. Synchronous growth helps studying particular stages or the cell division cycle and their interrelations.

In most of the bacterial cultures the stages of growth and cell division cycle are completely random and thus it becomes difficult to understand the properties during the course of division cycle using such cultures. To overcome this problem, the microbiologists have developed synchronous culture techniques to find synchronous growth of bacterial population.

Synchronous culture is that in which the growth is synchronous i.e. all the bacterial cells of the population are physiologically identical and in the same stage of cell division cycle at a given time.

A synchronous culture can be obtained either by manipulating environmental conditions such as by repeatedly changing the temperature or by adding fresh nutrients to cultures as soon as they enter the stationary phase, or by physical separation of cells by centrifugation or filtration.

An excellent and most widely used method to obtain synchronous cultures is the Helmstetter-Cummings Technique (Fig. 19.4) in which an unsynchronized bacterial culture is filtered through cellulose nitrate membrane filter.

The loosely bound bacterial cells are washed from the filter, leaving some cells tightly associated with the filter. The filter is now inverted and fresh medium is allowed to flow through it.

New bacterial cells, that are produced by cell division and are not lightly associated with the filter, are washed into the effluent. Hence, all cells in the effluent are newly formed and are, therefore at the same stage of growth and division cycle. The effluent thus represents a synchronous culture.

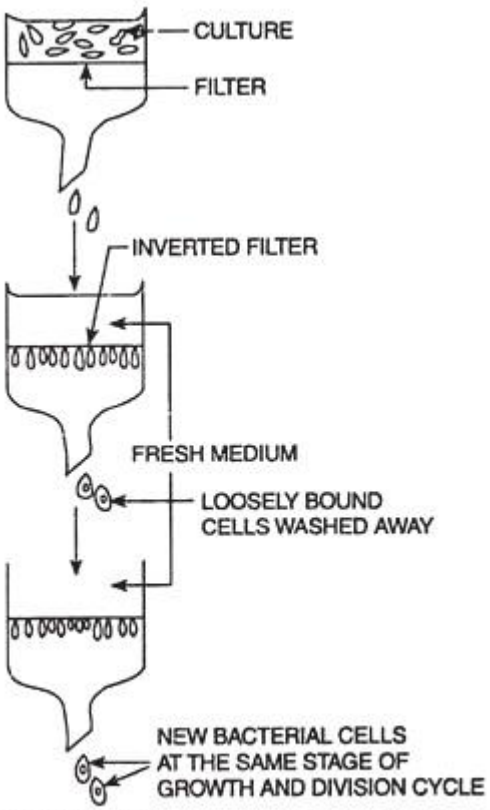

FIG. 19.4. Helmstetter-Cumming technique of obtaining synchronous cultures.

Type # 3. Continuous Growth: Chemo- Stat and Turbidostat:

Contrary to the studies in batch culture where the exponential growth of bacterial population is restricted only for a few generations, it is often desirable to maintain prolonged exponential growth of bacterial population for genetical and biochemical studies, and in industrial processes.

This condition is obtained by growing bacteria in a continuous culture, a culture in which nutrients arc supplied and end products continuously removed.

A continuous culture, therefore, is that in which the exponential growth phase of bacterial population can be maintained at a constant rate (steady state growth) for over a long period of time by continuously supplying fresh medium from a reservoir to growth chamber and continuously removing excess volume of culture medium of growth chamber through a siphon overflow.

By doing so the microbes never reach stationary phase because the end products do not accumulate to work as inhibitory to growth and nutrients arc not completely expended.

Continuous culture systems can be operated as chemostats or as turbidostats. In a chemostat (Fig. 19.5) the flow rate is set at a particular value with the help of a flow rate regulator and the rate of growth of the culture adjusts to this flow rate. That is, the sterile medium is fed into the vessel at the same rate as the media containing microorganisms is removed.

In a turbidostat (Fig. 19.6), the system includes an optical sensing device (photoelectric device) which continuously monitors the culture density in the growth vessel and controls the dilution rate to maintain the culture density at a constant rate. If the culture density becomes too high the dilution rate is increased, and if it becomes too low the dilution rate is decreased.

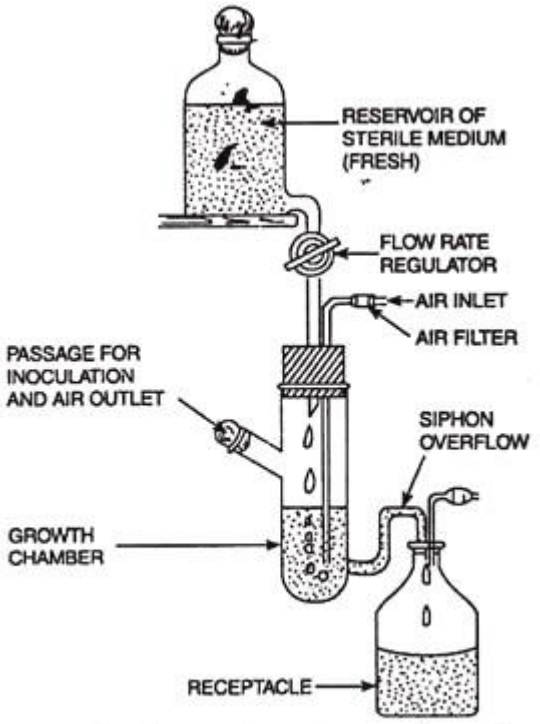

FIG. 19.5. The chemostat. a continuous culture system.

The turbidostat differs from the chemostat in many ways. The dilution rate in a turbidostat varies rather than remaining constant, and its culture medium lacks a limiting nutrient. The turbidostat

operates best at high dilution rates; the chemostat is most stable and effective at low dilution rates.

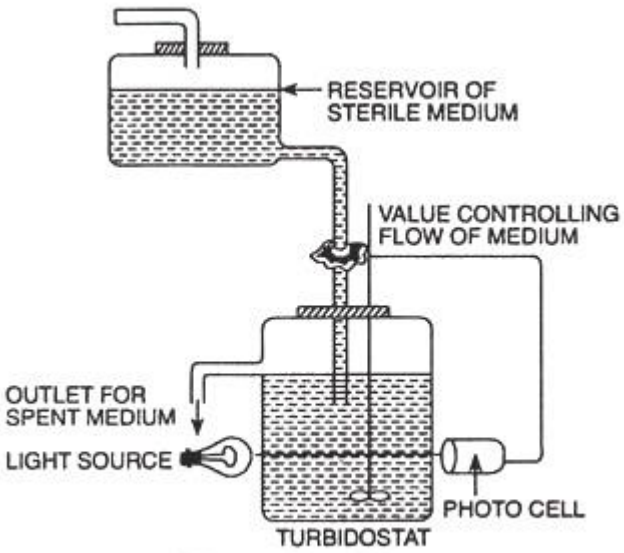

FIG. 19.6. The turbidostat.