Lecture 9: Microbial Growth Kinetics

Introduction-Studying growth of a microorganism is the basis of biotechnological exploitation of microflora for production of desired product. Optimization of growth of microorganism in a particular media is desirable due to economical and availability of particular growth constituent in a region. Despite this, some microorganisms have specific requirement and they grow in a particular growth media. Common media for growth of different microorganism, yeast and animal cells is discussed in future lecture. In today's lecture we will discuss bacterial cell division, methods of measuring growth, different phase in bacterial growth and growth kinectics.

Modes of Bacterial Cell Division-

1. Binary division-binary division is the most common mode of cell division in bacteria (Figure 9.1). In this mode of cell division, a single bacteria cell grows transversely with the synthesis of chromosomal DNA. A transverse septum appears in the middle of the cell body that divides the bacterial cell into the two with a distribution of chromosomal DNA, ribosome and other cellular machinery.
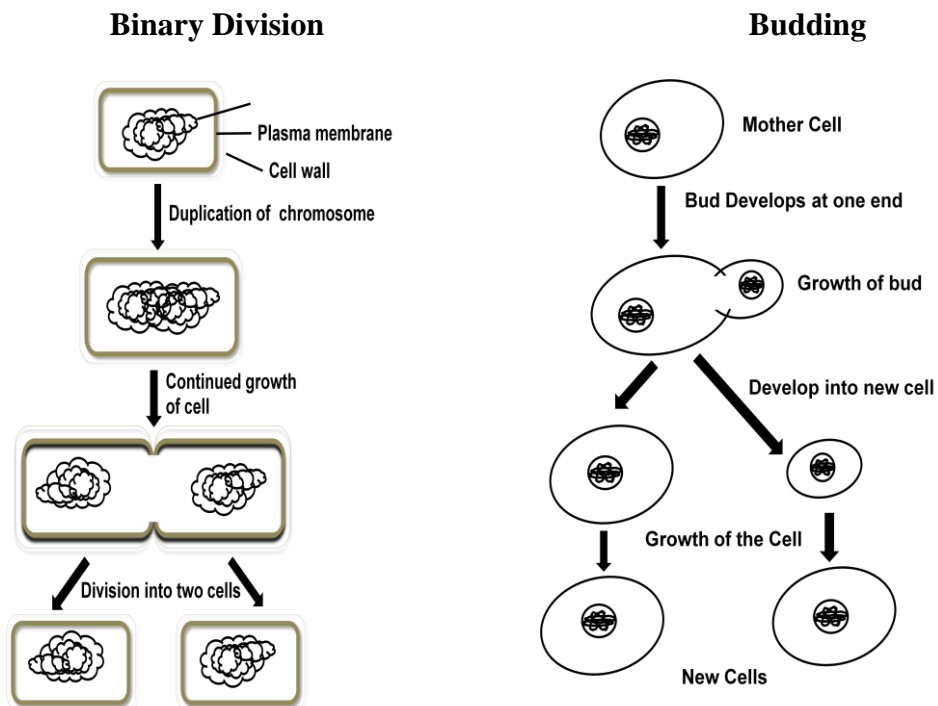

Figure 9.1: Different modes of cell division in bacteria.

2. Budding-In this mode of cell division, chromosomal DNA divides to form two copies. Sister chromosomal DNA moves to the one side of the cell and this portion of the cells protrude from main body to form bud. Eventually bud grows in size and get separated from main cell to develop a new cell.

3. Fragmentation-This mode of asexual division is more common in filamentous bacteria. In this mode, filament of the growing cell gets fragmented into small bacillary or coccoid cells, these cellular fragments eventually develop into new cell.

Measuring Bacterial growth- A number of methds have been developed to measure bacterial growth in liquid media and in solid support media. A few are discussed below:

Microscopic count-bacterial cells can be counted easily on a "petroff-hausser counting chamber" (Figure 9.2). The chamber has a ruling to make square ($1/400$ mm^2) of equivalent volume. A glass slide is placed ($\sim 1/50mm$ height) to make a chamber filled with bacterial cell suspension. Volume of each chamber is $1/20,000$ mm^3. This chamber can be used to observe bacteria with phase contrast microscope. For example, if each chamber has 8 bacteria then there are $8 \times 20,000,000$ or 1.6×10^8 bacteria/ml. A very high or low concentration of bacterial sample can not be counted accurately.

Plate count method-In this method, a defined amount of bacterial culture suspension is introduced onto solid support media to grow and give colonies. If number of colonies on solid media is too high, then serial dilution of original stock can be plated on solid media and number of colony can be counted with a colony counter. A manual colony counter has lamp at the bottom, a grid to divide the bacterial culture plate and a magnifying glass to visualize and count single colony. A plate with colony count of 30-300 can be used to determine the number of bacteria present in original stock.

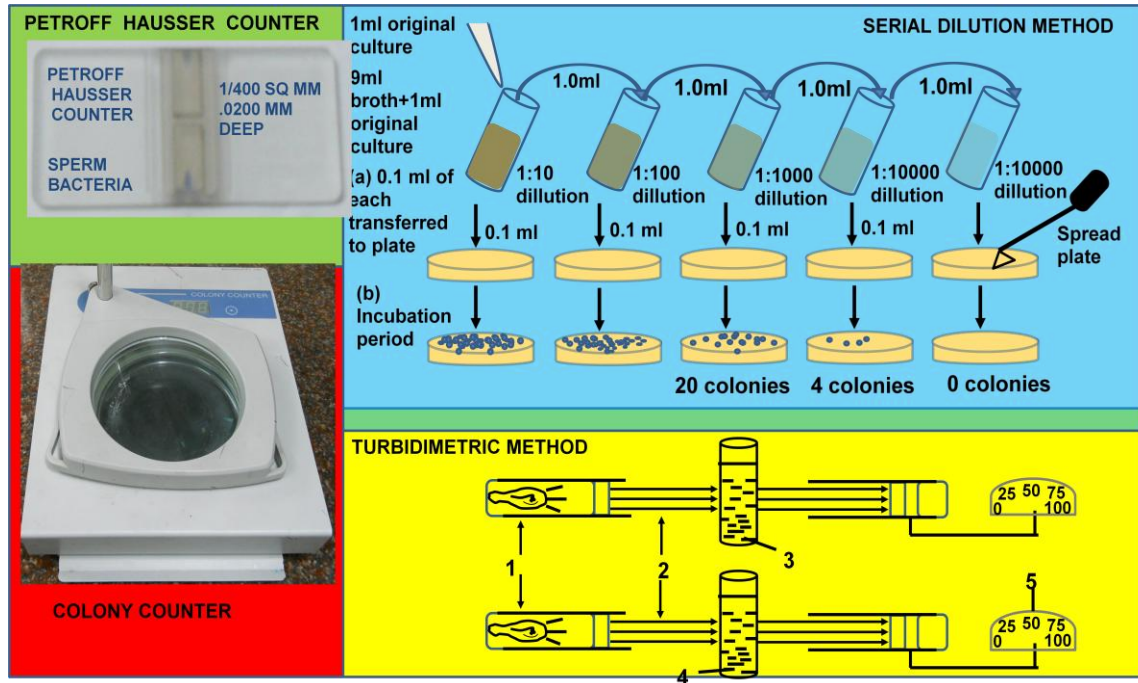Number of bacteria per ml=   Number of colonies counted on plate X dilution of sample

Figure 9.2: Different methods of bacterial counting.

Turbidimetric methods-This method is based on light scattering principles of particulate matter such as bacteria. A bacteria cell suspension is placed in test cuvette and corresponding media in reference cuvette. The optical density or absorbance of the bacterial suspension is used to measure the number of bacteria number. This method can not distinguish between live or dead bacteria as both form contribute to the turbidity.

Nitrogen content and Dry weight- A bacterial cell mass can be measured by direct measuremenof dry weight of culture or nitrogen content.

Growth cycle of bacteria- As discussed earlier, the most common method of bacteria division is binary fission and by this method, one bacteria cell gives two daughter cells. The time a bacteria takes to complete one division is called as generation time and it depends on bacteria species and media properties.

Hence, if we start from one bacteria, it divides after every generation time as follows-

Generation (n)	0	1	2	3	4	5	6	n
No. of bacteria	1	2	4	8	16	32	64	
No. of bacteria	1	2^1	2^2	2^3	2^4	2^5	2^6	2^n

Hence, After n generations, no of bacteria will be

N=1 x 2^n…………………………………………. Eq 9.1

But assume if number of bacteria at time 0 is N_o, then

N=No X 2^n…………………………………………..Eq 9.2

Log N=Log N_o+n log_{10} 2…………………………...Eq 9.3

n= 3.3 (Log_{10} N-Log_{10} N_o)…………………………….Eq 9.4

Eq 9.2 can be used to determine number of bacteria, if initial number of bacteria and number of generation is known where as Eq 9.4 can directly been used to calculate number of generations.

Bacterial growth in a liquid media is given in Figure 9.3 and it has 4 distinct phases:

1. Lag Phase-The single cell inoculation into the liquid media doesn't start dividing as per its generation time. During this phase bacteria gets adjusted to the new media and grow in size instead of dividing into daughter cells. In this phase, bacteria synthesize the most crucial enzymes or co-enzyme present in traces and required for optimal growth and multiplication. In addition, cell is metabolically active and be busy in synthesizing large amount of protoplasm. At the end of this phase, each bacterial cell divides and enter into the next phase of active multiplication.
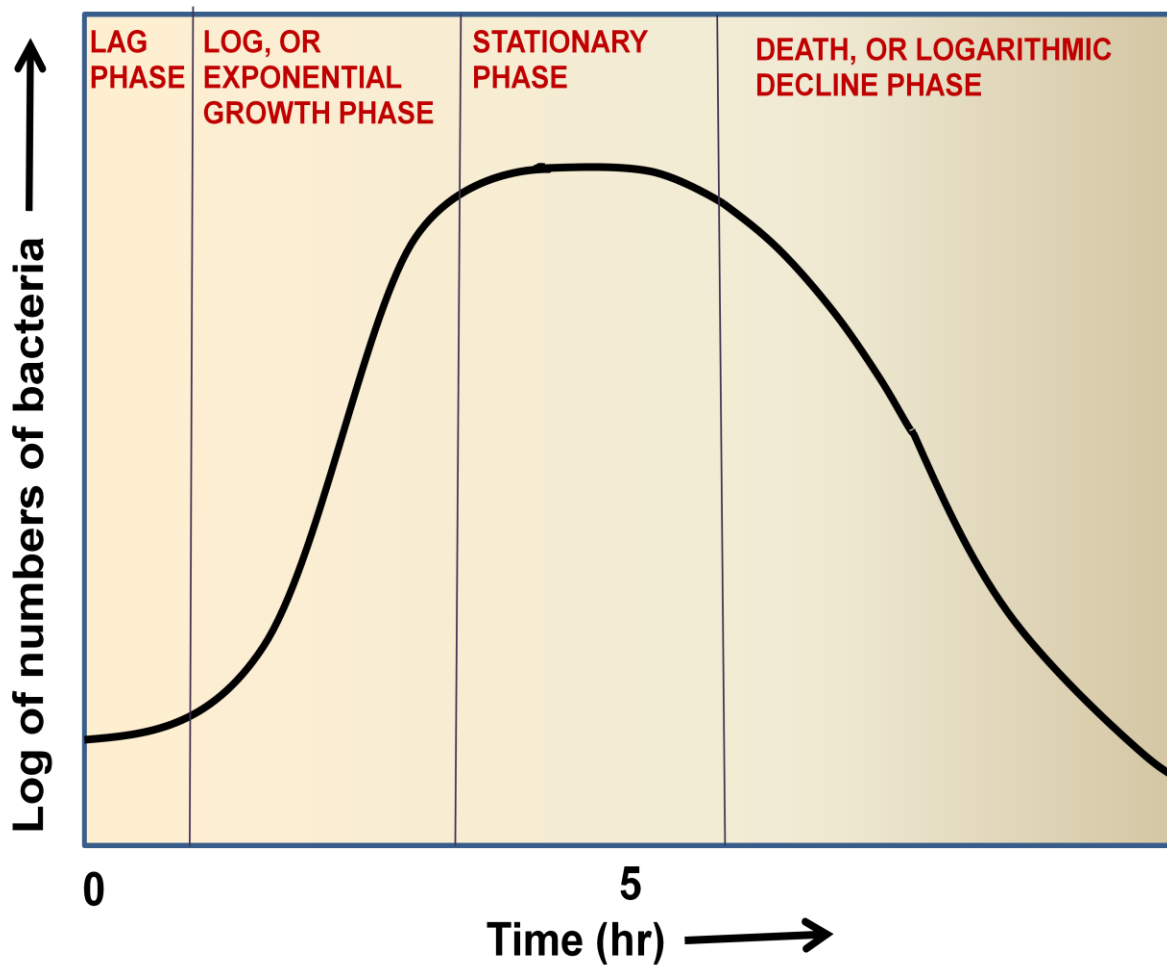

Figure 9.3: kinetics of Growth of bacteria.

2. Log Phase-In this phase, bacterial cell population is involved in active division and whole cell population is more or less homogenous in terms of chemical composition, physiology and metabolic activity. A plot of number of cell (in log scale) against time gives straight line. The growth of bacterial cell population is increasing at a constant rate and continues until substrate concentration is not limiting.

3. Stationary Phase-Once substrate is limiting, the logarithmic phase of growth begins to decline gradually with a constant number of cells to give a staright line. The population remains constant because number of divisions are equal to the number of death events. As substrate is limiting, death of old cell provides enough nutrient for remaining cells to grow and multiply to maintain the constant number.

4. Death Phase-When substrate is not sufficient from dying cells, death rate of bacteria superseed rate of growth and as a result number of bacteria declines sharply.

Quiz

Q1: In a culture of bacteria, a sample is taken at 10:00AM and contains 1000 cells per ml. A second sample at 8:00PM has 10,000 cells per ml. what is the generation time ?

Q2: A scientist wants 20,000/ml *E.Coli* cells for his molecular biology experiment, he has inoculated 1000 cells at around 8:00PM. The generation time of *E.coli* is 20 mins. Please tell when he should harvest the culture?

Q3: What is the significance of lag phase in bacterial growth curve?

Q4: Which mode of cell division is most common in bacteria?

Q5: The unit of measurement in the turbidimetric method of measuring bacteria growth ?