Environmental Biotechnology
Prof. Pinaki Sar
Department of Biotechnology
Indian Institute of Technology, Kharagpur

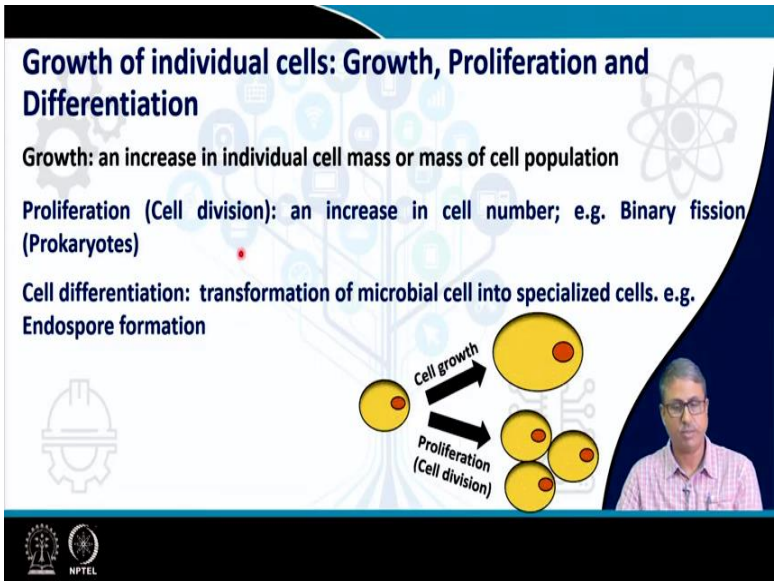
Lecture – 20
Microbiology of Environmental Engineering System (Contd.,)

(Refer Slide Time: 00:33)

Welcome to the next lecture on microbiology of environmental engineering system and in this lecture we are going to cover the following concepts which basically will focus on the growth of individual cells and population and this will include the growth proliferation and differentiation examples of transformation of microbial cells into specialized cells, cell cycles, coordination between the cell division cycle DNA replication cycle.

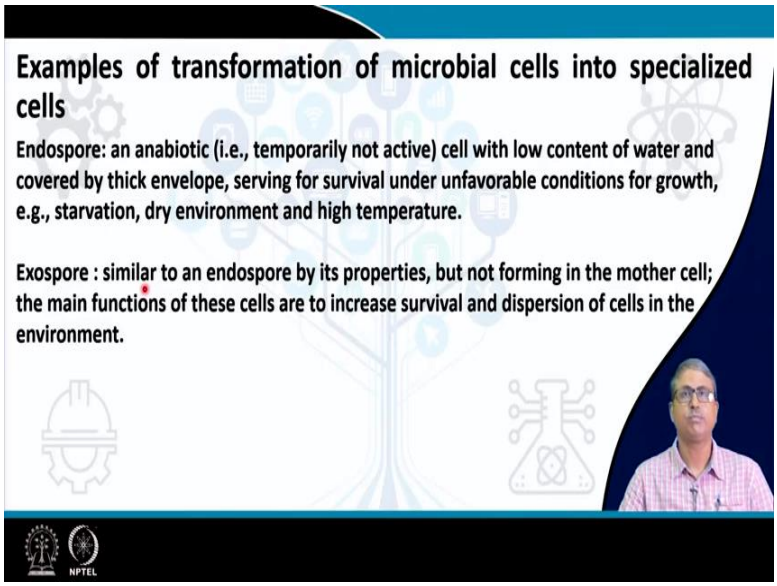
Growth of population will include exponential microbial growth, growth yield and growth efficiency, microbial growth curve in closed system batch culture and continuous culture will also be discussed and finally we will talk about the effect of environmental factors on microbial growth and also will mention briefly about microbial death.

(Refer Slide Time: 01:25)



So, starting with the growth of individual cells, growth is basically an increase in individual cell mass or mass of cell population whereas proliferation accounts for increase in cell numbers for example the binary fission for prokaryotes which is facilitated by the cell division process. And in cell differentiation transformation of microbial cell into specialized cells for example the endospore formation are included. So, this diagram represents the process of cell growth as well as cell division.

(Refer Slide Time: 02:12)

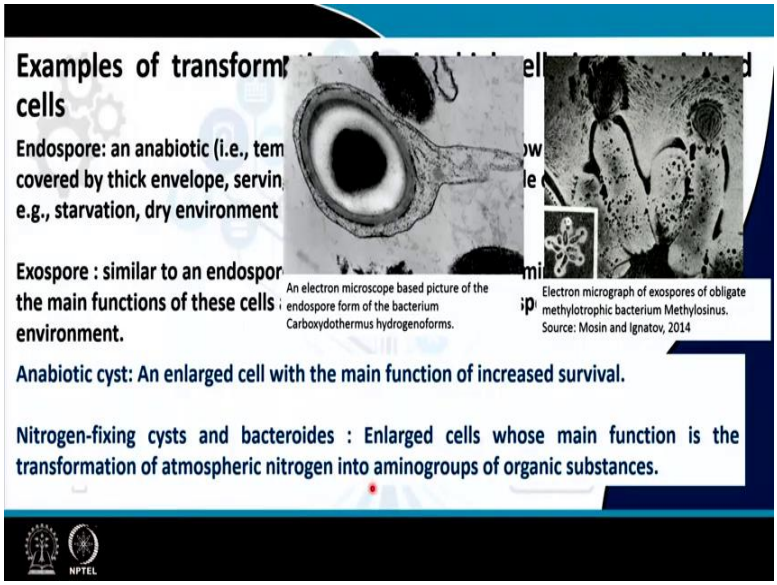


Now here we talk about the examples of transformation of microbial cells into specialized cells. And we may find that endospore is one of the most predominant such specialized cell structure or types of cells which are kind of anabiotic which refers to temporarily non active form of the

cell with low content of water and covered by a thick envelope. Serving for survival under unfavorable conditions for growth for example starvation dry environment like desiccation, high temperature and a combination of all these and other extreme and adverse conditions.

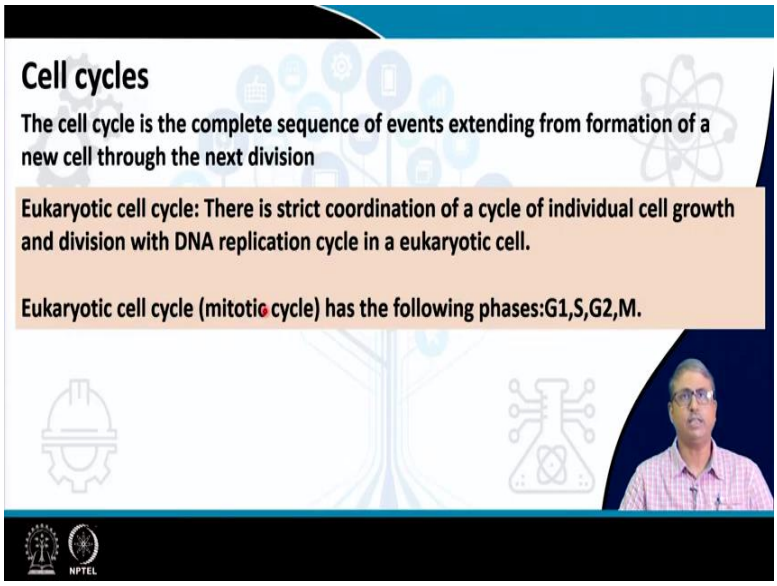
There are also the formation of the exospores by some microorganisms. The endospore is similar to an endospore by its properties but not forming in the mother cell. So, it is not formed inside the mother cell rather it is formed outside the mother cell. The main function of these cells are to increase the survival and dispersion of cells in the environment.

(Refer Slide Time: 03:38)



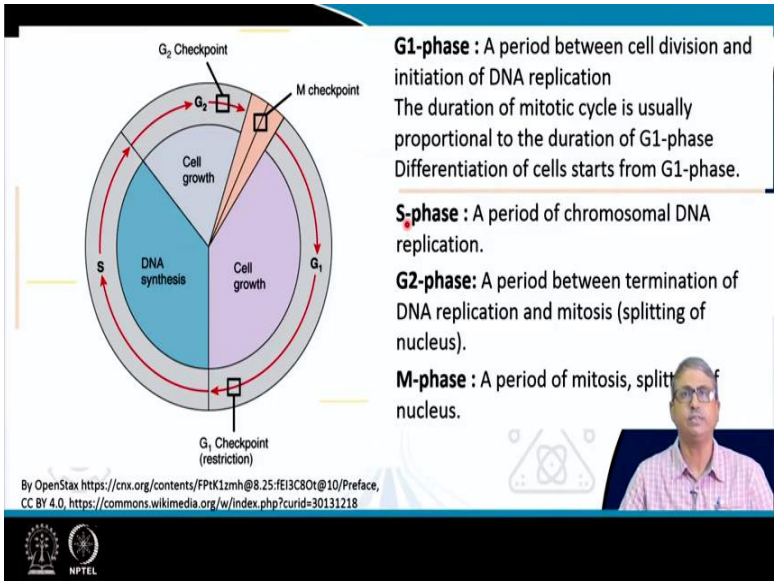
The following are the two electron microscopic pictures of the endospore formed by a temperature tolerant organism carboxy dotharmas and also the exdospore formation by methylosinus species. Apart from these two types of specialized cells we may have anabiotic cyst which is formed as an enlarged cell with the main function of increased survival and also nitrogen fixing cysts and bacteriodes which are enlarged cells whose main function is the transformation of atmospheric nitrogen into amino groups of organic substrates.

(Refer Slide Time: 04:29)



Now moving to cell cycles the cell cycle is the complete sequence of events extending from the formation of new cell through the next division. And in case of eukaryotic cell cycle there is a strict coordination of a cycle of individual cell growth and division of the DNA replication cycle which is very frequent in the eukaryotic cells. Eukaryotic cell cycle that is the mitotic cycle has the following phases G1, S, G2 and M.

(Refer Slide Time: 04:59)



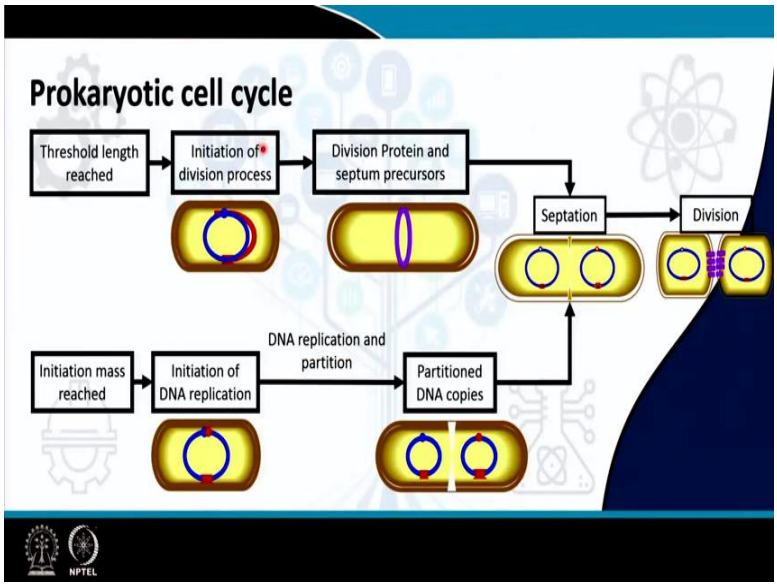
And as we can identify in this diagram the G1 phase is basically a period between the cell division and initiation of the DNA replication. The duration of the mitotic cycle is usually proportional to the duration of the G1 phase and differentiation of cell starts from the G1 phase next come the S phase which is basically the synthesis phase and in this phase where the

chromosomal DNA replication or the DNA replication occurs.

Then the cell cycle enters into its another gap phase or G phase that is called G2 phase and it is a period between the termination of the DNA replication and the mitosis. So, before the cell enters into the cell division or the mitosis this G2 phase is a kind of a final stage or final gap stage where we can have a check point before the cell finally decides and proceeds towards the mitotic event.

And finally we have the mitotic phase or the M phase which is a period of mitosis that means the splitting of nucleus into two daughter nucleus or nuclei occurs in this phase.

(Refer Slide Time: 06:23)



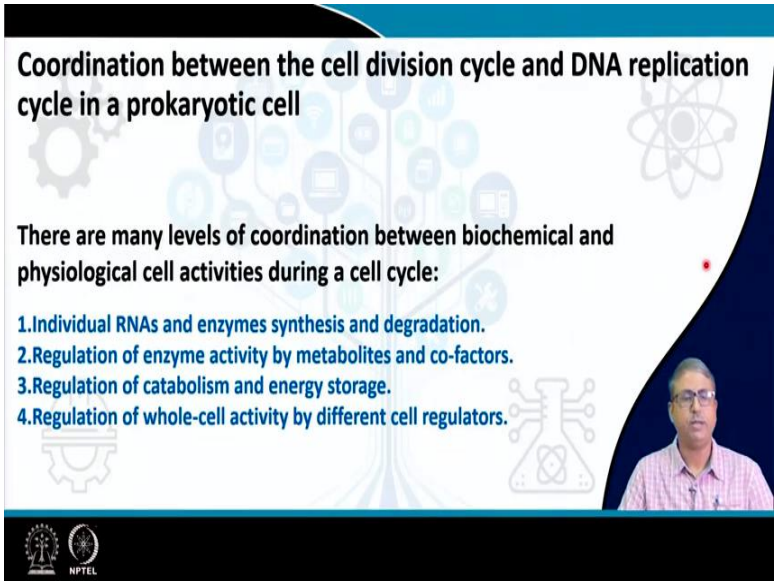
Now moving into the prokaryotic cell cycle, prokaryotic cell cycles are somewhat different from the eukaryotic cell cycles. And as you can see there are two distinct lines of events which proceed parallelly almost parallely or maybe simultaneously as the cells proceed towards division in one of the steps or the line of processes. We see that the initiation mass once the mass is reached that is cell reach to a kind of a threshold mass.

The initiation of DNA replication occurs and the DNA replication proceeds and the partition of the two DNA molecule the dotted DNA molecules took place inside the mother cell. So, that is called the partitioned DNA copy. So, ideally the cell still remains as a single cell but the DNA

duplicates into two copies. So, the DNA replication is completed. On the other hand a similar line of process or similar con sequence of process occurs where the initiation of division process took place which is facilitated by the division proteins and septum precursors.

And finally when both these septum precursor for and the septum formation facilitated by the division protein multiple division proteins and the partition DNA copies they converge the separation of the two daughter cells begins and eventually the division takes place.

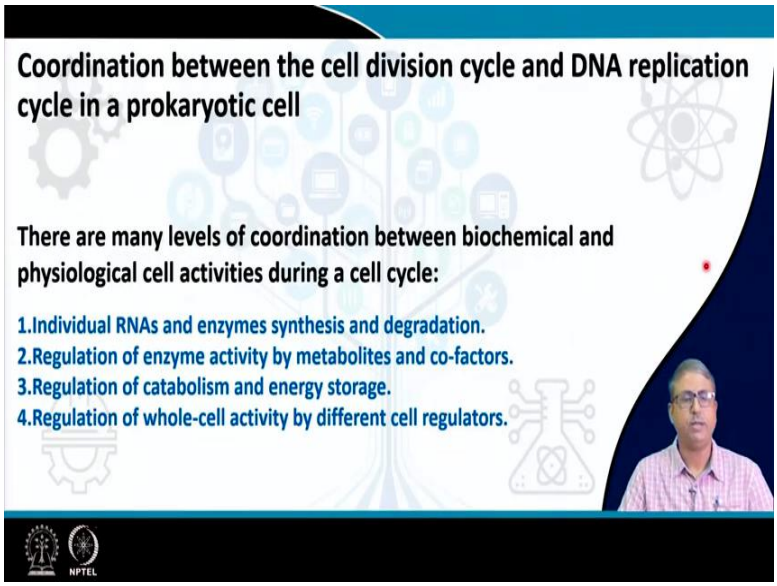
(Refer Slide Time: 08:03)



Now coordination between the cell division cycle and the DNA replication cycle in a prokaryotic cell is very important in order to understand their importance and their role or function within any environmental engineering or environmental biotechnology processes or systems. So, this coordination between the cell division cycle and the DNA replication cycle could be achieved at many levels. So, there are many levels of coordination between the biochemical and physiological cell activities during the cell cycle.

One of them is the individual RNA's and enzyme synthesis and their degradation regulation of enzyme activity by metabolites and cofactors, regulation of catabolism and energy storage and regulation of whole cell activity by different cell regulators. So, in case of bacteria and archaea the coordination between the cell division cycle and DNA replication cycle are relatively complex and it proceeds through a number of regulatory mechanisms which are mentioned.

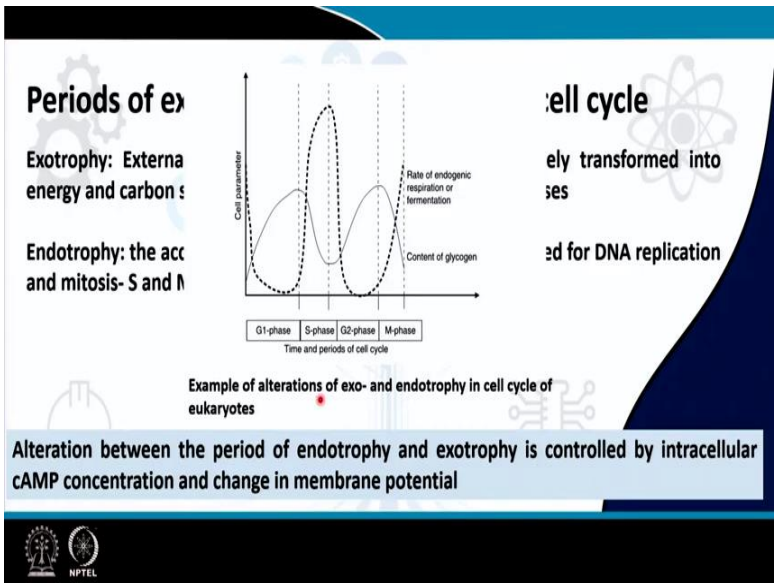
(Refer Slide Time: 09:13)



Now, periods of exotrophy and endotrophy inside cycle. Exotrophy is the process or a kind of an event where an external source of carbon and energy is extensively transformed into energy and carbon storage molecule like the glycogen starch lipids inside the cell. And it is mainly it is it is mainly occurring during the G1 and G2 phases when the cells are in the kind of gap phases or the within the cell cycle gap phases.

Whereas endotrophy is the acumen is the utilization of the accumulated stored energy carbon sources for the DNA replication and the mitosis that is the S and M.

(Refer Slide Time: 09:59)



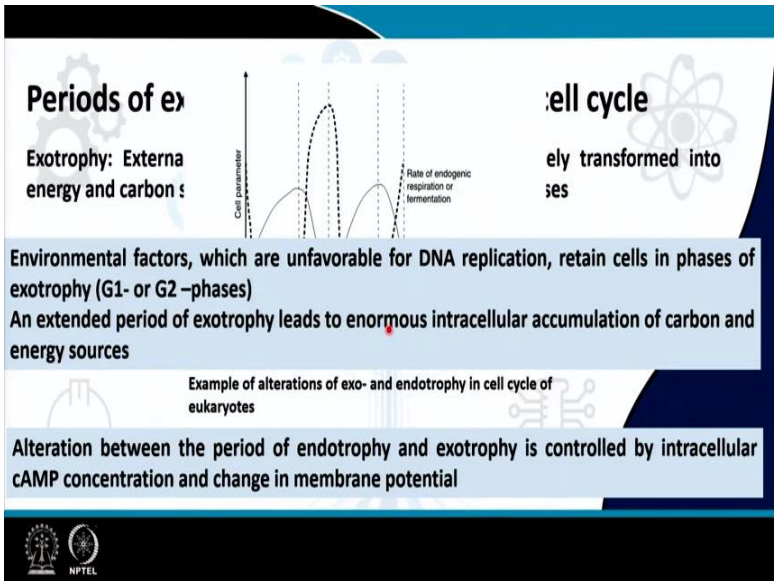
So, if we look at the four distinct phases of the cell cycle in eukaryote for example the G 1, S, G 2 and M. We may find out that there is a very distinct correlation with the switch between the exotrophy and endotrophy. As you can see that from this figure that if we monitor the content of the glycogen as an indicator of the cell storage. So, during the G1 phase as well as in the G2 phase we can find out the content of the glycogen as a storage molecule increases inside the cell.

What this means this basically refers to the fact that the cell is acquiring carbon and energy resources from the external environment that is the it is running through exotropic mode of nutrition and due to this excess supply of the carbon and energy the cells are able to convert the extra carbonyl energy into storage molecule. However during the synthesis phase like the s phase where the DNA replication took place and also during the mitosis phase we can we can we can find that the glycogen level declines very sharply this indicates that during this DNA replication phase or the DNA mitotic phase or the DNA division replication phase.

The cells are relying more on the endogenous storage material for example the glycogen level and they are not relying on external carbon sources or energy sources. Now the alteration between the period of endotrophy and exotrophy is controlled by the intracellular cyclic amp concentration and change in membrane potential. Because it is again a matter of tight regulation by the cells that cell tries to sense the concentration of the substrates and the nutrients like carbonyl energy sources available in the exterior environment.

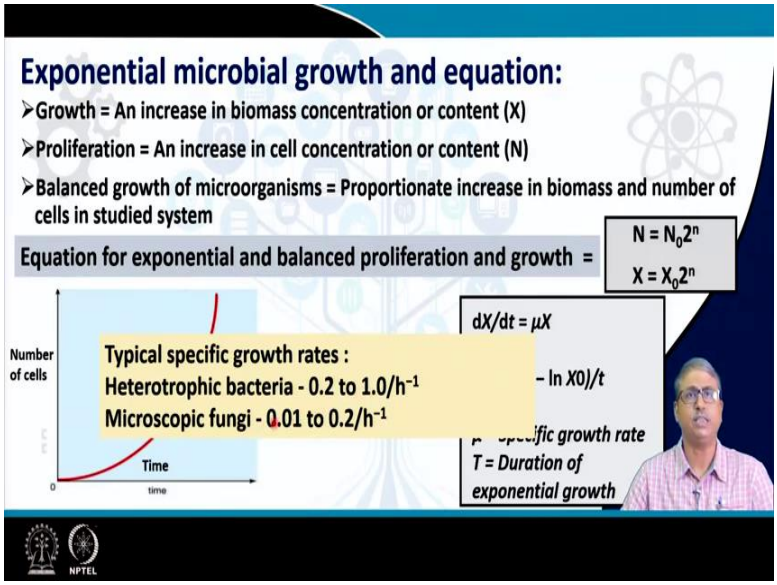
And their growth requirement and growth behaviour and which is coordinated through this kind of signaling molecules.

(Refer Slide Time: 12:17)



Environmental factors which are unfavorable for DNA replication retains the cell in phases of exotrophy. So, they remain as G1 or G2 phases and an extended period of exotrophy leads to enormous intra cellular accumulation of carbon and energy sources. So, these are again having significant contribution or importance with respect to environmental biotechnology and other environmental engineering processes and applications.

(Refer Slide Time: 12:46)



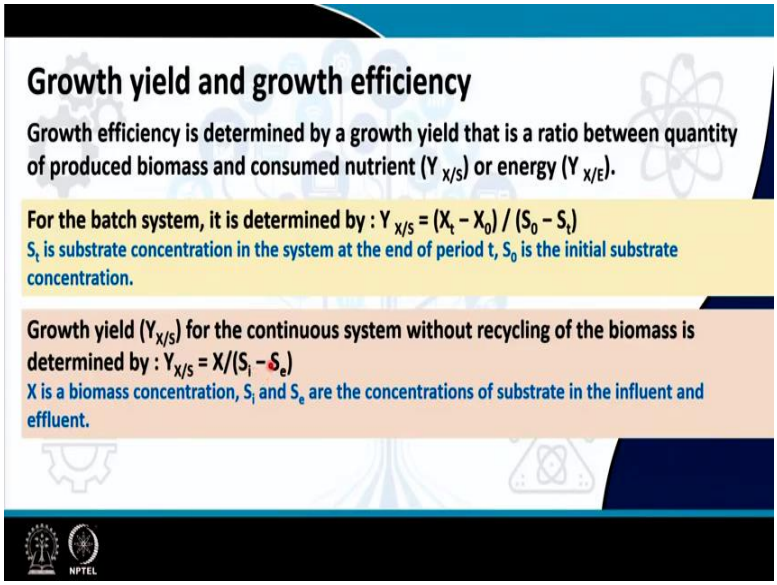
Next is the growth of the populations exponential microbial growth and its equation will be followed. So, as we mentioned earlier growth is an increase in biomass concentration or content which is which is represented as capital X. Proliferation is an increase in cell concentration or the content which is represented as capital N. During the balanced growth of microorganisms that

means where the there is a proportionate increase in biomass and number of cells within a studied system.

We can actually obtain the following equation for the exponential and balance proliferation and the growth which is capital N equal to N naught 2 to the power N or X equal to X naught to the power N and if we follow the exponential microbial growth pattern where time versus number of cells could be plotted easily and we can derive this very well known equation dx by dt equal to mu math mu x or the mu equal to ln x - ln x naught divided by t, mu refers to the specific growth rate T is the duration of the exponential growth.

Typical specific growth rates can be determined for many microorganisms or any microorganisms which are subjected to a controlled growth experiment for heterotrophic bacteria it ranges between 0.2 to 1.0 per hour whereas for microscopic fungi it is range between 0.01 to 0.2 per hour.

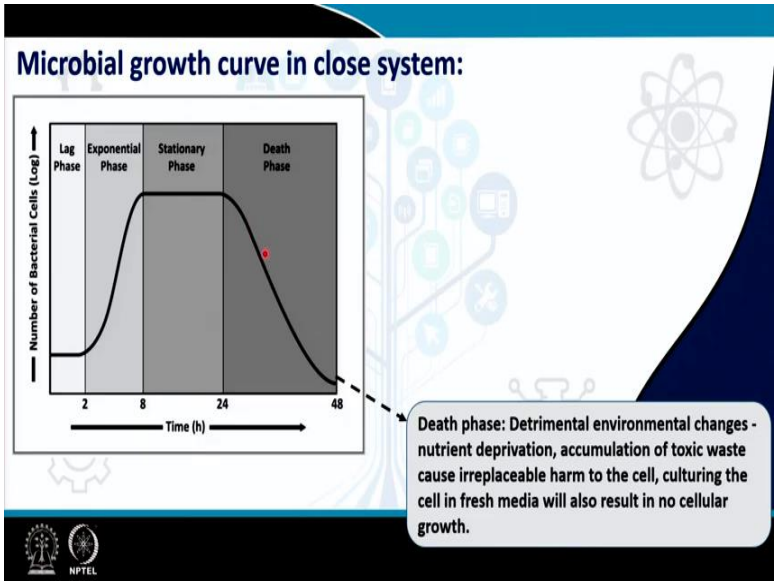
(Refer Slide Time: 14:24)



Growth yield and growth efficiency: Growth efficiency is determined by a growth yield that is a ratio between the quantity of produced biomass and the consumed nutrient or the energy. So, it could be represented by Y x by s or energy Y x by E. For the batch system it is determined by y x by s equal to X t - X naught divided by S naught - S t whereas S t is the substrate concentration in the system at the end of the period time t and s 0 is the initial substrate concentration.

Similarly the growth yield or y x s for the continuous system without recycling of the biomass can be determined by the following equation of Y x by s equal to x divided by S i - S e where in x is a biomass concentration S i and S e are the concentrations of the substrate in the influent and the effluent.

(Refer Slide Time: 15:37)



Similarly if we want to plot the microbial growth curve in a closed system we will be able to find out following very distinct phases lag phase, exponential phase or the log phase stationary phase and death phase. Here the number of bacterial cells it are plotted with respect to time. Now as you can see that there is a kind of a distinct lag phase where in the microorganisms introduced into a fresh culture medium and no immediate increase in cell number is generally observed.

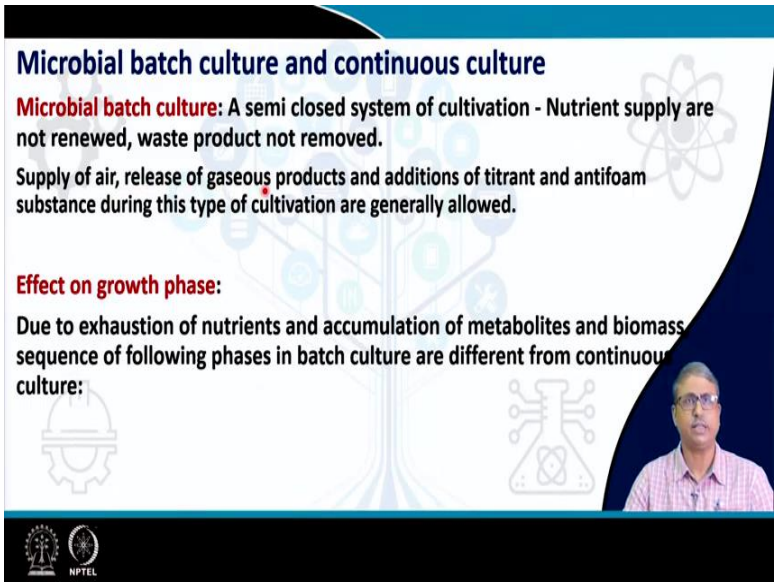
Cell synthesizes ATP, ribosome and different cofactors and gradually proceeds towards the exponential of the log phase. So, in the log phase microorganisms grow and divide at a maximal rate possible by utilizing the substrates carbon and energy and other nutrients available from the nu from the medium or the environment. The rate of growth is constant as microbial cells are dividing and doubling in number at regular interval.

This is followed soon by the stationary phase which is occurring because of the different conditions which started prevailing in the system like nutrient limitations, oxygen availability

accumulation of toxic waste as microbial population reach high density cell density cells to enter stationary phase in a closed system. So, generally it is a closed system. So, the oxygen and other essential nutrients or compounds will be reduced whereas the toxic substances metabolites will start accumulating in the system which will interfere adversely with respect to the cells metabolism.

And finally the cells are going to enter into the death phase where the detrimental environmental changes with in terms of the nutrient deprivation accumulation of toxic waste at higher concentration will cause irreplaceable harm to the cell. Culturing the cells in fresh medium from the dead phase may not yield further cellular growth because cells are damaged severely and they enter into a kind of a death phase.

(Refer Slide Time: 18:03)



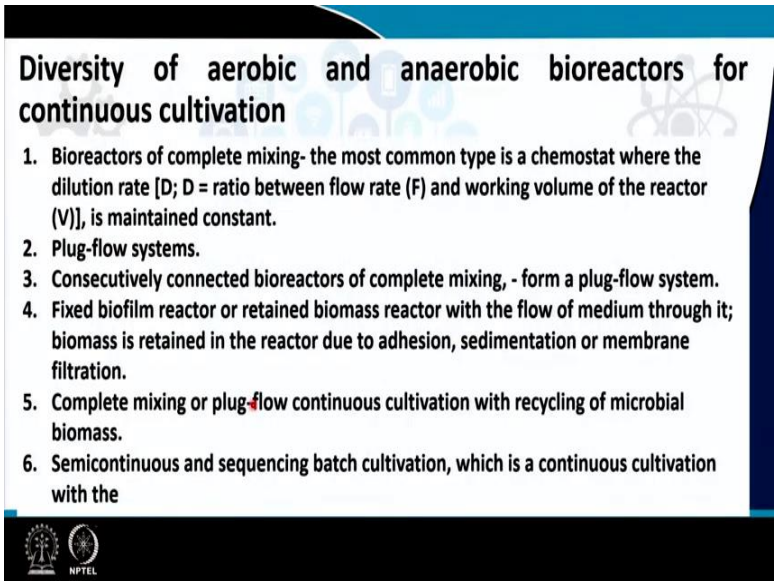
Now microbial batch culture and continuous culture microbial batch culture is a kind of a semi closed system of cultivation whereas the nutrient supply are not renewed waste products are not renewed. However the supply of air release of gaseous products like carbon dioxide methane or hydrogen and additions of titrant and antiform substances during this type of cultivation are generally allowed. So, that is why it is called semi closed system.

Now effect on the growth phase due to the exhaustion of the nutrients and the accumulation of metabolites and the biomass of course the sequence of following phases in the batch culture are

different from the continuous culture. In case of continuous culture generally an open system is allowed with constant environmental condition inside the reaction vessel or the reactor vessel which is maintained with continuous provision of nutrients and waste removal.

So, there will be always a feed and an effluent moving in and moving out of the reaction vessel where the cells are allowed to grow. Now with respect to effect of such culturing that is the continuous culturing on growth the microbial population can be maintained in exponential growth phase for a extended period of time and a constant biomass concentration for an extended period of time can also be obtained.

(Refer Slide Time: 19:32)

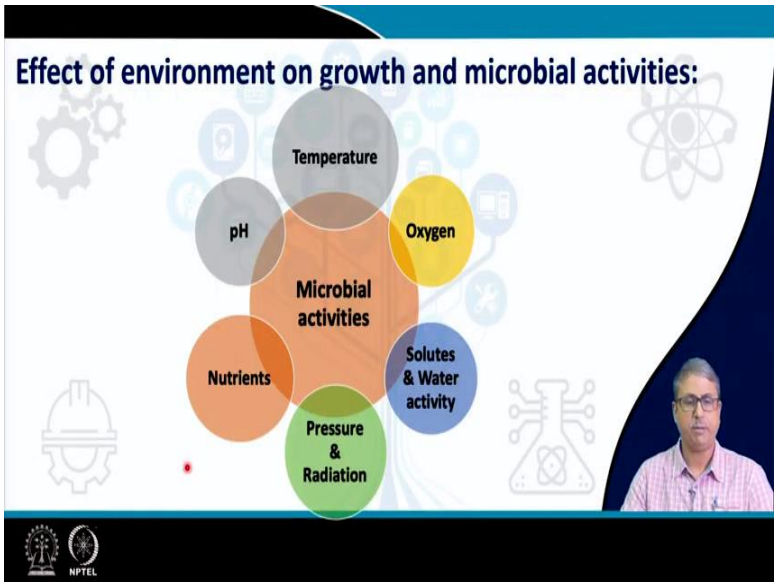


Now diversity of aerobic and anaerobic bioreactors for continuous cultivation is briefly mentioned. There could be a number of reactor types which are useful for aerobic and anaerobic cultivation of the cells for different environmental and other purposes which includes the bioreactor of complete mixing the most common type is a chemostat type plug flow system. Consecutively connected bioreactors of complete mixing form a plug flow system again fixed biofilm reactor or retained by biomass reactor with the flow of medium through it.

Biomass is retained in the reactor due to the adhesion sedimentation or membrane filtration complete mixing or plug flow continuous cultivation with recycling of microbial biomass and the semi continuous and sequencing batch cultivation which is a continuous cultivation with

different modifications.

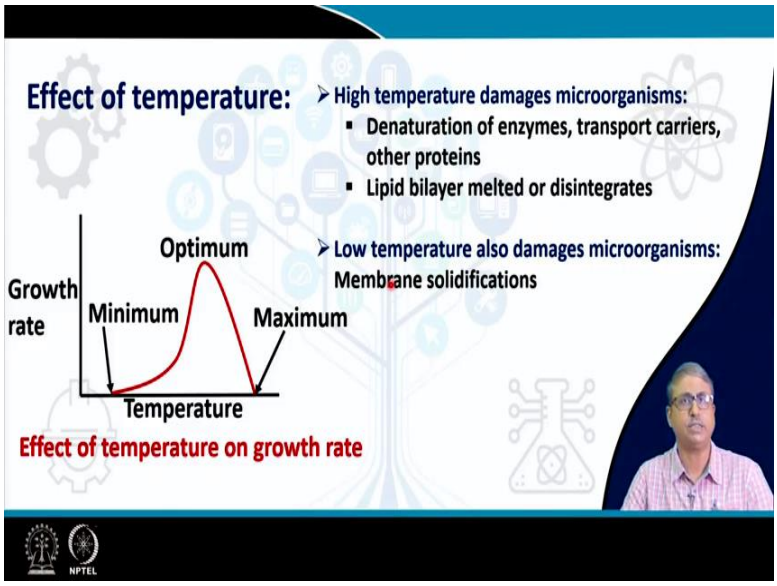
(Refer Slide Time: 20:29)



Now with respect to the effect of different environmental parameters on growth and microbial activities we will discuss briefly about the different parameters or different factors which are predominated in the environmental conditions or environmental systems which might affect the microbial growth and activities. So, as you can see there are a number of factors some of them are included in this diagram like temperature, pH, nutrients, pressure and radiation, solutes and water activity and oxygen.

These could be the major factors present in the environment which affects the microbial activities but there could be many other factors like the concentration of different ions and etcetera and the interaction between this physico chemical parameters itself.

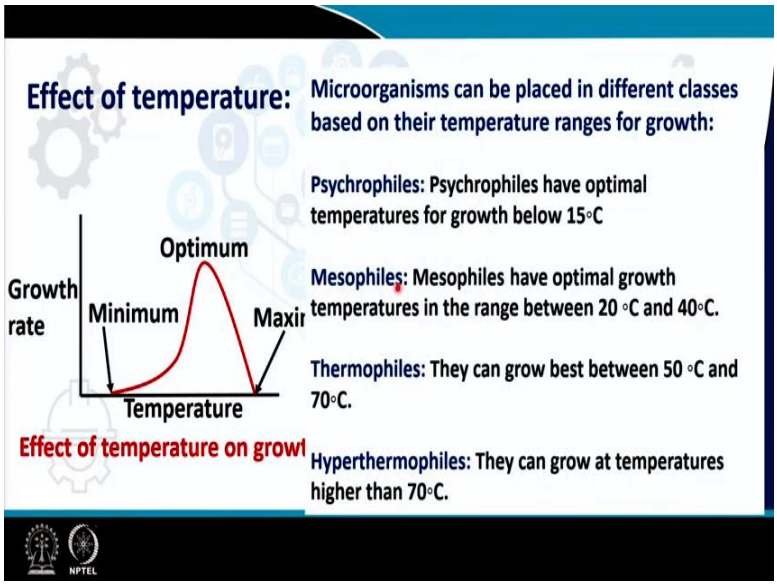
(Refer Slide Time: 21:27)



Now with respect to effect of temperature, now it is well known that temperature has a very significant effect on microbial growth and if we plot the temperature versus microbial growth rate we can find out that there could be a minimum optimum and maximum which is called cardinal temperature or cardinal plot can be obtained. At high temperature the cells are damaged normal stress cells which are which are having a kind of optimum temperature of 30 to 37 degree centigrade can be damaged severely if they are grown or exposed to high temperature like 50 degree or 60 degree temperature.

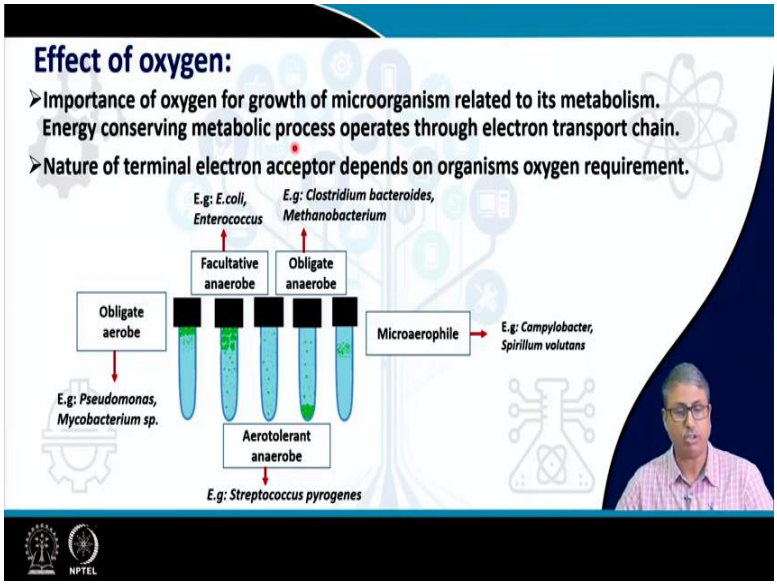
This damage caused by the high temperature is because of the denaturation of enzymes transport carrier molecules other proteins and also the melting of the lipid bilayer. Similarly at low temperature like less than four degree temperature one degree or a sub zero temperature that also causes damage serious damage to the cells the microbial cells unless some cryo-protectants are added into that one of the major cause of these damages is the membrane solidifications.

(Refer Slide Time: 22:38)



Now, microorganisms can be placed in different classes or groups based on their temperature tolerance temperature ranges for growth. For example the psychrophiles which have the optimal temperature for growth below 15 degree centigrade, mesophiles which have the optimal growth temperature between the 20 degree and 40 degree, thermophiles between 50 to 60, 70 degree and hyper thermophiles they can grow at temperature higher than 70 degree centigrade.

(Refer Slide Time: 23:06)

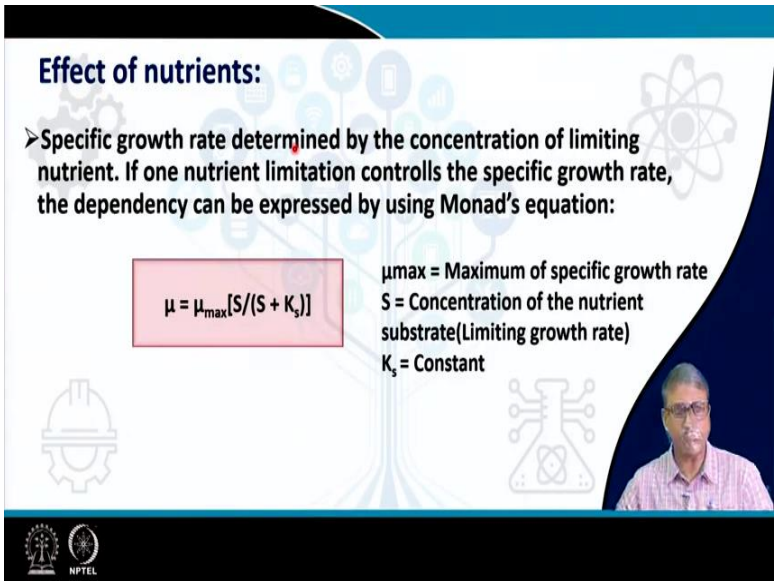


Next is the effect of oxygen: Importance of oxygen for growth of microorganisms related to its metabolism as we have already discussed in earlier lectures that oxygen could be acted as a kind of a terminal electron acceptor. And in energy conserving metabolic processes through electron transport system this oxygen plays a very important role in yielding a maximum amount of

energy through the transport of electrons. Now nature of terminal electron acceptor depends on the organism's oxygen requirement.

So, for example if we; try to look at the different type of oxygen requiring or oxygen sensitive microorganisms. So, there could be obligate aerobe like micro bacterium or pseudomonas. There could be facultative anaerobe like E coli or enterococcus. There could be an aero tolerant anaerobe like streptococcus pyrogenes or obligate anaerobe that is the clostridium and methanol bacterium like archaea and finally there are micro aerophiles. For example the campylobacter or spirulum pollutants like organisms.

(Refer Slide Time: 24:19)



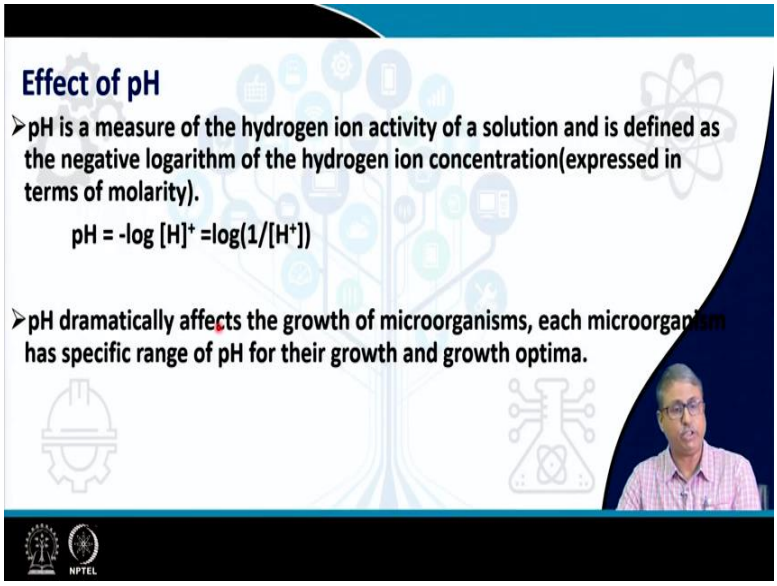
The effect of the nutrients is another very important factors because nutrients are essential however they may play a negative role sometimes if a specific nutrient is deprived. Or in reduced concentration or its metabolism is going to produce some intermediates which are toxic to the cells. Or some components which are metabolized happily by the microorganisms at certain concentration but they might results into a toxic effect like many of the hydrocarbons including phenol or alkane molecules.

They are utilized metabolized rapidly by many of the hydrocarbon degrading bacteria at a lower concentration but as the concentration of those substrates like the alkanes or the the aromatic compounds increases. So, the microorganism they experience different kind of toxic effects. So,

specific growth rate determined by the concentration of the limiting nutrient if one nutrient limitation controls the specific growth rate.

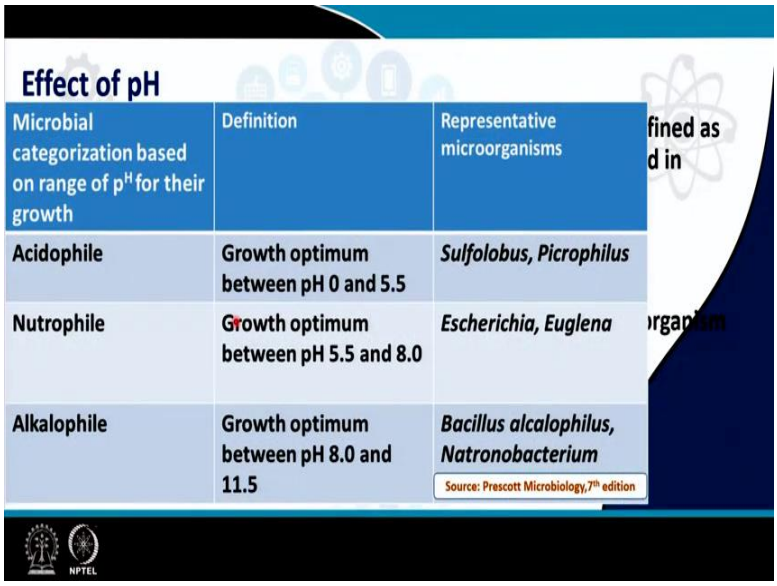
The dependency can be expressed through the mono very well known well studied monarchs equation which is mu equal to mu max S by S + K s.

(Refer Slide Time: 25:33)



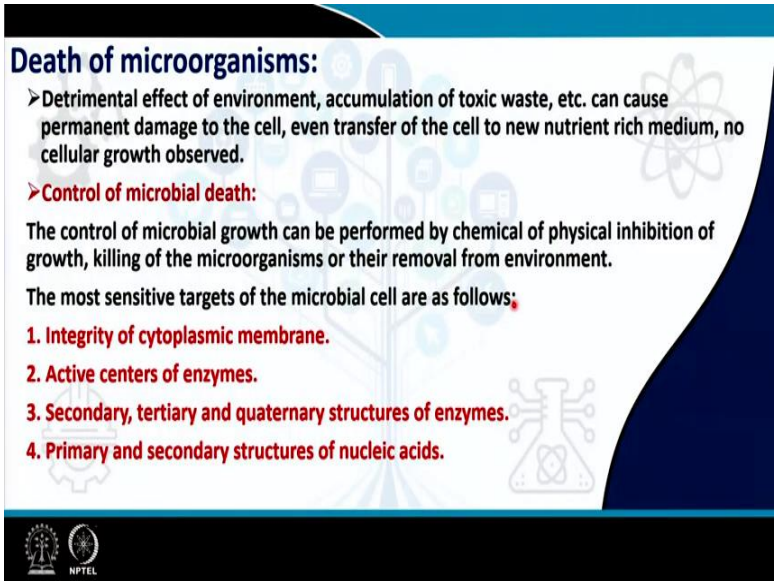
And similarly the effect of pH can be well elucidated because pH is basically the negative measure of the hydrogen ion activity of the solution and is defined as the negative log of the hydrogen ion concentration expressed in terms of the molarity. And pH dramatically affects the growth of microorganisms each microorganism has their specific range of pH for their growth and growth optima similar to the oxygen or threshold the there could be a very sharp ph threshold or ph optima for maximum growth by each of the organisms.

(Refer Slide Time: 26:09)



And based on that we can actually categorized organisms as acetophile, neutrophil or alkalophile where for acetophile the ph of the growth optimum between 0 to 5.5 for neutrophil it is 5 to 8 and for alkalophile it is between 8 to 11.5 and the examples of such organisms are acidophile for example the sulphur lobus or picrophyllus neutrophil are these chirichia and euglena and alkalophiles are the different type of bacillus species and natural bacterium.

(Refer Slide Time: 26:44)



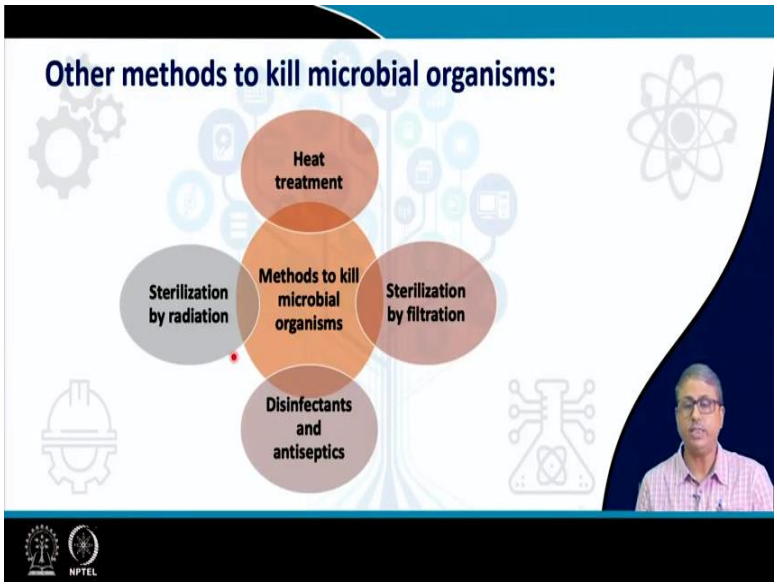
Death of the microorganisms: Now detrimental effect of environment, accumulation of toxic waste etc can cause permanent damage to the cell. Even transfer of the cell to new nutrient rich medium may not yield cellular growth because the cells are in a depth phase or the cells are dead. Now the control of microbial death is a very important factor for their utilization their

successful deployment in any kind of system.

The control of microbial growth can be performed by chemical or physical inhibitors of growth, killing of the microorganisms or the removal from the environment the most sensitive targets of the microbial cells are as follows. The integrity of the cytoplasmic membrane, so, any chemical compound which affects the integrity of the cytoplasmic membrane or active centers for the enzymes secondary tertiary and quaternary structure of the enzymes.

If they are affected by any kind of substances or any kind of physical changes, primary and secondary structure of nucleic acid these are all the targets for different treatments different chemical or physical treatments that are that actually leads to cellular death.

(Refer Slide Time: 28:09)

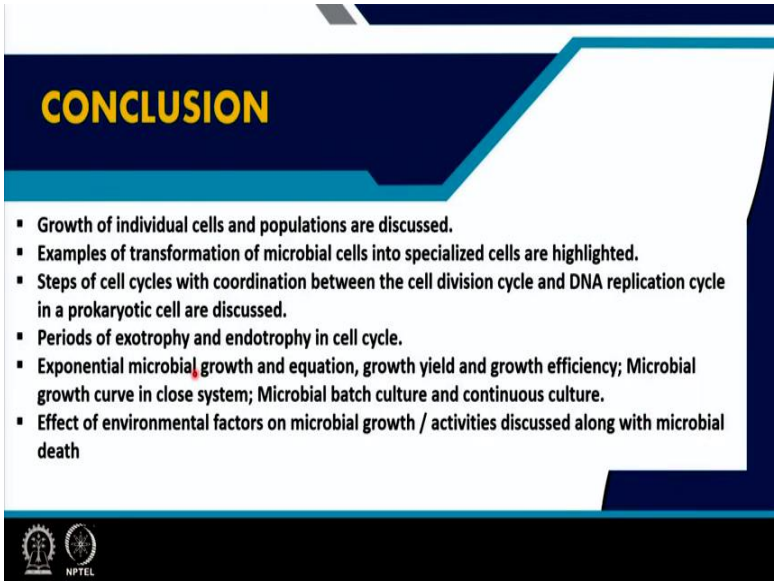


Other methods to kill the microbial organisms include heat treatment sterilization by radiation, disinfectants and antiseptics and sterilization by filtration.

(Refer Slide Time: 28:20)

For this part of the lecture the following are the references which includes the environmental biotechnology book by the Wang et al, Prescott microbiology book. Molecular cell Biology by Harvey Lodish.

(Refer Slide Time: 28:37)



And finally in conclusion the growth of individual sales and populations are discussed. Examples of transformation of microbial cells into specialized cells are highlighted. Steps of cell cycles with coordination between the cell division cycle and DNA replication cycle in a prokaryotic cell are discussed. Periods of exotrophy and endotrophy in the cell cycle are mentioned. Exponential microbial growth and equation growth yield and growth efficiency, microbial growth curve in closed system.

Microbial batch culture and continuous culture are discussed effect of environmental factors on microbial growth activities are discussed along with microbial death, thank you.