Environmental Biotechnology
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Lecture – 19
Microbiology of Environmental Engineering System (Contd.,)

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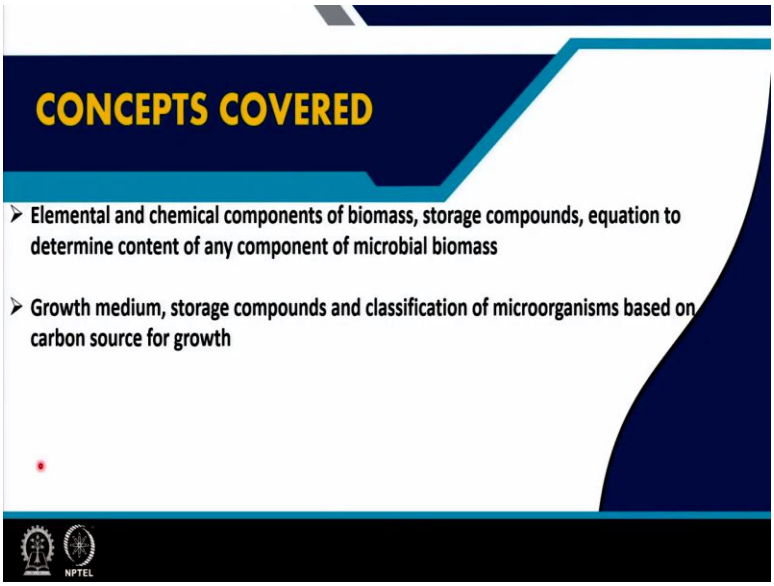

Welcome to the next lecture on microbiology of environmental engineering system. And in this particular lecture we are going to cover the following concepts elemental and chemical components of biomass, storage compounds, equation to determine the content of any component of microbial biomass, growth medium, storage compounds and classification of microorganism based on carbon sources for their growth microbial.

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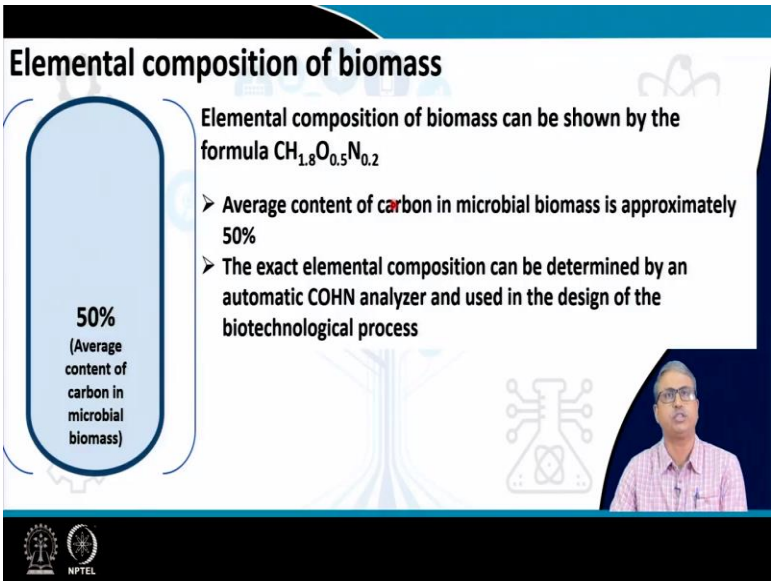

Growth nutrients and media elemental composition of the biomass. Now as we can understand that elemental composition of biomass can be shown by the following formula and average content of carbon in microbial biomass is found to be quite high and it is approximately 50%. The exact elemental composition of the microorganisms can be determined by an automatic carbon, oxygen, hydrogen, nitrogen or carbon, hydrogen, nitrogen CHN or COH and analyzer.

And used in the design of the biotechnological process it is interesting to note that the content of the carbon or other important constituents of the microbial biomass is significantly controlled by their growth and the environment in which they are growing.

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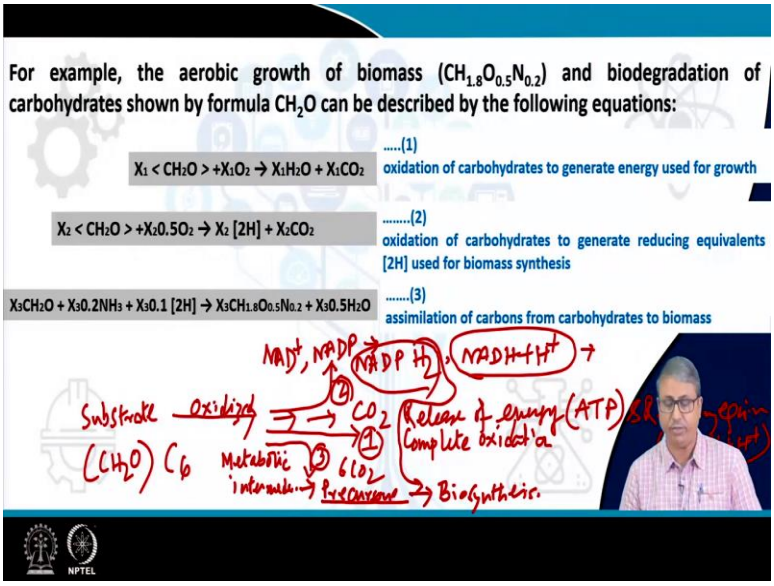

For example the aerobic growth of biomass and bio degradation of carbohydrates is shown by the formula in this following section by CH 2 O can be described by the following equation. As you can see that during the process of complete oxidation of the carbohydrates or the bio degradation of the carbohydrates which are available to the microorganisms in presence of the oxygen as the terminal electron acceptor we can generate maximum amount of energy.

As we mentioned before because all the electrons from the reduced carbon substrates are released and oxygen is used as a terminal electron acceptor allowing the generation of the large amount of energy in the form of mostly in the form of ATP and thereby allowing cellular growth and cellular metabolic activities mostly the cellular activities because the carbon is completely oxidized to carbon dioxide and water.

So, leaving behind very less amount of or NO metabolic intermediates between this the oxidation of carbon. However there could be some other mechanisms of oxidations or other types of oxidations as well. In the second reaction as you can see that the bio degradation of this carbohydrate or the oxidation of the carbohydrate molecule is achieved through the production of reducing equivalent that is the electrons are released as the complex carbon is degraded to carbon dioxide.

And these electrons are eventually received by the electron acceptors which are present in the cellular system that is the we call them reducing equivalent like NADP or NADH, H+ and they settle this electrons they transport these electrons to other electron acceptors like the terminal electron acceptors oxygen or other electron acceptors in case of anaerobic respiration. Or they may trans for this electrons to the electron requiring reactions of the anabolic pathways.

So, this is a very important part of the entire carbon metabolism by the cells particularly in terms of the cells growing in an environmental engineering system that the electrons can be this can be given to the reducing equivalent. And the reducing equivalent can take the electrons to the anabolic process or they can transport these electrons to the electron acceptor. So, if they transport the electrons to the electron acceptor maximum amount of energy can be generated which is coupled to the oxidation of the substrate to the carbon dioxide.

If they transfer the electrons to the anabolic pathway then biosynthesis of other macromolecules are achieved. So, thereby this shuttling of electrons to the anabolic pathway facilitates the biosynthesis reactions thereby allowing subsequent growth of the cells. The reaction 3 demonstrate that the oxidation of the carbon substrate and simultaneous utilization of nitrogen reduced nitrogen compound.

And where the both the biosynthetic type of reactions as well as the oxidation reduction reactions are able to allow the cells to assimilate the carbon from the substrate which is basically subjected to bio degradation and eventually it goes to the biomass. So, that means that means that the substrate which is available to the biomass might have three routes to follow. One of the roots could be if we have the substrate that is the carbon substrate.

Here we are abbreviating at CH 2 O the substrate can be oxidized and following oxidation we can expect that it will be converted into carbon dioxide. However there could be one route where it is completely oxidized. So, this will write as root one complete oxidation of the substrate like if we are considering glucose the glucose C 6 H 12 O 6 is completely oxidized. So, 6 carbon of the glucose is converted to 6 CO 2 molecule.

So, 6 CO 2 molecule eventually it is a complete oxidation. So, we can write here complete oxidation and this facilitates the release of energy how because as the glucose or the carbon substrate is completely oxidized maximum amount of energy can be obtained in the form of ATP for example and also the reducing equivalent like NADP, NADH + H+ for example now this NADH+ or its counterpart like the NADP can be utilized for biosynthesis also.

So, that is option 2. So, in option one we have the complete oxidation of the substrate and generation of huge amount of energy. In option 2 we can have more number of NADP reduced to NADP H 2. So, electrons are given to the reducing equivalent like NAD+ or NADP and resulting in the production of NADP H 2 or NADH+ H+. These NADH+ can donate the electrons to membrane carriers or membrane transporters and facilitate transport of ions and create the proton motive force and facilitate other reactions whereas the NADP H 2 can donate the electrons to the

biosynthetic reactions.

And so, the strategy number three is oxidation of the substrate with the formation of metabolic intermediates. So, we generate a number of metabolic intermediates through the degradation of the substrate like the substrate to carbon dioxide might be a 20-25 steps reaction. So, you have intermediates and these some of the intermediates are actually important precursor molecules. So, these precursor molecules are taken away.

So, we have 6 carbon molecules within the glucose out of these it may be possible that 4 carbons were taken away in the form of the metabolic intermediates which will eventually lead to the production of precursors. And these precursors when they are supplied with the reducing equivalent of NADP H will allow the biosynthesis reaction. So, what we can see or what we can try to understand that the substrate oxidation might follow these three parts.

Now how the cell adjusts themselves with respect to choosing one or the other it depends upon the cellular requirement and the substrates which are available. When the cell is requiring high amount of ATP perhaps the complete oxidation that is the route number one or the reaction number one complete oxidation will be allowed to produce more ATP to transport something or for certain other functions like flagella movement etcetera.

However when they require a kind of a balance thing some amount of energy and some reducing equivalent they might go for the step number 2 or if they rely try to rely more on the biosynthetic reactions that production of the precursor. So, they will take away the metabolic intermediates coming out of the glycolytic reactions or the TCA cycle and eventually use them as their starting molecule or the precursor molecule for the biosynthesis reaction where of course eventually the reducing equivalent like NADP H 2 is going to play a very important role.

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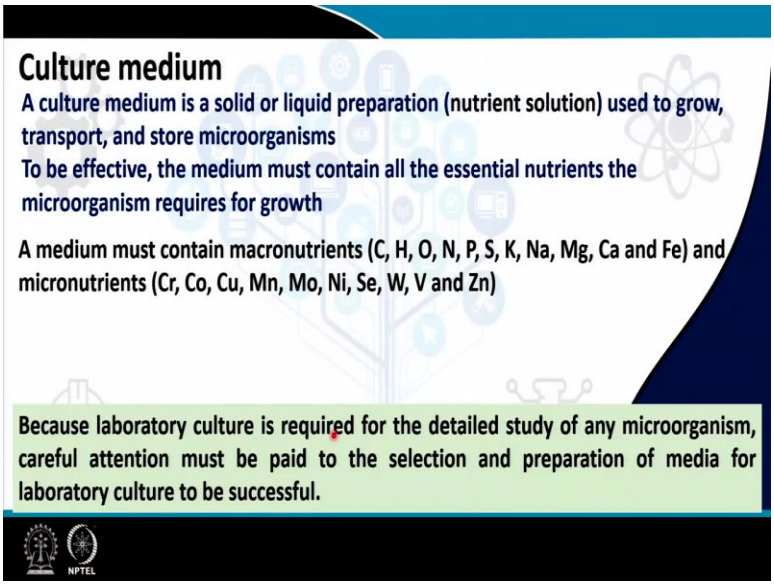

Next is the culture medium: A culture medium is a solid or liquid preparation which is used for the growth transport or storage of microorganisms. So, it is a kind of a liquid preparation or solid preparation. So, basically made as a liquid then converted to solid by adding some solidifying agents and basically its basically nutrient solution where all the nutrient molecules are added. Now to be effective the medium must contain all the essential nutrients the microorganism requires for their growth.

A medium therefore must contain the macronutrients like the carbon, hydrogen, oxygen, nitrogen, phosphorous, sulphur, potassium, sodium, magnesium, calcium and iron and a broad range of micronutrients including cobalt, copper, manganese, molybdenum, nickel, selenium etcetera. Now the nature of this micro nutrients might vary and that their requirement that how much the micronutrients are nutrients which are required in a very small amount and often we do not add them they often are present in the water that we use.

Or as a contaminant chemical contaminant into the other salts that are being used for supplying the macronutrients like carbon, oxygen, nitrogen or phosphorous, sulphur, potassium etcetera.

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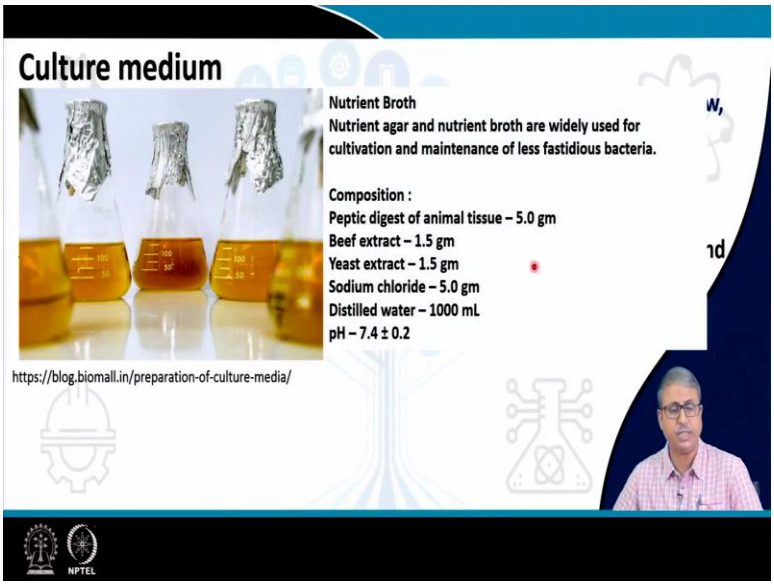

So, here is representative picture of the broth or the nutrient broth medium that we prepared in the laboratory for the growth of different type of organisms or the heterotrophic types of organisms. Now because the laboratory culture of the organisms is required for the detailed study of the microorganism or any type of microorganism careful attention must be paid to the selection and preparation of media for laboratory culture to be successful.

Particularly in terms of or with respect to the environmental surveillance when we try to grow cells in the laboratory to assess whether a particular type of bacteria or archaea mostly bacteria are present in a particular environment or not many a times we rely on cultivation based method that is we try to grow the cells under the laboratory condition. As we try to grow the cells in the laboratory condition utmost care should be taken to identify or select the appropriate medium.

Because the selection of the appropriate medium is the only means by which you can provide the necessary chemical ingredients as far as our understanding allows us to do that. Because still there will be some chemical factors or chemical requirements which are not known to us and perhaps that could be one of the reasons that many of the microorganisms are still uncultivable. Because they we cannot grow them in the laboratory.

Nevertheless we are able to grow a substantial or some amount of microorganism microbial species in the; laboratory condition and that is by virtue of the detailed information about their

nutritional requirement. And therefore the careful attention must be paid into the selection of and the preparation of the medium for example if you want to study the lithotrophic organism or the autotrophic.

Now lithotrophic organisms are organisms which are capable of utilizing inorganic electron donors like ammonia or sulfides or nitrite. So, during the preparation of such media we must pay attention that we are not adding any other chemical compound like yeast extract or glucose which might provide them organic electron source. Because in case of lithotrophy we are focusing only of inorganic electron donors.

So, the medium should not be or must not be contain any organic electron donor similarly for phototrophic organisms we must be ensuring or the autotrophic microorganism we must be ensuring that there is no heterotrophic substrate like the reduced organic carbon is included in the medium.

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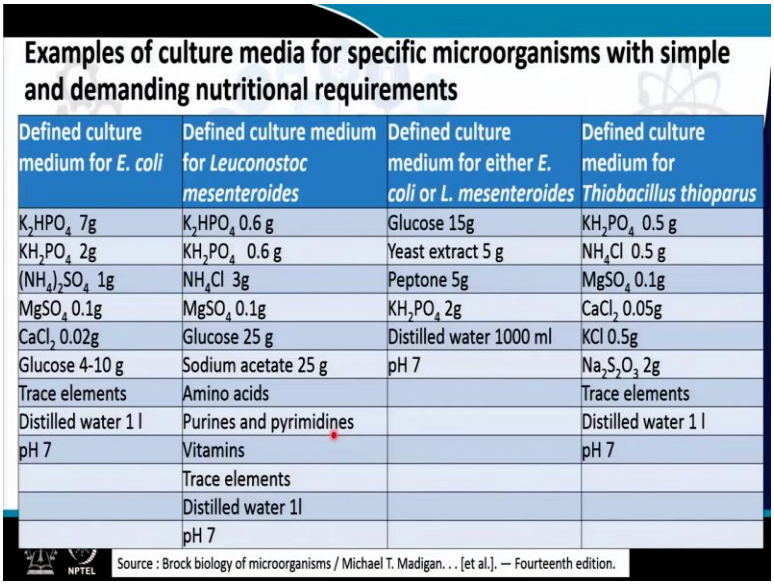

So, here is a brief summary of some of the or the examples of some of the culture media for specific microorganisms with simple and demanding nutritional requirements. The first one is for the E coli it is a defined medium and we will we will discuss about this defined medium little later. The defined medium or media medium in which the all the ingredients the chemical compounds which are added are known and they are highly quantified.

So, we can actually measure them each of the compounds and prepare the medium accordingly. Whereas in case of the complex medium the ingredients individual ingredients might be known to us quantitatively but the composition of those ingredients like peptone for example or the yeast extract for example. We are unable to define exactly how much carbon is there in the medium through Easter extract it is very difficult to delineate or determine that.

But if you see that the medium or the media that are being presented over here that you can see clearly that with respect to organisms for example E coli to leukonostoc. In one case we have the sufficient amount of glucose in other case we have the the level of the glucose varying or in some other cases we are adding substances like peptone or in some other cases we are adding no such compounds like organic carbon or any carbon substrate because we do not allow any kind of heterotrophic activity.

So, thiobacillus or thioparus for example is an autotrophic organism. So, we want to allow microbial growth the growth of thyroid vascular species in this case by utilizing the atmospheric carbon dioxide. So, we are not adding any kind of carbon substrate over here whereas we can you can see that there are carbon substrates like glucose Easter extract, peptone. So, this is a kind of to some extent a kind of a medium which is which is rich in terms.

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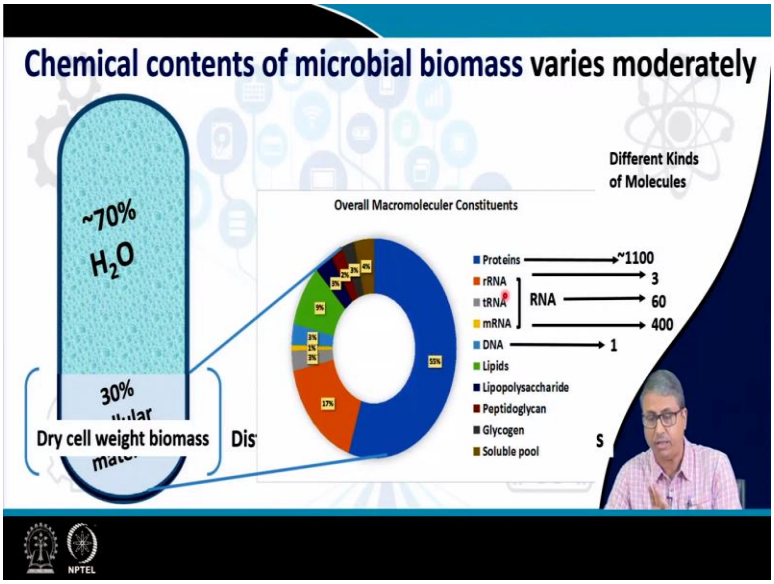

Chemical contents of microbial biomass however varies moderately and what we see that within a microbial cell it is around 70% is water and leaving 30% of the cellular material representing the dry weight of the biomass. Now this 30% of the cellular material is distributed mainly among a number of molecules or macromolecules and these macromolecules are protein representing around 55% followed by RNA's different types of RNA's in which you have ribosomal RNA transfer RNA and messenger RNA.

We have DNA which is corresponding to around 3% followed by 9% or so, lipids and then lipopolysaccharide, peptidoglycan, glycogen and many other molecules representing the soluble pool. And the different kinds of molecules which are representing these proteins and RNA's and etcetera are also mentioned over here.

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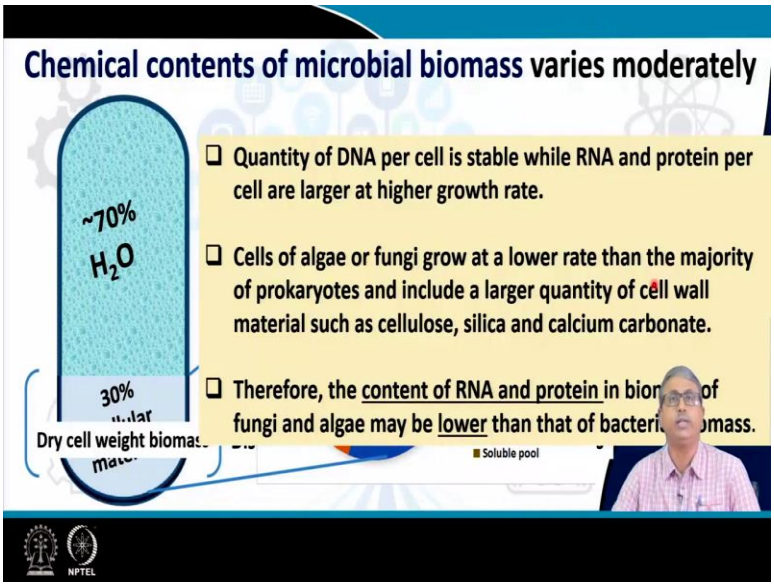

Now what is interesting is that the quantity of DNA parcel is stable because generally for prokaryotic microorganism it is the chromosomal DNA and plasmid DNA which may be more than one sometimes or there may not be any plasmid DNA. So, the quantity of the DNA per cell is relatively stable compared to RNA and protein per cell particularly at higher growth rate. Because when the cells are growing at higher rate the cells must be producing more proteins more enzymes because lot of biosynthesis activities are going on.

And in order to facilitate that the protein concentration is expected to be high and also since the

proteins are produced or translated from the mRNA's. So, it is expected that a high number of RNA is also there not only as mRNA but also as ribosomal RNA because for the translational processes ribosomes are very important critical and ribosomes are made up of ribosomal RNA. Cells of algae and fungi grow at a lower rate than the majority of the prokaryotes and include a larger quantity of cell wall material such as the cellulose silica and calcium carbonate.

Therefore the content of RNA and protein in biomass of fungi and algae may be lower than that of bacterial biomass.

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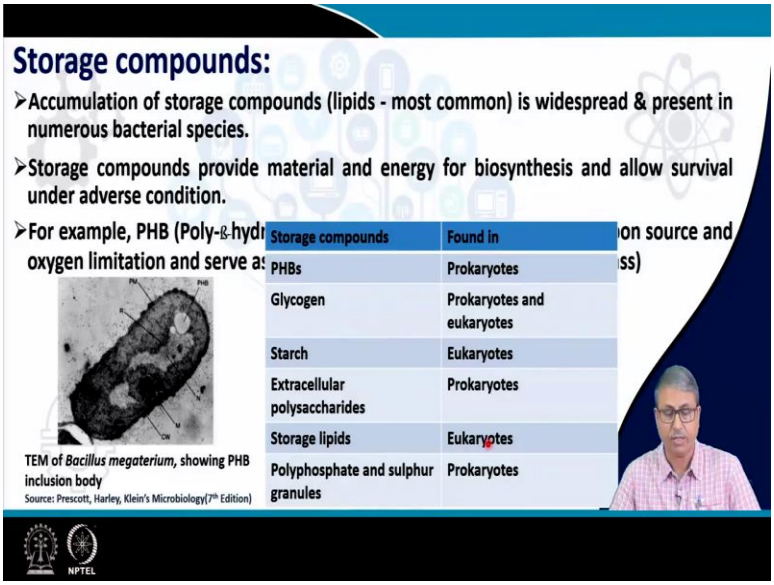

Storage compounds many bacteria particularly they produce a large number of storage compounds and the accumulation of the storage compounds mostly lipids but along with lipids there may be carbohydrates there may be the phosphate containing bodies like polyphosphate bodies. But the lipids are the most common is widespread and present in numerous bacterial species. Storage compounds provide material and energy for biosynthesis and allow survival under adverse condition.

So, basically the bacterial species or bacterial taxa those are capable of producing storage compounds. In fact most of the members they can produce storage compounds in order to store certain reserve material for their future use particularly during the unfavorable condition. So they store the carbon basically in the form of lipid or other polymers or the energy in terms of the

polyphosphate bodies where multiple phosphate bonds are there.

So, it is kind of a storage of material like carbon or and an energy because as you oxidize the carbon which is present in the lipid bodies or in the carbohydrate like glycogen or the polyphosphate bodies you release the energy. So, it is the material as well as the energy which will be utilized during the time of scarcity. So, many microbes; what we see that during their growth under different circumstances they accumulate lipids and other storage molecules.

For example the poly hydroxybutyrate the PHP accumulates under excess carbon source and oxygen limitation conditions and service carbon storage reserve up to 80% of the dry biomass. And here is the picture for the bacillus magnetarium which indicates the presence of the polyhydroxybutyrate like deposits. Now the storage compounds are as I mentioned that storage compounds might vary from polyhydroxybutyrate present in many prokaryotes, glycogen present in both prokaryotes and eukaryotes starch present mostly in eukaryotes.

Extracellular polysaccharides present in prokaryotes storage lipids present in eukaryotes and polyphosphate and sulphur granules which are present in different type of prokaryotic organism.

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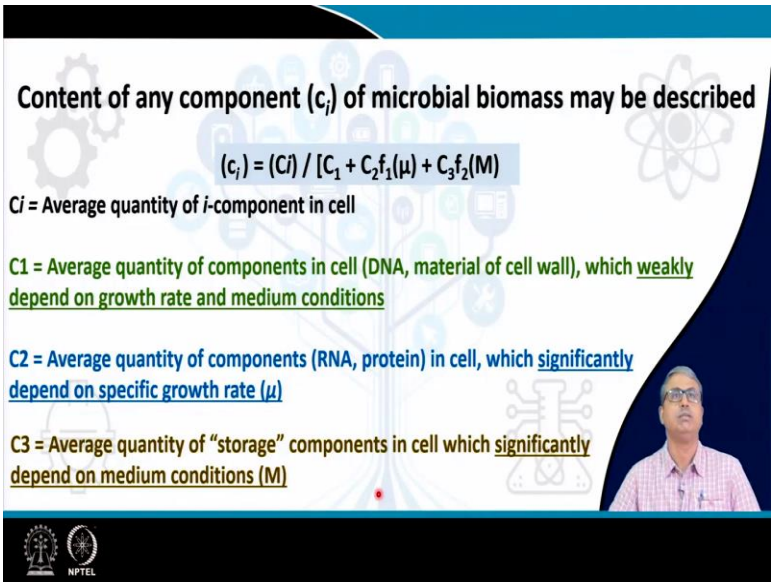

Now next is the content of any component which is designated at c i of microbial biomass may be described and as we try to describe the content of any component within this cell. We will

find or will realize that this is actually controlled or constrained by three factors. So, it can be determined by the following formula which is capital C i divided by c 1 plus c 2 f 1 mu + c 3 f 2 m. Now what is c 1, c 2 and c 3.

C 1 is the average quantity of components in a cell like DNA and materials of cell walls which weekly depend on growth rate and medium conditions as we have mentioned the DNA is quite stable the proportion of the DNA is always consistent within a chromosomal DNA. So, the contribution of the particular compound or particular element through DNA is not subjected to or subject to any kind of variation with respect to growth rate and medium condition generally.

C 2 which is subjected to the or the controlled by the growth rate constant is the quantity of macromolecules like the RNA and proteins that they represent the c 2 which is a function of growth rate because as I mentioned earlier when the cells are growing actively we can expect more amount of RNA and protein. So, c 2 is under the regular control of the function of the growth rate constant or growth rate c 3 is basically the average quantity of the storage components in the cell which significantly depends on the medium conditions again.

So, as we mentioned earlier during the carbon rich condition when the cells are grow growing under carbon rich condition more amount of carbon can be assimilated or when they are growing in energy reach condition more amount of energy can be converted in the form of the phosphate bonds which are present in the phosphorous star bond of the polyphosphate bodies. So, eventually what we find that the content of any component that is the c small c i is actually controlled by the average quantity of the ith component in the cell.

And the average quantity of the stable component and the components which are controlled by the growth rate and the medium composition.

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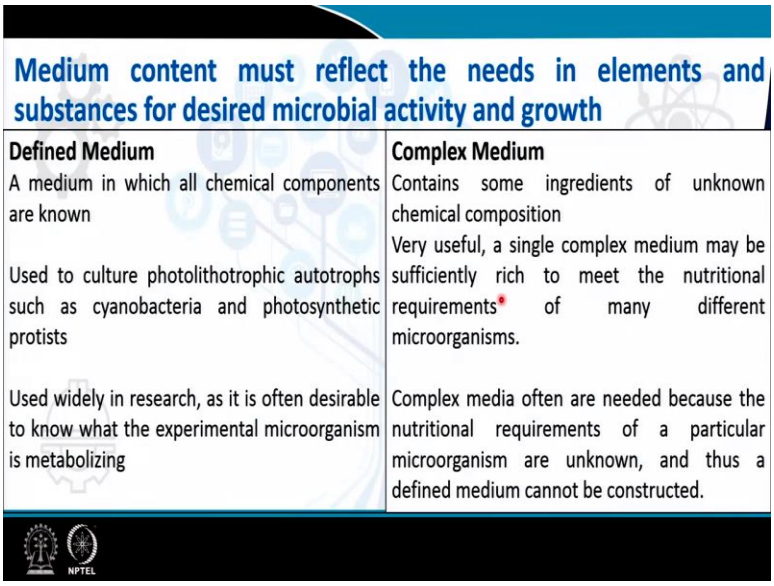

Therefore the medium content must reflect the needs in elements and substances for desired microbial activity and their growth. So, 2 types of medium as we mentioned earlier are there one is the defined medium that is a medium in which all chemical components are known. Used to culture the photolithotropic autotrophs such as the cyanobacteria and photosynthetic protist and also the lithotrophic other lithotrophic organisms used widely in research.

As it is often desirable to know what the experimental microorganisms is metabolizing. Complex medium these contain the ingredients of unknown chemical composition like the Easter extract or the peptone very useful in as in a single complex medium may be sufficiently rich to meet the nutritional requirements of many different microorganism like we often we use the nutrient broth or medium like Luria bartending medium.

So, a single medium can be useful for supporting growth of many different type of heterotrophic microorganism. Complex media are often needed because the nutritional requirements of the particular microorganisms are unknown and thus a defined medium cannot be constructed. So, when we are unable to figure out or unable to define that exactly what are the requirements of the particular type of bacteria that are the organisms that we are trying to grow.

Often we opt for the complex media because during this when we provide a complex medium many of the requirements are simultaneously satisfied but again. So, we may miss out some of

the organisms which are solely like the lithotrops and autotrophs it is very hard to grow them with respect to the with providing the by providing the complex medium.

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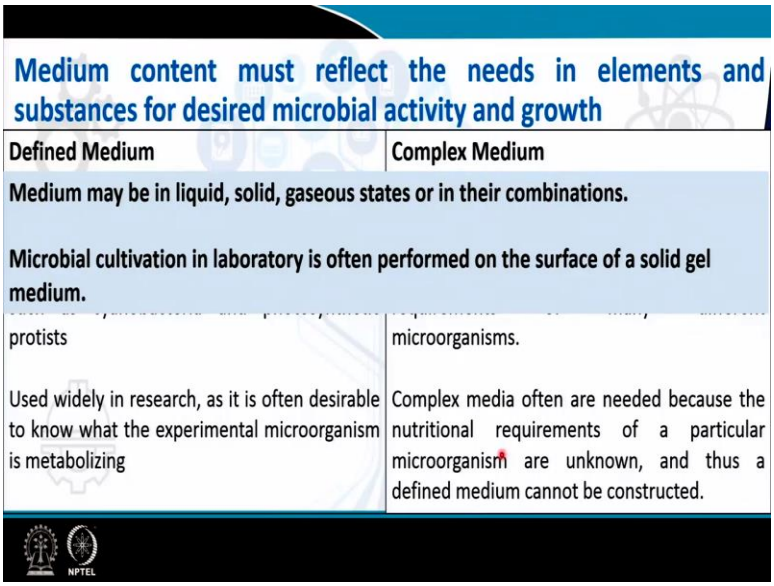

No, matter whether we are using the defined medium or the complex medium. The medium may be made in a liquid form the solid form in a gaseous state or they are combinations. Also microbial cultivation in laboratory is often performed on the surface of the solid gel medium that is a agar plate most often we use the agar plate or sometimes during the growth of the anaerobic bacteria we use the roll tube method.

For example where the organisms are grown in a kind of a semi solid agar or similar solidifying agent under a semi solid condition.

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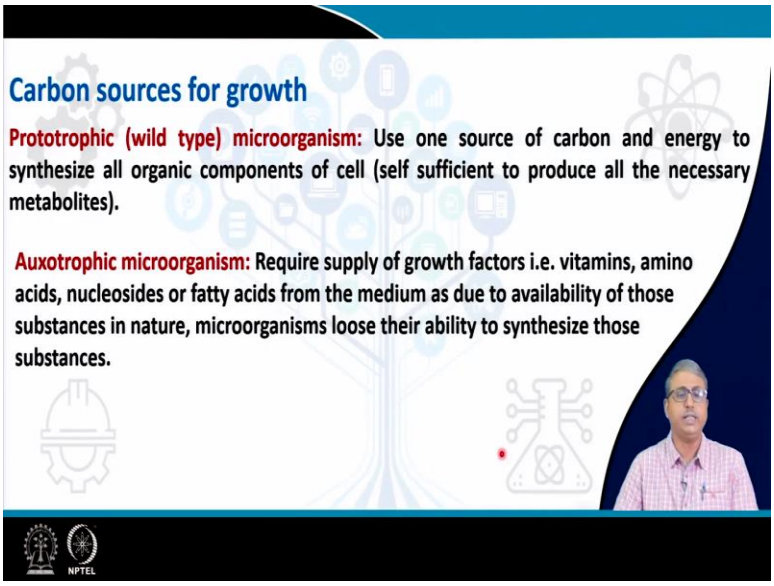

Regarding the carbon sources which are used for growth there are again 2 type of organisms. One is the prototropic organism these are called prototroph because they are wild type organisms they use one source of carbon and energy to synthesize all organic components of cell. Self-sufficient to produce all the necessary metabolites, so, all the necessary requirements the cofactors vitamins the amino acids everything can be produced or is produced by these microorganisms by themselves.

So, they use a carbon source may be glucose or Easter extract or some kind of other organic compound or some may be inorganic compound but they produce everything that they required by themselves on the contrary the oxotrophic microorganisms require the supply of the growth factors. For example the vitamins or the amino acids nucleosides or the fatty acids which they require essentially for their function like their cell mass synthesis from the medium.

Why they need to supply this in the medium the oxotrophic organisms because these oxotropic microorganisms are incapable of producing these growth factors, vitamin amino, acid fatty, acid etcetera by their and why they lost why they are unable to produce this by their own it is found that they lost this ability of biosynthesis of these cofactors or these small molecules that is essentially required for many many activities in the cell because due to their availability or the availability of these substances in nature where the microorganism these microorganisms are naturally living.

So, they lose their ability to synthesize these substances. So, it is a kind of an sacrifice that they have made in order to save certain maybe metabolic potential or certain other things they opted out to remove those synthetic abilities. So, oxotrophic organisms are organisms who are unable to produce the necessary amino acid vitamin nucleoside etcetera from the carbon source that we provide to them.

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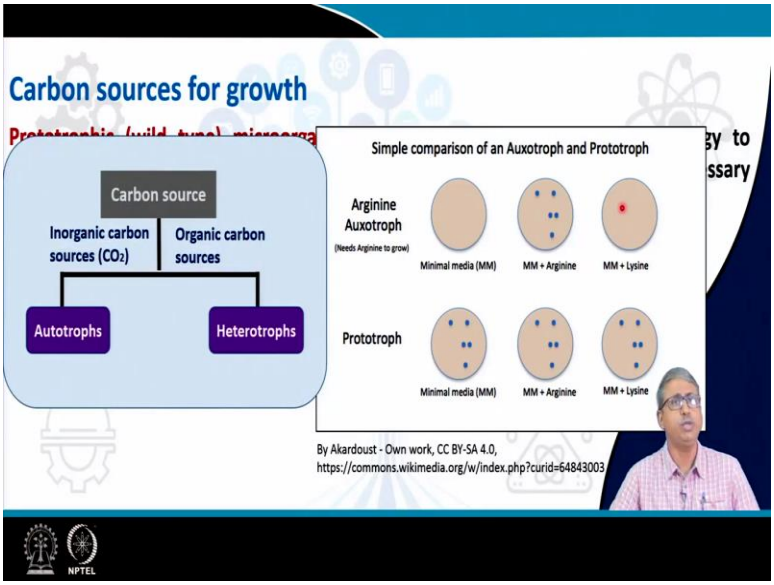

So, this following picture will help us to understand let us see that prototropes. So, prototroops if we put the prototrophic cells on agar plate minimal and medium aggregate plate. We will see that they are able to form colonies. If we add amino acid like arginine or lysine for example they form the similar colonies in that because they are prototroph they are capable of producing the amino acids by their own.

So, they do not bother whether amino acids are added or not if you have the pro suitable carbon source they will produce the necessary amino acids and other things by their own. But if they are for example we have obtained some of the some oxotrophic variant of the same organism and these oxotrophic variants are for example with respect to arginine metabolism they are defunct. Defunct in the sense they are unable to produce arginine amino acid by themselves from the carbon source or the substrate that we provide them.

That means in other words the needs arginine to grow. So, they need arginine in their medium to grow them. So, minimal medium only minimal medium will not have any colony appearing because unless you add arginine into that the cells are not going to appear or going to grow because they lost their ability they do not have the ability to produce arginine and arginine and essential amino acid.

So, without synthesis over the availability of arginine they cannot survive they cannot grow. But if you add arginine into the agar medium you see growth happens and the colonies are appearing that means externally available arginine must be there. However instead of arginine if we provide lysine to them we do not see any growth of the cells that again confirms that they lost the ability to produce the arginine. So, these are called arginine oxide.

So, similarly; for other vitamins and nucleoside and other fatty acids that there could be oxotrophic variants. Now with respect to carbon sources as we have discussed earlier the carbon sources can be of organic and inorganic type and therefore there could be autotrophic organisms and there could be heterotrophic organisms.

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So, for this part of the lecture we have followed the environmental biotechnology by Wang et al and we have also partly followed the Prescott microbiology 7th edition.

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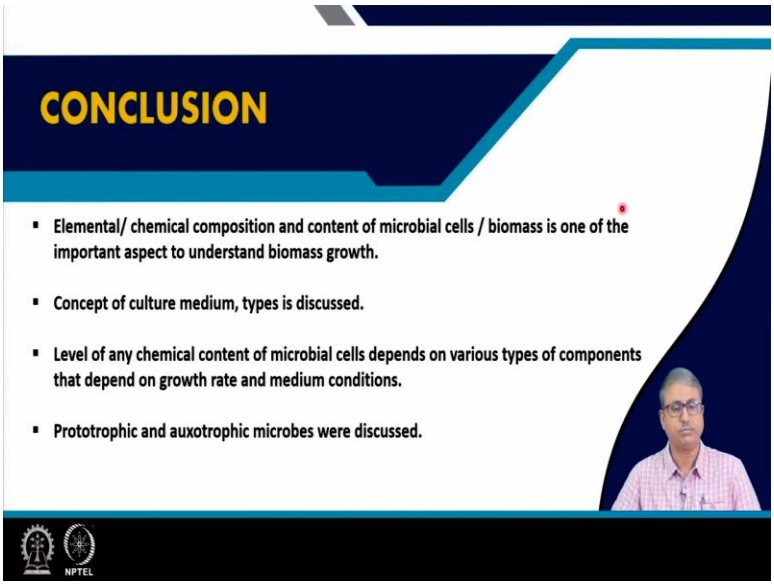

So in conclusion elemental or chemical composition and content of microbial cells or biomass is one of the important aspect to understand biomass growth. Concept of culture medium types etcetera are discussed, level of any chemical content of microbial cells depend on various types of components that depend on the growth rate and medium conditions as well and prototrophic and oxotrophic microbes were discussed thank you