Genetic Engineering: Theory and Applications.
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Module II
Basics of Biological System.
Lecture-5.
Growth Media for Different Expression System.

Hello everybody, this is Doctor Vishal Trivedi from Department of Biosciences and Bioengineering, IIT Guwahati. And let us continue our discussion about the host cells and how to, the the basic information about the cells. So, in the subsequent and the previous module we have discussed about the structures of the prokaryotic as well as the eukaryotic cells. And in the previous lecture we have discussed about the different types of cellular metabolism which these cells are running. And the purpose of these metabolic pathways is to sustain the life of these organisms.

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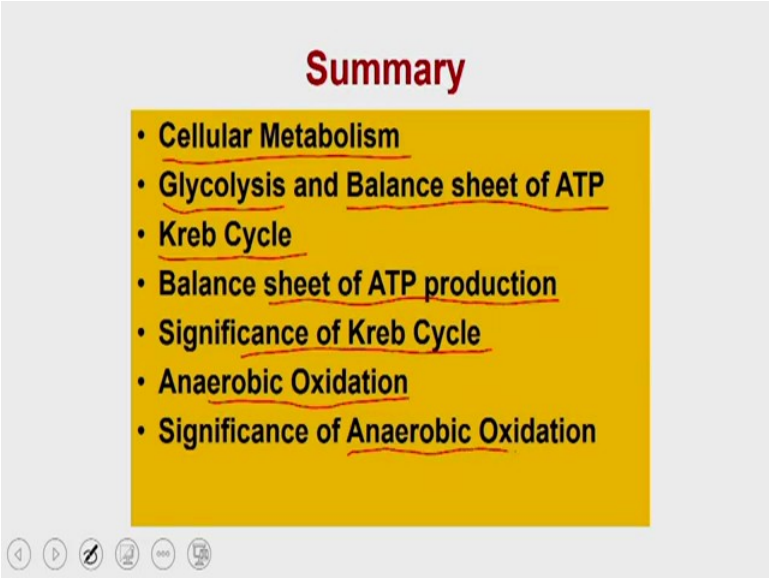

So, what we have discussed so far in the previous lecture, let us summarise what we have discussed in the previous lecture. So, in the previous lecture we have focused on to the different types of cellular metabolism. So, what we have discussed, we have discussed about the different types of cellular metabolism. We have discussed about the glycolysis in which the glucose is getting converted into the pyruvate and we have also discussed what is the balance sheet of this ATP production.

And we have we have discussed how the different intermediates are providing the ATP or how they are utilising the different ATP molecules for glucose activation. Then we have

discussed about the Krebs cycle and we have also discussed how the ATP is being produced at different steps within the Krebs cycle. Then we have also discussed the connection of Krebs cycle as a central metabolic pathway and how the Krebs cycle is connected to the other metabolic pathways in the cell.

And then at the end we have also discussed the anaerobic oxidation which is happening when there is no oxygen present in the environment. And then the cell is not going through the the Krebs cycle or oxidative phosphorylation, then it is going through the anaerobic oxidation to generate or to regenerate the NAD plus. We have also discussed what is the significance of anaerobic oxidation in terms of the cellular physiology as well as for the industrial relevance.

So, in this context we have also discussed how the anaerobic oxidation or the organism which are performing the anaerobic oxidation are helping the human beings to produce the different types of products such as the curd, or the ethanol as well as the bread. There are many other molecules which are being produced by the anaerobic oxidation occurring in the different organisms. So, if you want to do a (meta) if you want to do a biotechnology related events or biotechnology related processes, you also and if you want to exploit the metabolites which are being produced in the anaerobic oxidation or the metabolites which are being produced in the other kind of (meta) cellular metabolism, you have to grow these organisms in large quantities.

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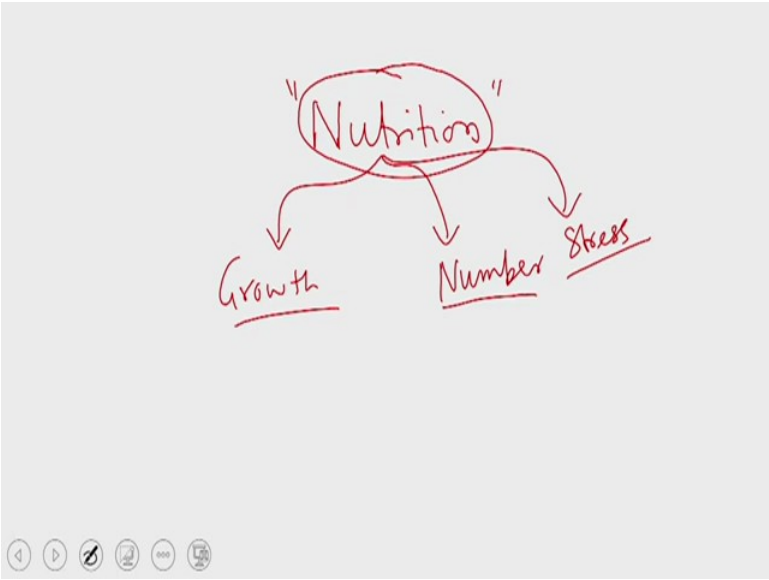

And to grow an organism, the organism requires the nutrition and the nutrition is required only for two purposes. So, the organism requires the nutrition if you want to grow these

organisms and this nutrition is required for two purposes, one you have to grow these organisms, so it is required for going through the different phases of the growth cycle. And then it also requires for the increasing their number. So, it is also requires for dividing and increasing the number and the nutrition is also required to withstand the stress or the stress linked factors.

So, (it) how to provide the nutrition into the different organisms depends on the different types of metabolic pathways or metabolic reactions which these organisms can run, so that you will provide the nutrition in in that form which will be (uet) which will be useful for that particular type of organisms. And because of that the nutrition or the the media what you are going to prepare to provide the nutrition to a particular organism so that they will grow as well as they will increase their number and they can be able to withstand the different types of environmental as well as the biotech stresses is very very different.

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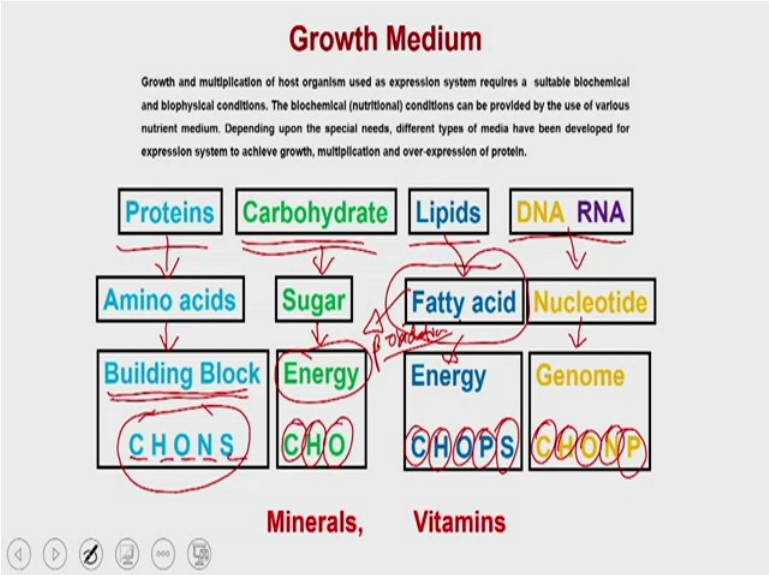

And the Nutrient what you are going to use to provide the nutrition are has to be provided in that form which the organism is going to accept. Let us see what other different (orga) different nutrition which you have to provide. So, for a particular organism you (ha) or for every organism, you have to provide the basic biomolecules. What are these basic molecules? You need to provide the proteins, you need to provide the Carbohydrate, you need to provide the lipids and then you have to provide the nutrients or the the the resources so that it can be able to synthesise the DNA as well as the RNA.

Protein is made up of amino acid and the amino acids are considered to be the building block and the amino acids are made up of the items which are of mostly carbon, hydrogen, oxygen, nitrogen and sulphur. Which means you need to provide the nutrition or the nutrients which can provide all these items so that the organism will use that particular kind of nutrients and utilise these items to first produce the amino acids and then these amino acids are going to link to each other to produce the protein.

Similarly the carbohydrates, it will if it has to synthesise the Carbohydrate, it has to synthesise the monosaccharides or the smaller sugar molecules and the monosaccharide is made up of carbon, hydrogen and oxygen. Again, for the lipid molecule, if the organism has to synthesise the lipid molecule, lipid molecules are made up of fatty acids and these fatty acids are made up of carbon, hydrogen, oxygen, phosphorus and sulphur. Again, for DNA and RNA, they have to synthesise the nucleotides and nucleotides are made up of carbon, hydrogen, oxygen, nitrogen and phosphorus.

The purpose of these biomolecules is also very different as we discuss, the protein is considered to be the building block which actually makes the the body of the particular organisms as we discuss. The proteins present in the plasma membrane as well as the protein is present in the other organelles within the cell as well as the protein is also present in bacteria. Similarly the sugar, sugar is a source of energy, so that we have discussed in the previous class, how the Carbohydrate molecules are being oxidised in the glycolysis as well as the Krebs cycle to produce the energy.

Similarly the lipid is or lipid or the fatty acid is also been a source of energy for the cell and the fatty acid is producing the energy by a process called beta oxidation. So, the fatty acids are getting (convert) getting oxidised by by the beta oxidation to generate the energy. And the nucleotide which is actually a basic molecule for generating the genome or RNA which is actually been utilised for producing the protein. Which means you need to provide the nutrients which can provide all these constituents for the cell to for the organism to to make the the bigger (biomo) to make the big (bio) (bio) biomolecules and which help in (achi) help them to achieve the growth, multiplication as well as if you are suppose overexpressing a particular protein, it would also satisfy that particular type of purpose as well.

So, there are different types of media molecules and which can people can use and apart from these biomolecules, (the) you also need to provide the small amount of mineral as well as the

vitamin as a source, so that the organism can do the growth optimally. So, there are different constituents which which you can use to provide the different types of sources.

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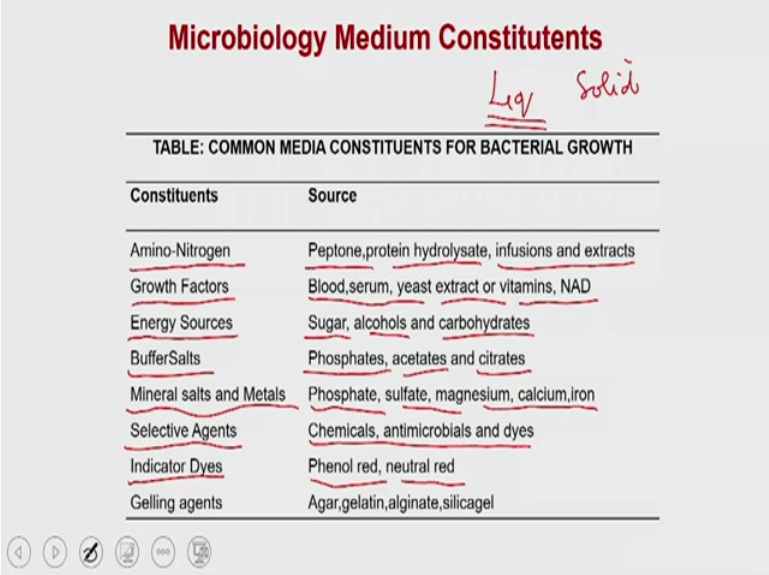

For example, in this in this I have shown the constituent as well as the source from which you can actually get these constituents. So, for example if you want to produce the amino acid, which is actually the nitrogenous (fac) molecule, you need to have the peptone, protein hydrolysates, and as well as the other kinds of extracts into the media what you are going to produce for the bacteria. Then you also need to provide the growth factor, so some of the bacteria also require the growth factors, which you need to provide in the form of blood, serum, yeast extract or the different types of vitamins, NADH.

Then you also need to provide the source of energy, source of energy could be sugar, alcohol or the carbohydrates. And then because we are talking about the media, when in the media when the organism is growing, it is producing a large quantity of or it is actually changing the pH of the media so that pH is also should be maintained. So, that actually you do mostly by keeping the phosphate, acetate or the citrate in the media. And then as I said you, in in a very small quantity you also need to provide the mineral or the different types of metals, that you have to provide by having the different types of salts of the these these mineral salts and then you also have to provide the selection agents.

For example if you want to screen a molecule for a particular type of antibiotic or a particular type resistance, then you have to keep those molecule as well as in the in the media. And then you also sometimes have to add the indicator dyes, which is actually phenol red or the

neutral red, so that it will also tell you if there is a change in the pH of the media. And at the end, this media could be in the liquid form or it could be in the solid form. This liquid media you have to constitute all these molecules and it will give you the liquid media.

Whereas for the solid media, you have to provide a gelling agent, for example agar, gelatin or the silica gel so that it becomes a solid media so that you can be able to generate the plates. Because some of the organisms are preferring to grow on a solid surface compared to the liquid (su) liquid media. So, let us see what are the different media recipes are available for growing the prokaryotic cells.

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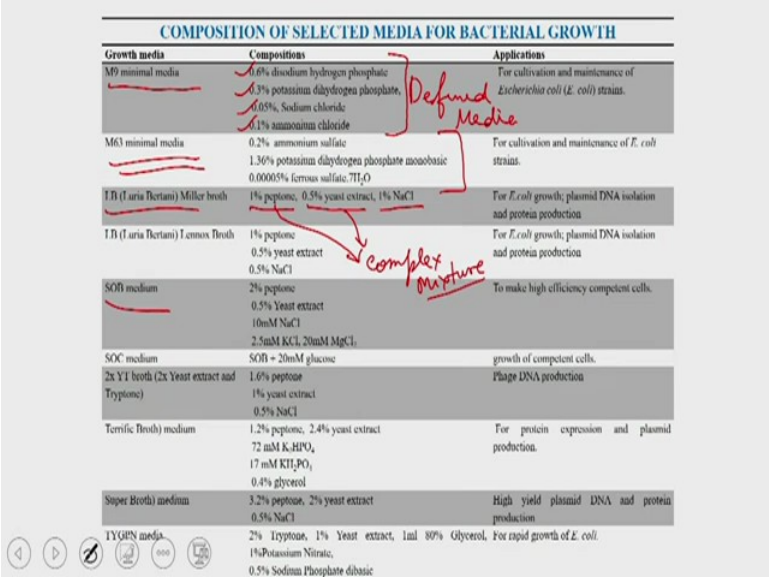

So this is the small table to show you the different types of media which people are using for the bacterial growth. You have the M9 minimal media, M 63 minimal media, these are the 2 media which are also called as the defined media. What is meant by the defined media is that it actually has the defined concentration of the salt as well as the other sources what you are providing in the media. So, because it is a pure media, so you can actually vary these are the vary the (cons) constituents and that is how you can actually change the media compositions in a very very controlled fashion.

For example, if you want to change the phosphate or if you want to change the NaCl or if you want to change the ammonium chloride, it actually very very precisely and there is no other contaminating complex mixture present. For example, if you compare the M9 media to LB media, what you will see is it contains the 1 percent peptone, 0.5 percent yeast extract and 1 percent NaCl. But what happen is the peptone is a complex mixture, it is actually a very very

complex mixture. So, you do not know what are the constituents are present in the peptone and what is their what is their relative abundance.

So, because of that and similarly the same is true for the the yeast extract as well. So, when you are putting the peptone, you are actually not adding peptone but you are adding the hundreds of molecules. That is why the LB or all other medias are not called as the defined media. And the advantage of having a defined media is that you could be able to manipulate these parameters and achieve the best production of your metabolites or the best production of your protein, whatever you would like to overexpress or whatever the downstream metabolites which you are producing.

So, these metabolites production or as well as the metabolic pathways are going to respond very nicely if you change these parameters. Whereas if you want to do the similar kind of optimisation in a other kind of media such as LB media or the SOB media, it would be difficult because the moment you add the peptone or the yeast extract, it becomes very very very complicated. So, let us let us see how to prepare a microbiology media.

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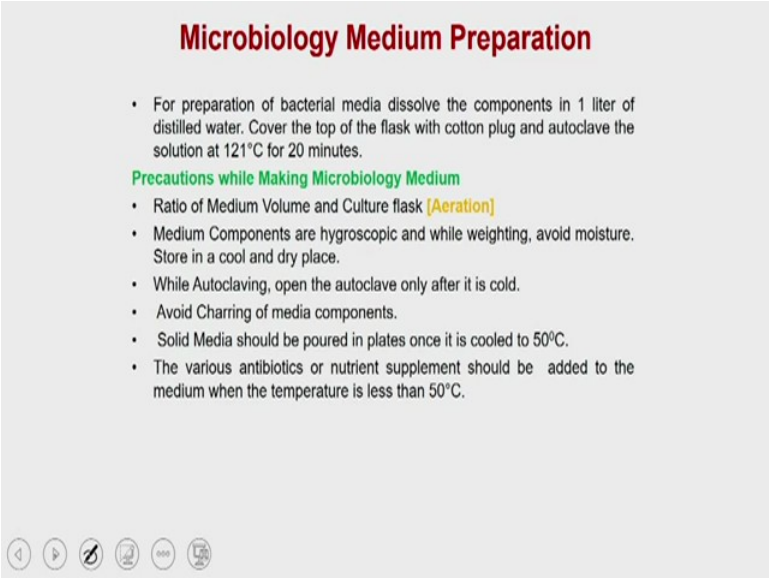

So, for preparing a microbiology media, what you are supposed to do is you have to take the media components and then you you dissolve the media components in the 1 litre of distilled water. And you cover the top of the flask with a cotton plug and autoclave the solution at 121 degree Celsius for 20 minutes. And here I have we have prepared a very small 10 minutes clip for you to show the different steps in very very detail. And this clip is being prepared in

our laboratory to demonstrate you how to prepare a microbiology media and this clip is being prepared by the one of my student, whose name is Suram Banesh.

And what you will see is that the Banesh has explained to you the many aspects of preparing the microbiology media and as well as what are the different precautions you should take.

Student presentation video:

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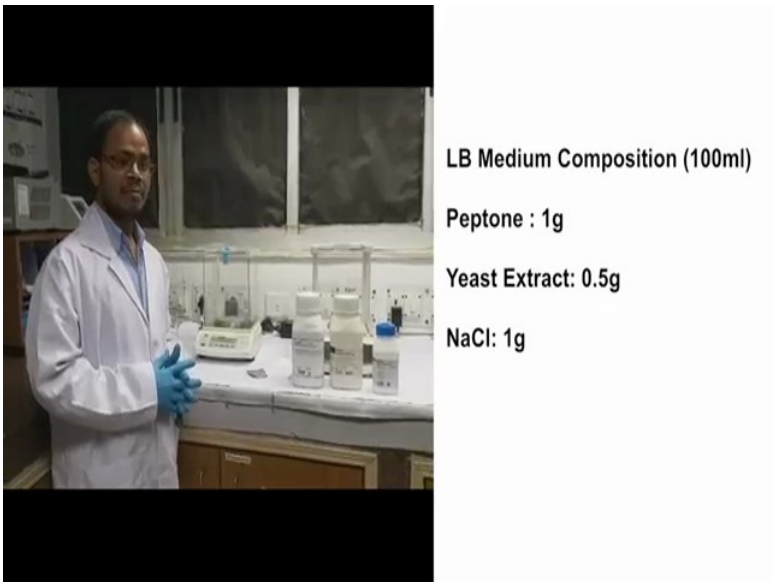


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In this video we are demonstrating how to prepare bacterial culture prapt. For preparing Culture prapt, we need 3 components, one is peptone, yeast extract and sodium chloride. For 100 ML of culture prapt, we need 1 gram of peptone, 0.5 gram of yeast extract and 1 gram of sodium chloride. I am going to way individual components and to dissolve it in double distilled water then we we have to autoclave the media. Before weighing, care should be taken spatula is clean and the balance is 10.

After weighing, we have to clean the spatula and keep it in original (posi) position. And during weighing, care should be taken to avoid contact with any of these media components. After weighing the media components, we have to dissolve them in double distilled water. So, initially we are dissolving in 80 ML of distilled water. Once the components completely dissolved, we have to make up the volume up to 100 ML. While it is stirring, we have to prepare cotton plug for the flask.

For preparing cotton plugs, you have to take one thick layer of sheet of cotton and with your two hands, fold like this. Once the media dissolution is complete, we have to pour into the flask. We have to pour up to 100 ML, so we will use only one third of the place, remaining space is empty. This is used for aeration purpose and also to ensure proper autoclaving. In order to check whether the components are autoclaved or not, the media is autoclaved or not, we use stability indicator, this is table-based stability indicator.

We have to paste it on the flask and we have to autoclave. If the autoclave is properly finished then we will see the white strips turning into the black ones. So, this is the indication of autoclave process is complete. Now, the media components are completely dissolved, now we have to pour into the flask. Cap the mouth with cotton plug and wrap with aluminium foil. Now, this is ready for autoclaving. Once the media preparation is complete, we have to sterilise the media in order to use for further applications.

This is the typical autoclave where you can see temperature and pressure indicator and these are the pressure knobs and this one is quick pressure release knob. You can use when you you (ha) you are in a hurry, you have to use this one but I will prefer not to use this one, let it go on itself. We have to turn on the autoclave, so you can see here is the bulb is glowing. Before keeping the media components into autoclave, make sure that the heater inside the autoclave is submerged with water.

Now, I am going to keep the media components in the basket which they will use for the autoclaving. You can keep this one inside the autoclave. While closing the autoclave, make sure that your closing in opposite directions. Once the pressure and temperature reaches 121 degree Celsius and 15 Kelvin pressure, you have to hold on that point for 20 minutes, then you have to turn off the machine, let it cool down and remove the components. The same procedure you have to, while opening you have to open in opposite direction.

To conclude the video demonstration, we have discussed how to prepare bacterial culture media and how to prepare cotton plugs and autoclaving. During (culture) weighing of the media we have to make sure that the components media components should not be exposed to air, because those substances are those substances absorb the moisture and become liquefied. So, another thing is that for cotton plug preparation, we have to take a single layer of cotton and then we have to fold it. And during autoclaving, we should not, we should not after autoclaving we should not release pressure in a single shut. Let it cool and come to normal pressure, then we have to open autoclaving.

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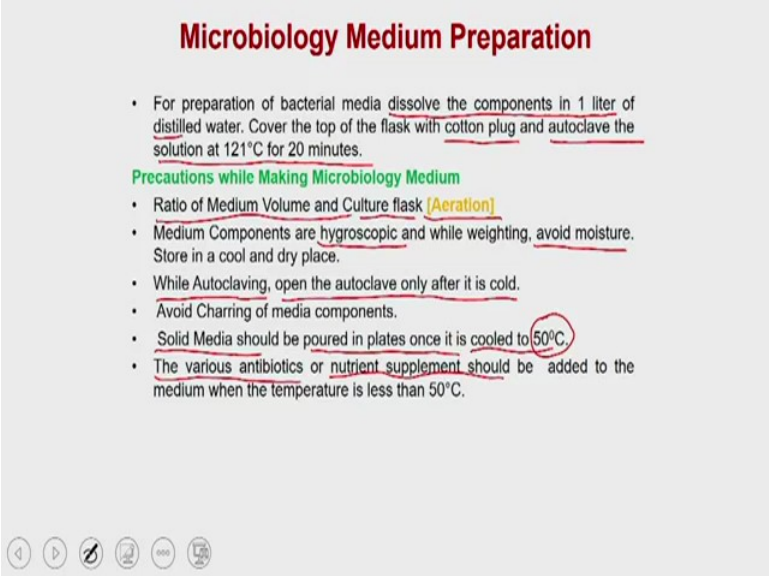

Professor:

In this movie what you have seen is that Banesh has discussed many precautions (how) (when) while you are preparing the microbiology media. One of the major thing what you have to keep under consideration is that there should be a fixed ratio between the culture flask what you have taken and the amount of media volume what you are going to take. This actually is important because you want to maintain the aeration in the system. Similarly the

media components are very hygroscopic, which means they are going to take up the water as soon as you open them in the air. So, that is why while you are weighing or while you are keeping them in a in in (in) while you are storing them, (it has) you should avoid the moisture because once the media is going to be take up the moisture, your media composition is going to vary from one batch to another batch.

And that actually is going to affect the downstream, the protein production as well as the growth of the bacteria. Whereas, you have to take a lot of precautions when you are doing the autoclaving. You only have to open the autoclave when it is completely cold because the autoclave has the inbuilt vapour inside inside the autoclaving machine. So, if you open the autoclave while it is hot, sometimes these vapours actually comes onto the face and that is why and also sometimes when there is a high-pressure inside the autoclaving machine, you also, the water which is present inside the autoclaving machine also comes out and these (auto) this water is very very hot, so it can actually hurt you or it can actually cause the severe burn.

Similarly the when, if you are preparing a solid media, the solid media should be poured in plate once it is very very cold. Means, it is cold to the temperature of 50 degree Celsius. And once it is cooled down to 50 degree Celsius, then only you add the antibiotics or other nutrient suppliments. For example, if you have if you have to add the carbon source, for example if you have to add the the glucose molecule as a carbon source, then you have to wait for the media to get cool down to 50 degree and then only you should add and then (you) use this media for the bacterial growth or as well as other the prokaryotic cells growth.

Just let us move onto the other category. So, in the eukaryotic cell, the eukaryotic cell as we discussed in the past also, the eukaryotic cells are much more complicated and they require the much more the sophisticated systems to not only to grow them but also to to monitor their growth as well as to provide the nutrients. So, let us see what are the different media (com) are available for the for the for the eukaryotic cells.

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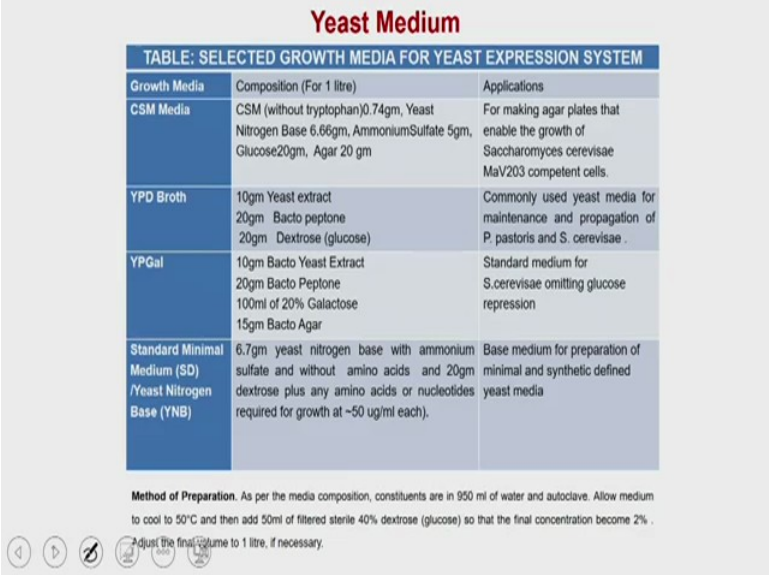

So, initially we will take up the the easy (pro) easy eukaryotic cell, which is called as a yeast cell. So, Yeast are the unicellular yeast unicellular eukaryotic cell and these are the selected media what we are can we can use for the yeast propagation. These are called CSM media, YPD media, YP Gal media and standard minimal media. Standard minimal media is a media which requires the yeast nitrogen base with ammonium sulphate and it is without (amin) amino acid. And 20 grams of dextrose plus any (antibio) amino acid or nucleotide required for the growth at 50 microgram per ml.

It is the preparation of yeast media is exactly the same as it was for the microbiology media. So, you have what you have to do is you have to prepare the media composition, you have to weigh and then you have to dissolved these constituents in the 950 ML of water and autoclave. You have to follow exactly the similar steps what we have what we have already discussed in the preparation of microbiology media and that is how you can use this media for propagating the some of the yeast molecule such as the S.cerevisae and other the other yeast molecules.

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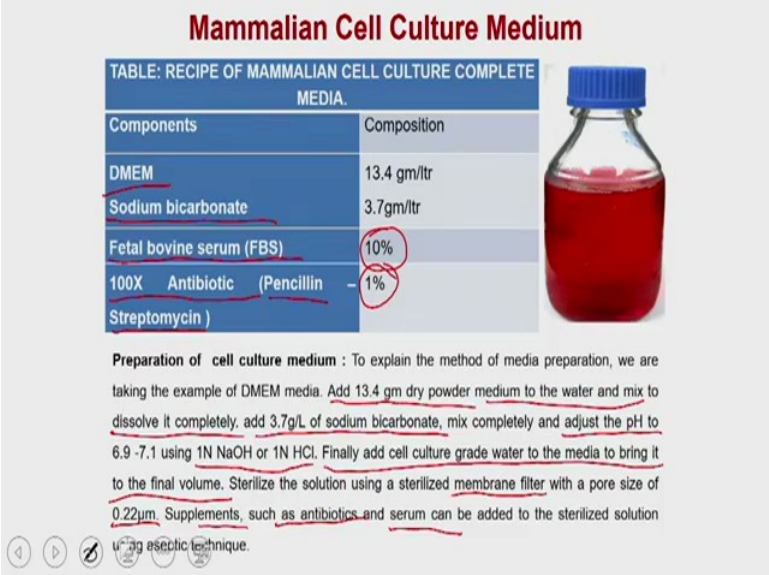

Let us move onto the mammalian system. So, in the mammalian cell culture media, what you are supposed to do is you have to have the media, which in this case is DMEM and then you have to add the sodium bicarbonate is actually a buffering agent and then because the the the mammalian cells are requiring the growth factors and other kind of the hormones. Then you have to add the the foetal bovine serum and which is actually, you have to add at the level of 10 percent and then because the mammalian cells are very prone for contamination, you also have to add the antibiotics.

So that so, what you have to add is you have to add the cocktail of penicillin and streptomycin sulphate and that is at the 1 percent. So, if you see the preparation of cell culture media, what you are going to do is for example in this case we have taken the example of D MEM, what you are going to do is you take the DMEM 13.4 grams then mix it to the water and let it be completely dissolved and then you add the 3.7 grams of per litre of sodium bicarbonate, makes it completely and then you adjust the pH with the help of NaOH or HCl , whichever is applicable and you can (adju) you can check the pH with the help of pH paper and finally you can make up the volume to 1 litre and bring it to the final volume ok.

Since the mammalian cell culture media is having the serum and as well as the other biomolecules, such as the amino acids and all those things, you cannot actually autoclave these media. Because if you autoclave, you are actually going to destroy the the amino acids and other heat labile biomolecules and because of that the mammalian cell culture media need to be sterilised by by using a membrane filter which is actually of 0.22 micron filter and once you are going to prepare the sterile media, then you can actually add the

supplements such as the antibiotics and serum and that actually will give you the sterilised mammalian cell culture media in a for for for your for your propagation of mammalian cells.

Preparation of mammalian cell culture media is very very complicated, we have also tried and we have also prepared a small movie to show you and to demonstrate you the different steps of setting up the preparing the media, how to maintain the pH, what are the precautions you have to take and also how to filter this media in a biosafety cabinets. Because most of these mammalian cell culture propagation or manipulation has to be done in a biosafety cabinets so that your sample is going to be protected from the contaminating bacteria.

And all these steps are being demonstrated by one of my students whose name is Mohammad Rafi and it will also going to explain you the precaution as well as the crucial steps what you supposed to do and what are the common mistakes, what people are doing when they are preparing the mammalian cell culture media.

Student presentation video:

In this video we are going to demonstrate how to prepare cell culture media for mammalian cells. For preparing cell culture media, there is a step-by-step process. First we need to weigh the components of the media and dissolve getting required amount of water. Then we need to set the pH using pH strip and then we need to filter the media using 0.22 micron filter to make it accepted. For further use, we can also adequate the media and store it in (())(34:28). So, let us get the video started.

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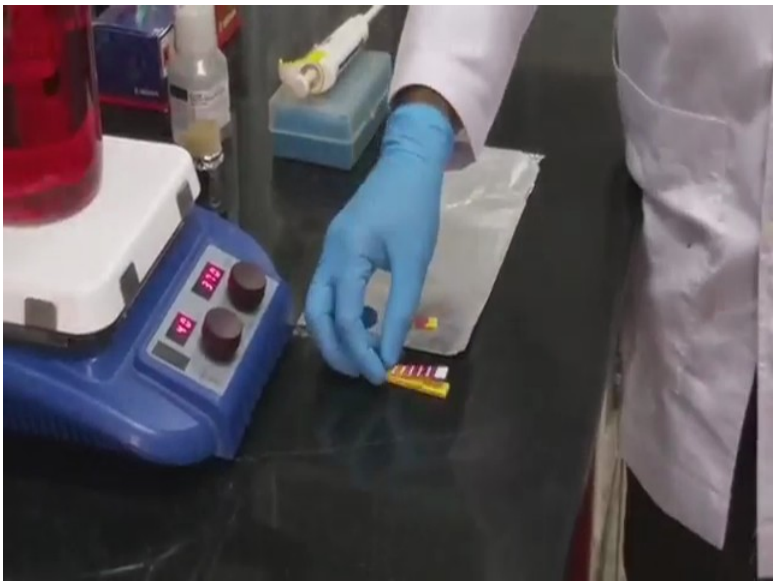
Step 2 : Dissolving Media Components



In this video we will be demonstrating how to prepare mammalian cell culture media. For that purpose we need the cell media, which is DMEM, developers modified (())(31:07) and we need FBS, foetal bovine serum and we need antibiotic cocktail, comprised of streptomycin and penicillin. The vessel media provides inorganic materials, amino acids which are required for basic development of cell and FBS is used for providing both factors to the cell. We cannot autoclave this media because it might degrade the components of the media. For that purpose we use 0.22 micron filters. This is a 250 ML bottle top filter, now I will be demonstrating how to prepare filters for media.

We have to pack it closely so that it does not allow any leakage. And after this we have to put it for autoclave. This is a autoclavable bottle top filter. After when we pack the filter, we have to keep it for autoclaving. For that purpose we use indicator to check whether our filter has

been autoclaved or not. When the lines on this strip turns black, it means that the filter has been autoclaved. In order to prepare media, now we will be adding to the cell media to the already autoclaved double distilled water.

We can use double distilled water or medique water for that purpose. After adding media, we need to stir with magnetic stirrer for the components to dissolve completely. We can either use double distilled water or medique water, but double distilled water is more preferable as it contains more ions than medique water. After the media components have dissolved completely, we need to set the pH of the media. For that purpose either we can use pH metre or pH strips. In this case we cannot use pH metre as the bulb of the pH is sensitive to to the media components and may get corroded.

After the media components are dissolved completely, then we were able to set the pH of the media. After the components of the media have been dissolved completely, we now need to adjust the media, pH of the media. The bright red colour indicates that the pH of the media is in the range of 7.2 to 7.4. If the colour of the media turns purple, then it indicates that that the media is (acidic) basic. If the colour of the media turns yellow, then it indicates that the media has become acidic.

Now we will be checking whether our media falls in the range of 7.2 to 7.4. After the media has been see, we now need to filter the media inside the biosafety cabinet as we have added the constituents in the non-acidic conditions. After the media components have been completely dissolved and pH has been set, we now need to (sterile) sterilise the media using (())(40:09) filter media. For that purpose we use class to biosafety cabinets which are used for handling mammalian cell cultures.

So, this is a typical biosafety cabinet. In which we perform the filtration for media. This is a control panel which (we) which is use to operate this machine, this is on and off switch, this is the switch for normal light, this is the switch for UV light. Now, I will be demonstrating how to filter the media. Now, we are going to filter the media, for that purpose we need a suction pump which can be connected to the bottle top filter. This suction pump is for the purpose of extracting the air from the bottle top filter so that we can filter the media.

Initially we need to check the media, we need to check the bottle top filter with less media, we checked whether if there is any leak or not. For that purpose we are going to add around 50 to 100 ML of media. As we can see that there is no leakage in the filter, we can proceed

with the filtration. After the media has been filtered, we now need to add FBS and antibiotic in order to make it complete media. The complete media comprises of serum, whereas the incomplete media does not contain serum.

We are adding 100 ML of foetal bovine serum in order to make it 10 percent FBS containing serum. With this we have prepared 1 ML of DMEM complete media comprising of 10 percent FBS and 1 percent antibiotic solution. So far we have seen how to prepare cell culture media for mammalian cells, although there are some precautions to be followed, like when we are (use) when we are setting the pH of (me) pH of the media we (needs) we need to use the strips instead of using the pH metre. There are some media that can log onto the bulb of the pH metre and reduce its efficiency.

Secondly when we use the media, we need to thaw the media from 4 degree to 37 degree, but we need to thaw it first to room temperature and then to 37 degree to avoid change in the pH of the media. And also if we are producing the media in larger quantities, we have to aliquot as per our requirements and then use in order to avoid contamination and change in pH of the media.

Professor:

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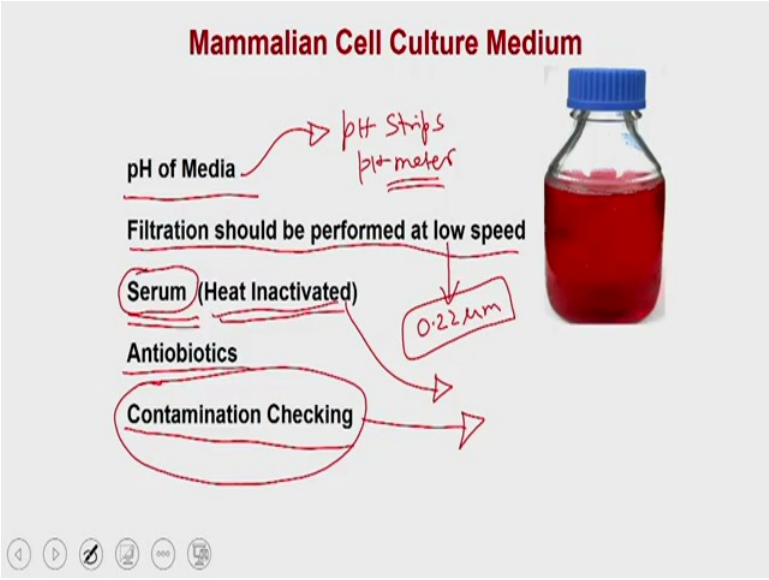

So, what Rafi has discussed is that you have to be very precise in maintaining the pH and once you maintain the pH, you have to wait for some time and always try to use the pH strips for maintaining the pH instead of pH PH metre, because the pH metre has a (tharow) narrow membrane at the base or in the probe and (that's) that membrane is susceptible for getting

clogged with the constituents of the media components. Then you also have to do the filtration as you might have seen that he was filtering the media with the help of a filtration system where he is using the vacuum pump.

So, the filtration should be performed at a very very low speed. And why it is important, because if you do a very high-speed high-speed filtration, you are going to compromise the the pore size or the pores of the membrane what you are using. Because the membrane what we are using is having a pore size of 0.22 micron metre and if you do it at a very high-speed, it actually going to make those pores slightly bigger and because of that some of the bacteria or the contaminating agent also can get passed through. Similarly the serum, the FBS or the foetal bovine serum what you have to use is you should use a heat inactivated serum.

Heat inactivated serum is important because you do not want to add the immunologically active molecules, so that you have to remove the compliments as well as the other (immunal) immuno agents, so that it should not destroy your cells. Because, every organism has some immunity for a particular set of cells. So, if you do not inactivate the serum, because what we need from the serum is only the growth factor as well as the hormone. We do not need the the the antibodies or the compliments from the serum.

And because of that these molecules has to be removed and that you will do by (doing) removing the heat inactivated serum. So, these heat inactivated serums are readily available from the vendors. Also because the mammalian cells are very susceptible for antibiotics, (for) is very (sensit) sensitive for the contaminations, you also have to add the antibiotic cocktail to to control the contaminations. Also when you start preparing the media, because suppose the first time you are preparing this media and you are not trained, then you always should prepare the incomplete media.

And once the incomplete media is prepared, you should check for the contamination. For example, you can take this incomplete media, make it filter sterile and then you keep it in 37 for maybe two days. If there is a contamination, if there is a mistake made by you, then this this bacteria is going to grow in that particular type of media. And because of that the you can be able to know whether the media is sterile or the it is having some kind of contamination which actually comes into the media because there is a mistake.

So, if you make any mistake and that (you) is very important to do in the in the initial stages, the the bacteria which has been added into the media is going to grow in another two days.

So, so what we have discussed, we have discussed about the growth different growth media for the prokaryotic as well as eukaryotic cells. We have also discussed the different steps what we are going to use to prepare the microbiology media as well as the cell culture media. And in the subsequent lecture what we are going to discuss, we are going to discuss how the how to monitor the growth of prokaryotic as well as the eukaryotic cell and how you can be able to count the bacterial cells or how to count the mammalian cells.

Because in many of the downstream biotechnology applications, you need to add the the definite number of cells into the into the reactions or sometimes you need to select the bacteria at a particular phase of its life cycle or particular phase of its growth cycle, so that you will be able to get the better transaction or better transformations. So, with this we would like to conclude our lecture here and in the next lecture we are going to discuss about how to monitor the growth of the (micro) prokaryotic as well as the eukaryotic cells. Thank you.