Waste to energy conversion
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Lecture – 37
Cultivation of Algal Biomass ans Treatment of Waste Water – 1

Good morning. Now we will start discussion on a new module Cultivation of Algal Biomass and Treatment of Waste Water. So far in this subject we have discussed on different types of waste to energy conversion roots, thermal biological and chemical roots. And in transesterification module that is chemical route we have mentioned that micro algae or algal biomass can also be used as a feed stocks for the transesterification process and biodiesel can be produced.

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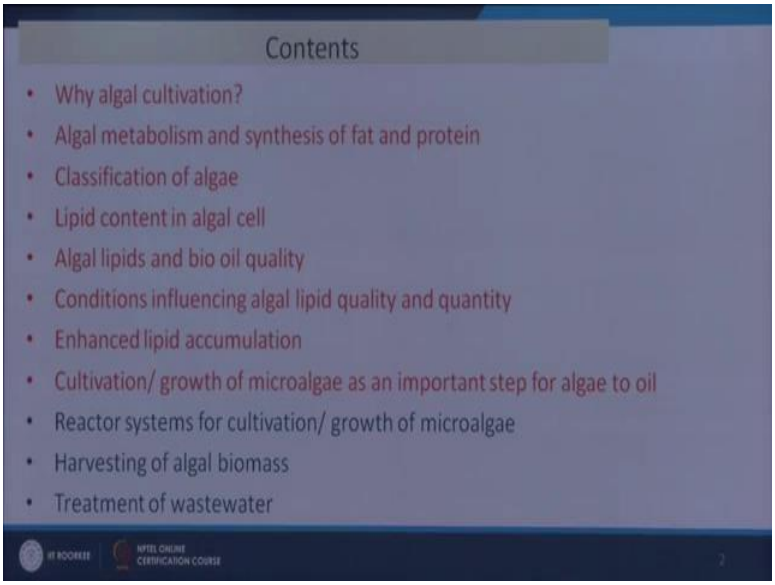

But this feed stock is micro algae or algal biomass. This is quite different from the other feed stocks like say vegetable oils and recycled grease and waste cooking oil. Because we have to produce this algal biomass and during this production, we can also will get an opportunity to treat waste water, but in other waste that is vegetable oils etcetera and cooking oil and recycled greased those are generated as a waste product. So, if we want to use the algal biomass through the transesterification process or any other process for the production of oil or fuels. So, our first step will be to produce algal biomass and the second step will be to convert it into energy.

So, in this module we will discuss the first part the growth of micro algal biomass and it is separation from the water media, that is called harvesting and then in other module we will be concentrating on the upgradation or the production of bio oil from the algal biomass and then it is upgradation to biodiesel. So, in this module the contents are why algal cultivation, algal metabolism and synthesis of fat and protein, classification of algae. Then lipid content in algal cell algal lipids and bio oil quality, then conditions influencing algal liquid quality and quantity, and enhanced lipid accumulation, then cultivation or growth of micro algae as an important step for algae to oil production, and reactor system for cultivation and growth of micro algae harvesting of algal biomass and treatment of waste water.

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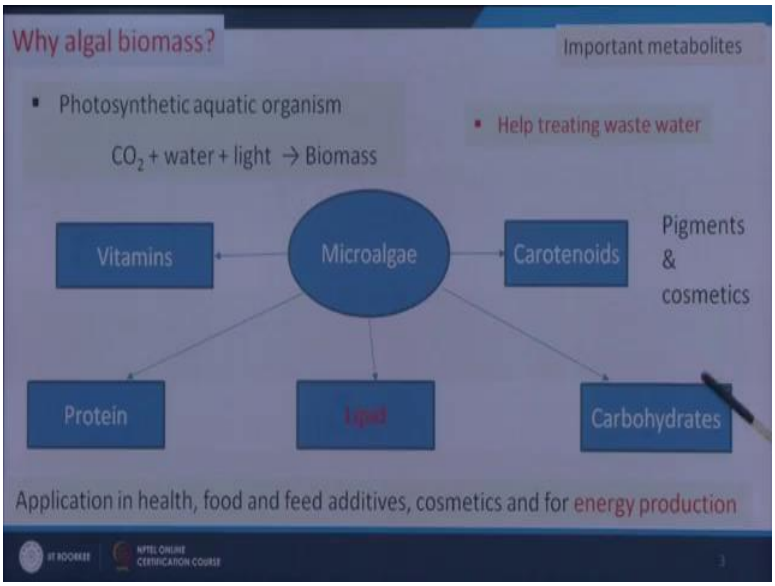

So, at first we will see why the cultivation of algal biomass. As I have discussed that we need to grow the algal biomass first and waste water treatment can be possible through this process. So, algae is aquatic plants like organisms. So, it captures carbon dioxide and in present of sunlight using water it forms the carbohydrates and those carbohydrates to other metabolites and growth of micro algal mass we get and side by side it helps treating waste water. So, it gives us two way benefits: one is waste water treatment and another is production of renewable biomass or energy feed stocks. So, this biomass or algal biomass during metabolite metabolism. So, different types of metabolites are formed like say vitamins it can form protein it can form lipid it can produce carbohydrates and carotenoids. So, this carotenoid are pigments and used in cosmetics.

So, now we are seeing here if we grow micro algal biomass, we can get different metabolites and we can separate these metabolites and we can use for various applications like say food applications for health care sectors or a feed additives and cosmetics and energy production. So, we are interested here with the energy productions, and lipid content present in this micro algae is basically responsible for the upgradation to biodiesel. So, algal bio oil to bio diesel basically the upgradations of the lipids present in it, but if we apply other methods then protein carbohydrates or vitamins etcetera they can also be converted to energy like say thermal or biological routes we can use.

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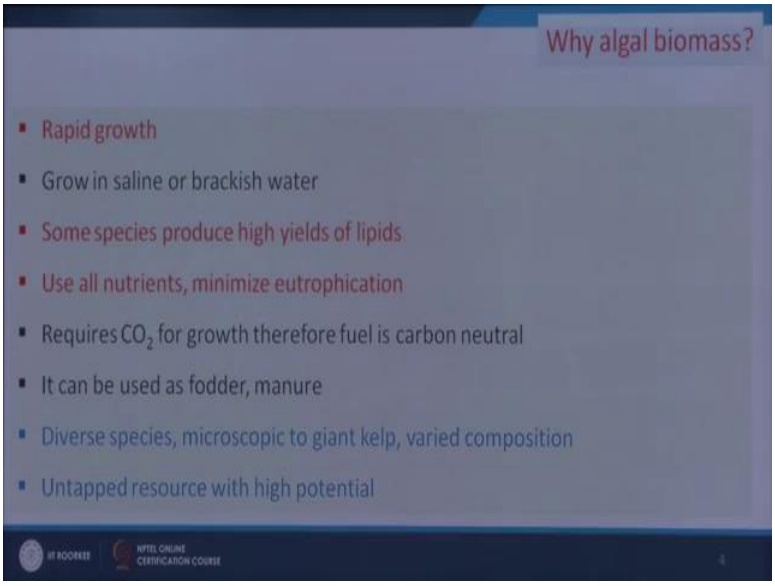

But now we will be seeing some other factors which helps us or which guides us to develop more algae. Because the algal growth is very first. So, rapid growth algae grows very fast. So, due to the growth of this it requires less area for the production of certain amount of algae with comparison to other plants. So, grow in saline and brackish water algae can grow both in sea water as well as in fresh water. And some species produce high yields of lipids. Now you see it can produce different types of metabolites, but these metabolites are not same in content in every species. Some species are there where lipid content is very high.

So, if we can identify some species which is having very high lipid content. Those species will be more suitable for the energy production and use all nutrients and minimize eutrophications that is waste water treatment it helps and requires C O 2 for

growth therefore, fuel rich carbon neutral and it can be used as a fodder and it can be manure and most important is that the algae are a very diverse species very small to giant kelp size and number of species are there which will be having immense potential. As you have discussed different types of metabolites are present and this can be separated effectively and can be used economically, but this resource has not yet been explored well. So, untapped high potential resource this is.

So, we can cultivate it and then we can use it for different applications although we will be concentrating here for the energy production.

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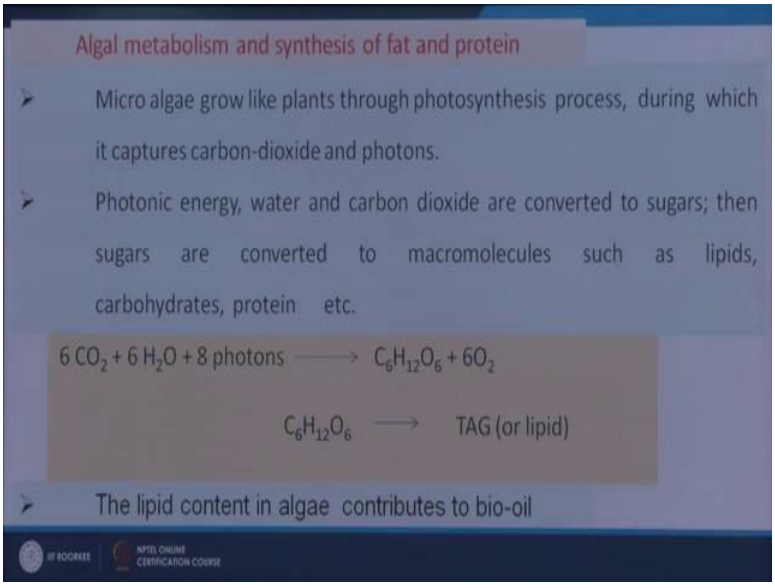

Now, we will see the how the lipid that is the more important component of the algae which is responsible for the biodiesel production through transesterifications after extraction of oil then transesterification. So, if we want to increase the lipid content in the algal biomass, then we have to understand the first it is mechanism or metabolism through which lipid is produced in the algal biomass. So, as we know this microalga are able to perform photosynthesis and produce a carbohydrate in the cell and then this carbohydrate is further converted to different metabolites. So, this is the basic reactions 6 C O 2 plus 6 H 2 O plus 8 protons and then C 6 H 12 O 6 plus 6 O 2 and then C 6 H 12 O 6 is further converted to trialcylglyceride. So, TAG or this is called lipid.

So, now we will see in detail how this lipid is formed a TAG is formed from this C O 2 H 2 and photons.

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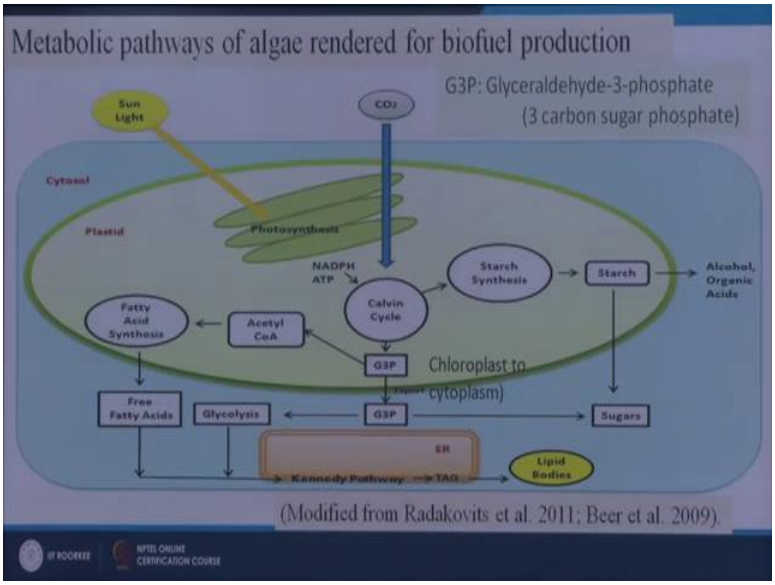

So, a microalga has chlorophylls and the chloroplasts are the center where the photosynthesis takes place. So, in this case in chloroplast, the sunlight comes and photosynthesis takes place, the pigment which is present in the plastids that capture the light and the photons and then produces NADPH and ATP. So, this ATP helps to capture C O 2 and through calvin cycle the starch synthesis takes place and 6 sugar 6 carbon sugar is produced, but part of this 6 carbon sugar is reduced to 3 carbon sugar that is G3P that is called that is glyceraldehyde 3 phosphate. So, glyceraldehyde 3 phosphate is produced in the plastid in presence of sunlight. So, this G3P which is produced here part of this G3Pree G3P is exported to cytoplasm that is cytosol as written here. So, it is coming to cytosols.

And this G3Pree G3P which is coming to cytosols that further goes through glycolysis and glycolysis helps to produce per weight and then per weight to acetyl COA coenzyme and then to fatty acids. So, in cytosols this reaction is going on. Similarly, in plastids also some G3P is converted to acetyl CoA and coenzyme then acetyl coenzyme to free fatty acids. So, fatty acids synthesis is taking place also. So, those free fatty acids are getting out from this cytosol to from plastid to cytosol. Then this free fatty acid which are produced in the cytosol that is going to ER endoplasmic reticulum. I have shown in this

figure. So, which are having these free acids that is coming to ER endoplasmic reticulum and where kennedy pathway is followed to produce triacylglyceride, that is called the lipid.

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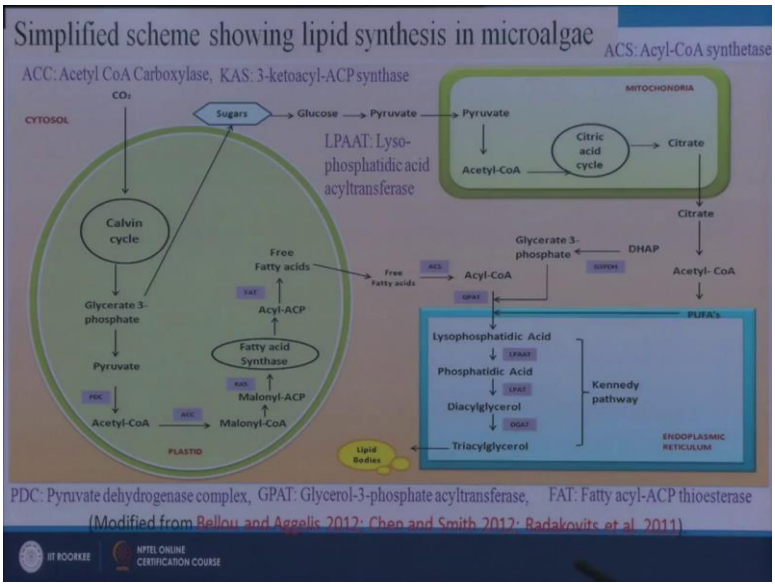

So, for more insight we will see the second figure, in that C O 2 is entering to the plastid and calvin cycle is giving us glycerol 3 phosphate and then glycerol di 3 phosphate is converted to pyruvate and pyruvate to acetyl CoA acetyl CoA. So, acetyl coenzyme is produced by PDC. What is PDC that is pyruvate dehydrogenase complex. So, pyruvate dehydrogenase complex and then acetyl CoA. Then acetyl CoA is further converted to malonyl CoA. So, this malonyl coenzyme is produced by the help of ACC. And ACC acetyl CoA carboxylase. So, this helps for the production of malonyl CoA. So, that is the first step of the acid synthesis. So, this malonyl ACP, COA is processed through malonyl ACP and then fatty acid synthase synthesis and then it is going acyl ACP and free fatty acids, so then free fatty acids are going out.

Now this is one route another sugar which is produced through this calvin cycle that is going from the plastid to cytosol then cytosol. So, it is converted to glucose then pyruvate and it is going to mitochondria and mitochondria again citric acid cycle is there. So, this citric acid cycle is converting to acetyl CoA coenzyme to citrate and cit after citrate formation. It is going out from the mitochondria to agai cytosol and then it is acetyl CoA enzyme is formed. And we get PUFAS poly unsaturated fatty acids poly

unsaturated fatty acids we are getting here. And then all the fatty acids which are produced in this cytosol are getting entry into ER endoplasmic reticulum, when the fatty acids through acyl COA are attached with the glycerol molecules and then it forms triglycerides.

So, now in this air the elongation of the chain of the fatty acids takes place, as well as in this air also some double bond formation takes place unsaturation takes place. So, the extent of unsaturation and extent of elongation of the fatty acid bond will depend upon different factors the presence of different enzymes etcetera. So, this is the overall process for the synthesis of lipid bodies in the algae. So, this lipid come produces in the endoplasmic reticulum and comes into the cytosol. So, as we have come to know about the detailed process of this the production of lipids in the micro algal cells.

So now, we can monitor the production of this lipid bodies by variation in the conditions that is the operating conditions or growing conditions as well as by using some molecular methods. So, if we apply some starvation the conditions that is adverse situations. So, though we have here 2 options you see G3P 3 produced in plastids when it is coming into this your cytoplasm, then cytosol there are 2 routes one pathway is for sugar formation another pathway is for pyruvate formation. So, pyruvate formation helps for the production of fatty acids. So, if the microalgae are grown in adverse situations then this sugar path is reduced and this lipid path is increased. So, that is why the people tried to increase the lipid content by generating starvation situations in the media growth media.

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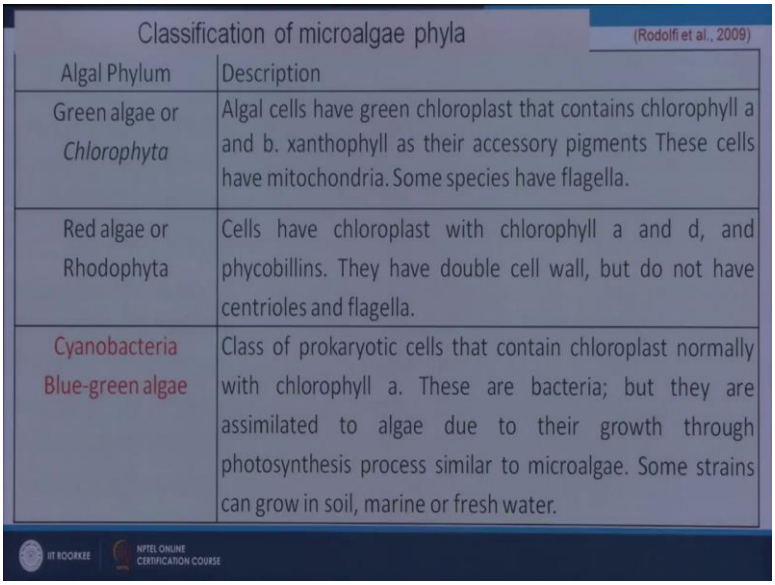

Now, we will see some different types of algae which have been reported and tried for the production of lipid as well as the biodiesel. So, we can classify the algal phylum into different categories like say green algae or chlorophyta. Then this green alga means the green in colour they are having some chlorophylls and the chloroplast basically and b chlorophylls are present in it. And this is xanthophyll as their accessory pigments and due to this xanthophyll the colour green colors appear other is red algae or rhodophyta.

So, rhodophyta is having some other pigments they are the red pigments that is phycobillins the phycobillins are responsible for the red colour and in this case and d chlorophylls are present. The cyanobacteria or blue green algae another type of algae that has been proved to have maximum capacity for lipid production. So, this cyanobacterium is having chlorophyll a these are basically bacteria, but they are assimilated to algae due to their growth through photosynthesis process similar to microalgae.

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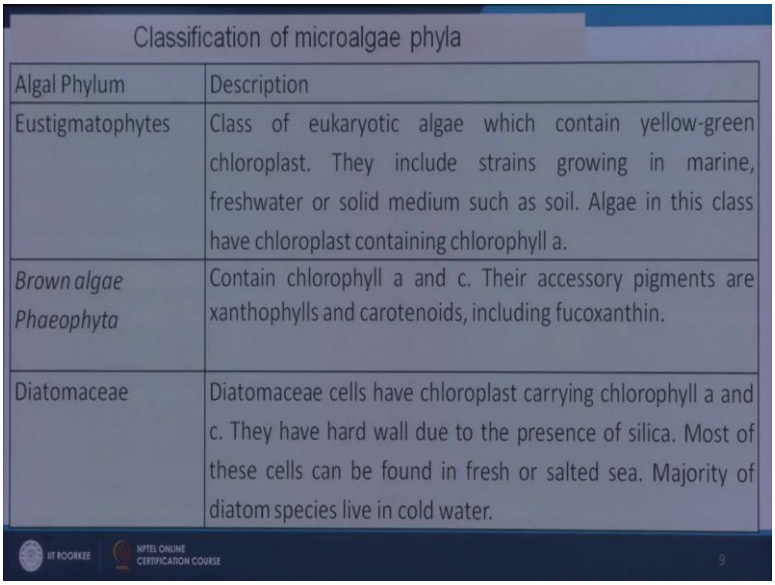

Apart from these three some other types are there like say diatomaceae brown algae and eustigmatophytes. So, these are the descriptions of these different types of microalgae. All this microalga are having different types of chlorophylls different colors. So, in brown algae that is phoeophyta with these contains chlorophyll a and c and their accessory pigments are xanthophylls and carotenoids including fucoxanthin.

So, this is the pigment which is giving the brown colour to the algae. So, diatomaceaes are type of cells algal cells those are having chroplast carrying chlorophyll a and c. So, these are different descriptions of different types of algae, which have been tested for the production of lipid and as mentioned the cyanobacteria are blue green algae having the more capacity to produce lipid.

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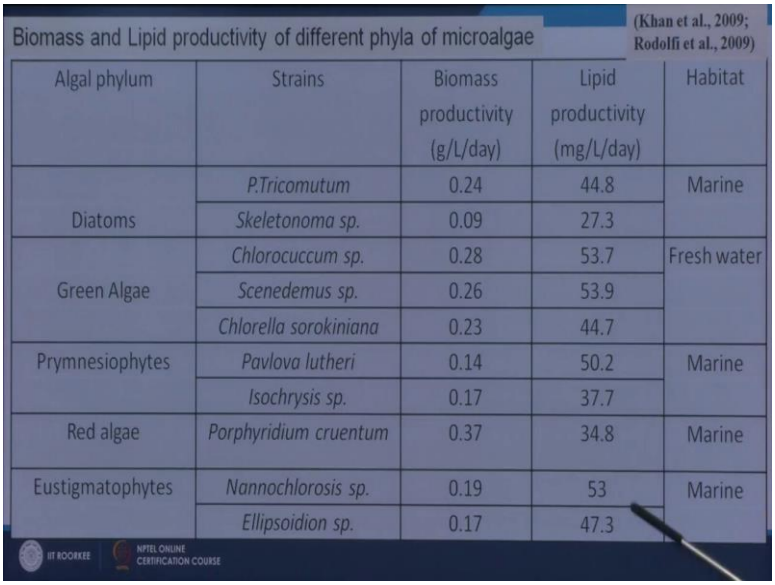

Now, this slide give us some comparison about different algal phylum and their biomass productivity and their lipid productivity. So, diatoms green algae, prymnesiophytes and red algae eustigmatophytes. So, they have has been given different strains and different biomass productivity and different lipid productivity. And all those algal phyla some of these can be grown in marine water some in fresh water. So, this is the advantage that algae can be grown in all type of water and this can be grown in waste water also. So, here the lipid productivity we are getting maximum around 53.9 for green algae.

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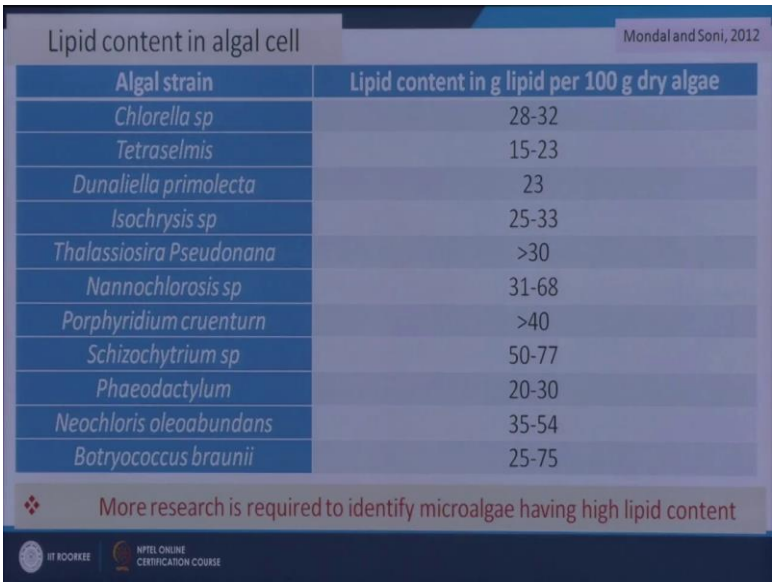

This slide gives us some idea that per unit mass of the algal biomass or unit body weight what is the lipid content. So, different types of algal strains are given here that is chlorella tetraselmis and different others are given here. So, we see somewhere 20 to 32 28 to 32 15 to 23 like these and maximum we are getting say 15 to 77 and 25 to 75. So, these 2 phylum are giving us the maximum lipid content per unit body weight of their of the algal biomass.

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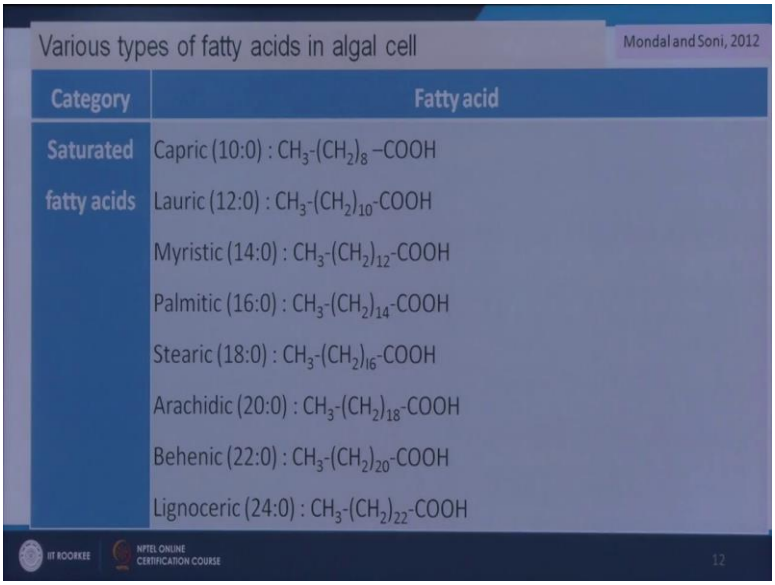

Now, in the lipid of the algal oil or algal biomass, the lipids which we can get that contains different types of fatty acids number of fatty acids are present in it. So, starting from C 10 carbon 10 carbon 12 protein 14 16 18 20 22 24 carbons fatty acids are there some examples has been given here. So, all those fatty acids in this slide are of the saturated there is no unsaturation all are fatty acids saturated fatty acids. So, capric acid C 10 lauric acid C 12 myristic acid 14 palmitic acid C 16 stearic C 18 arachidic C 20 and behenic C 22 and lignoceric C 24. So, now, we will see some unsaturated fatty acids.

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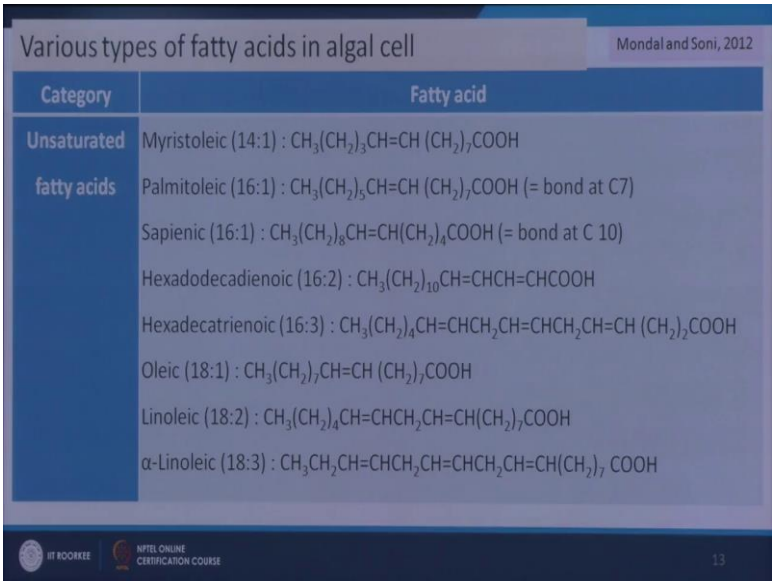

So, unsaturated fatty acids may be having 1 unsaturation may be having 2 unsaturation may be having 3 unsaturation and number of carbons also vary. So, 14 carbon 1 unsaturation 16 carbon one unsaturation as shown here.

But here you see both the cases 16 carbon 1 unsaturations one is, one is palmitoleic acid and another is sapienic acid. So, these 2 acids sapienic acids. So, we are having number one number of unsaturation, but the position of carbon is different in this case at C 7 the double bond is there, but in this case at 10 number of carbon it is having double bond. So, other. So, carbon 16 carbon 2 unsaturations 16 carbon 3 unsaturation. So, hexadodecadienoic acid this is hexadecatrienoic acid and this is a oleic acid 18 1 and linoleic 18 2 unsaturation and then alpha linoleic 18 number of carbon, but 3 unsaturations are shown here other unsaturated fatty acids are also there that is 4 unsaturation 4 double bonds 5 double bonds 6 double bonds. So, all are present.

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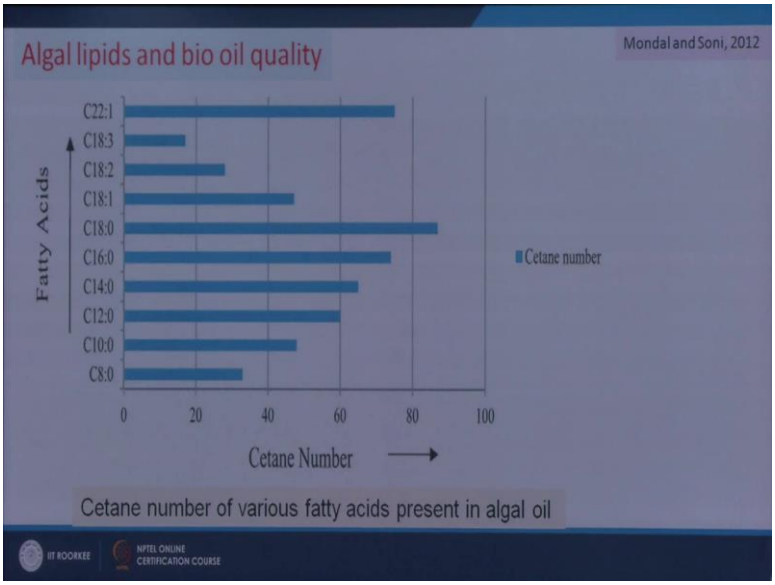

Now, the different types of fatty acids are present in the lipid which is produced in the algae and this lipid the type of lipids will be having different cetane number; that means, variations in the relative concentrations of these fatty acids will give us different quality of the biodiesel. From this slide it is very clear that if we increase the carbon number in the fatty acids cetane number is increasing. So, C 8 C 10 C 12 C 14 C 16 C 18 the cetane number is increased gradually, but when with C 18 if we increase the unsaturation that is 0 1 2 3 then the cetane number is increasing. So, it seems that higher carbon number fatty acids with lesser number of unsaturations will be giving us more cetane number, but you see here we get cetane number more than 18 if C 180 is present there; that means, C 18 fatty acid containing 18 carbon with no unsaturation in that case 80, but as per the standard we should have at least 50. So, this is say 50. So, all these fatty acids are suitable we do not need this C 18 C 0 with unsaturations can also be possible.

Now, this is very clear to us that the relative amount of this different fatty acids will influence the quality. So, how can we control the relative amount of these different type of fatty acids the people have tried on it.

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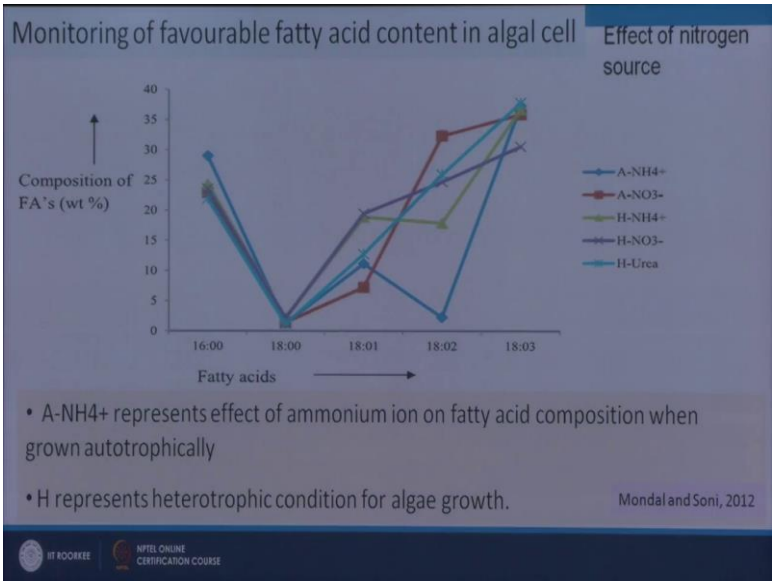

When you see here the variation in the nitrogen source somewhere we have used nitrogen from ammonia somewhere nitrate. So, somewhere urea. So, these are different source and the condition growth conditions algae can be grown in heterotrophic as well as autotrophic condition. So, they can produce energy or they can also consume organic carbon for their growth. So, if they grow using organic carbon as shown in here hydrogen H N O 3 minus H urea H indicates that heterotrophic conditions or algae will use organic carbon for growth and this is a N H means indicates this represent the effect of ammonium on a fatty acid composition when grown autotrophically; that means, they are not using organic carbon.

So, here what we see the C 0 the X carbon 16 carbon continuing fatty acids without any unsaturation we are having maximum amount here for a N H 4, but if we use that is heterotrophic condition the double bond are increasing. So, these 2 cases this one and this one in both the cases we are getting more double exit means 18 if fatty acid containing 18 carbon is produced is available in the in the microalgae then that those are basically unsaturated. And this unsaturation extent increases when the algae is grown in heterotrophic condition. So, that way we can monitor to some extent the relative ratio of different types of saturated and unsaturated fatty acids in the microalgae as a result we can monitor to some extent the quality of the lipid which is produced and the biodiesel which will be produced through this through the up gradation.

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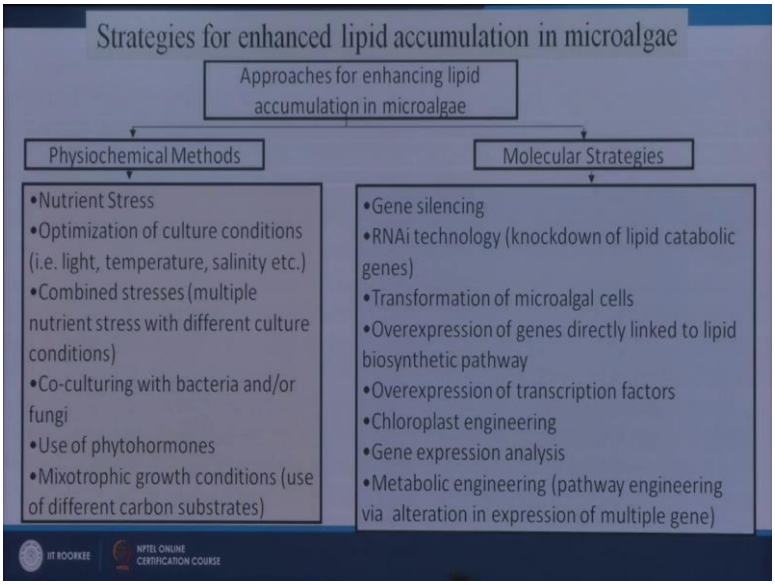

Now, here we will see some strategies for enhanced lipid accumulation for micro algae. As I have discussed that the variations in the growing conditions growth conditions the lipid content can be increased that is one way that is physicochemical methods. So, by physicochemical methods we can increase the lipid content in the micro algal biomass. And molecular strategies are also there through which we can be able to increase the lipid content. So, a physicochemical method includes nutrient stress that is under starvation that I have just discussed.

And then optimization of culture conditions like temperature salinity etcetera and combine stresses multiple nutrient stress with different culture conditions. That also helps for the production of more lipids and co culturing with bacteria and or fungi; that means, there will be some competitions. So, once the competitions arises, the cells prefers to increase the lipid metabolic path use of phytohormones can also help for the getting more lipid and then mixotrophic conditions use of different carbon substrates, that also helps to get more lipid content in the algal biomass. So, these are the physical physicochemical methods now molecular strategies increased in includes many things and as mentioned here basically the gene expressions genetic modification etcetera.

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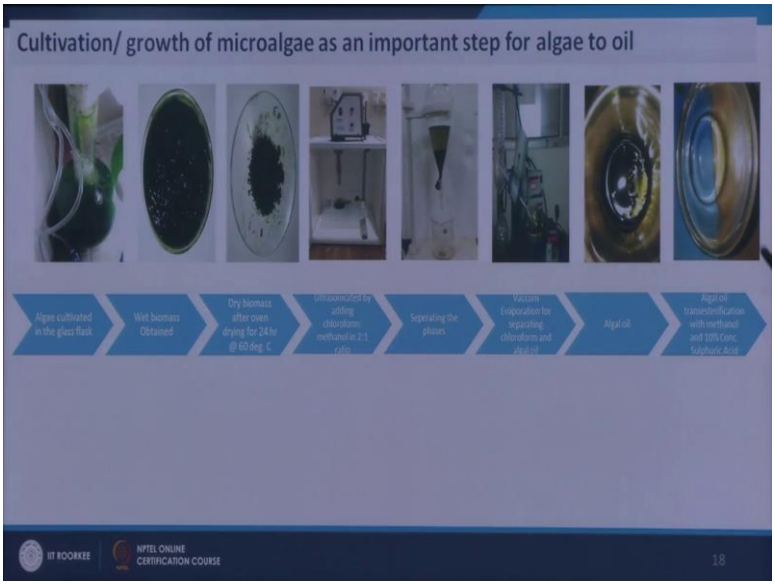

Now, we will see the how the microalgae can be grown how the micro algal biomass can be separated and then how the micro algal biomass can be used to produce algal oil and then how algal oil can be converted to biodiesel. So, this slide us a complete idea where we are growing the micro algal cells, we are growing the micro algal cell here. Then we are separating it we are getting the biomass of it wet biomass then wet biomass to we are getting dry biomass dry biomass to we are going for extraction of oil, then oil is extracted here then separation of the solvent and the algal oil, then algal oil we are getting here.

So, algal oil is converted to biodiesel. So, we are here this module is dedicated to discuss up to this. So, we are here we are growing the microalgae in the media or in waste water. So, for this how can we measure how much biomass is produced in the media.

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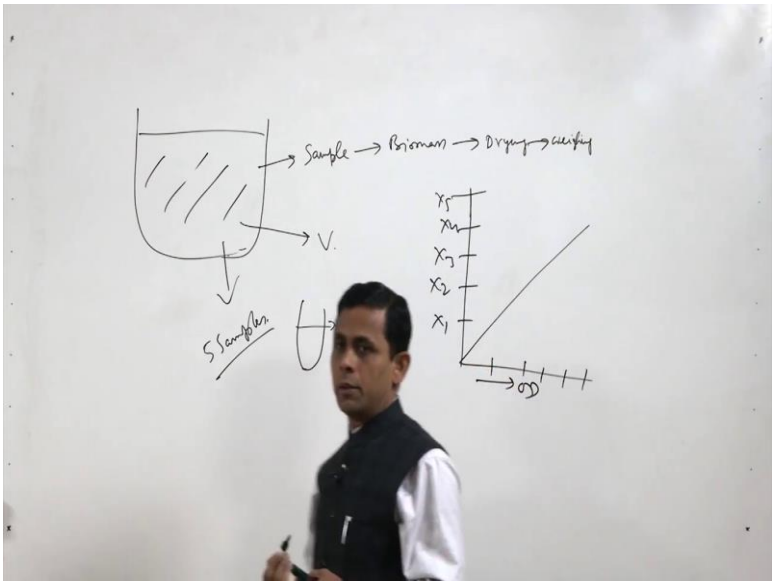

If we have one media. So, we are growing some micro algal mixture here. So, how can you get what is the concentration of microalgae here.

There is may be possible that each time we are getting the sample we are getting the biomass separating the biomass from the water we are drying it, and weighing then how much liquid we have taken how much amount of dry mass, we are getting from that we can get the concentration here. So, each time experimentation is not possible or it is very difficult. That is why a system is calibrated, a system is calibrated first number of exam number of experiments are done to calibrate in that in this case, what happens we take sample from it certain volume say volume v sample we are taking from it then we are using we are using the sample in a spectrophotometer and we are measuring the OD optical density. So, optical density is measured.

So, sample we have taken OD we are measuring in u vs visible spectrophotometer u v is equal to spectrophotometer. So, that OD we have taken say 5 samples here. So, 5 samples we have got 5 OD. And this 5, for 5 samples we have follow the same process sample. Then we separate the biomass from the sample dry it then weigh it then we will get the amount of biomass and volume we have taken we will get the concentration. So, that way we will be adding having some X one will be having X 2 X 3 X 4 X five. So, are we are having. So, by this data we will put in this graph.

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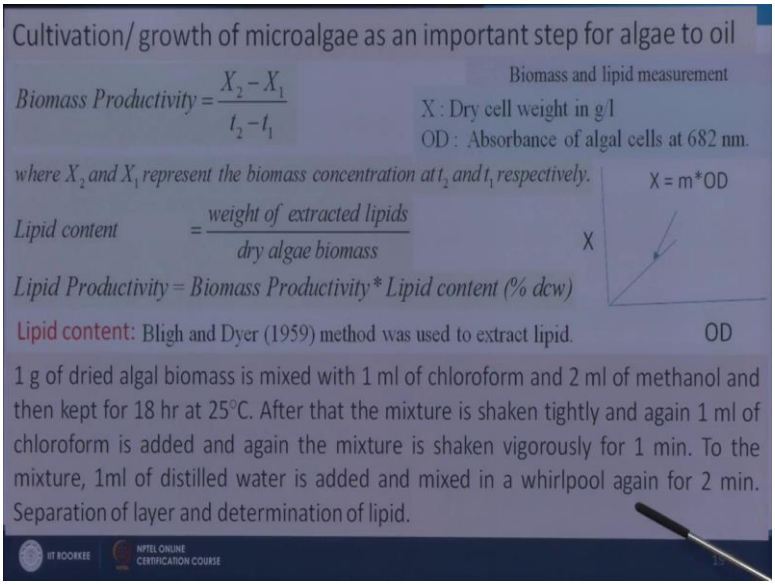

And we will get one relationship here X is equal to m into OD, m into OD, m value we will calculate from this the m value will be known. Now, once m value is known now for any sample for first 5 samples we will be getting the value of m then thereafter for any sample we will take the OD value and we will multiply it with the m and we will get the value of X. So, that way we can get the X content that is the biomass content concentration of biomass in the slurry and then biomass productivity say within time t 1 to 2 we have got X 2 minus X 1 of biomass growth say X 2 minus X 1 by t 2 minus t 1 is the biomass productivity. And lipid content of this biomass, the biomass which we are getting here, the biomass which we are getting here, what will be the lipid content for maximum lipid content has been determined by bligh and dyers method 1959 they have reported this.

See in this case the one gram dried algal biomass is mixed with 1 ml of chloroform and 2 ml of methanol and then kept for 18 hour at 25 degree centigrade. And then it is shaken tightly and again one ml of chloroform is added and again the mixture is shaken vigorously for 1 minute to the mixture one ml distilled water is added and mixed in a whirlpool again for 2 minute then the separation of layers takes place then the lipid amount is measured, the how much lipid is got that is the maximum lipid content and lipid content is equal to weight of extracted lipids by dry algal biomass and the lipid productivity is the biomass productivity into lipid content.

So, that way when we are growing a micro algal biomass a species of microalgae what will be the biomass productivity what is the lipid productivity and lipid content we can determine. So, up to this in this part of this module and we will discuss the rest part in the next part of this module.

Thank you very much.