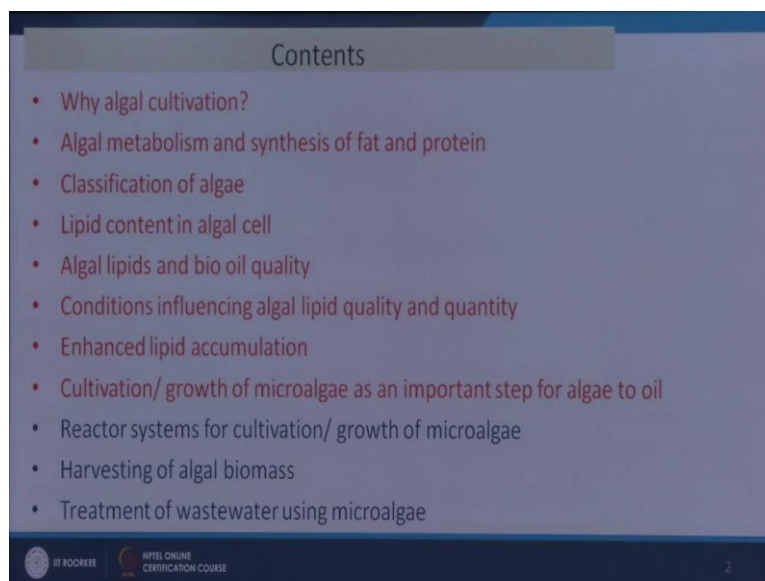


Waste to energy conversion
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Lecture – 38
Cultivation of Algal Biomass and Treatment of Waste Water – 2

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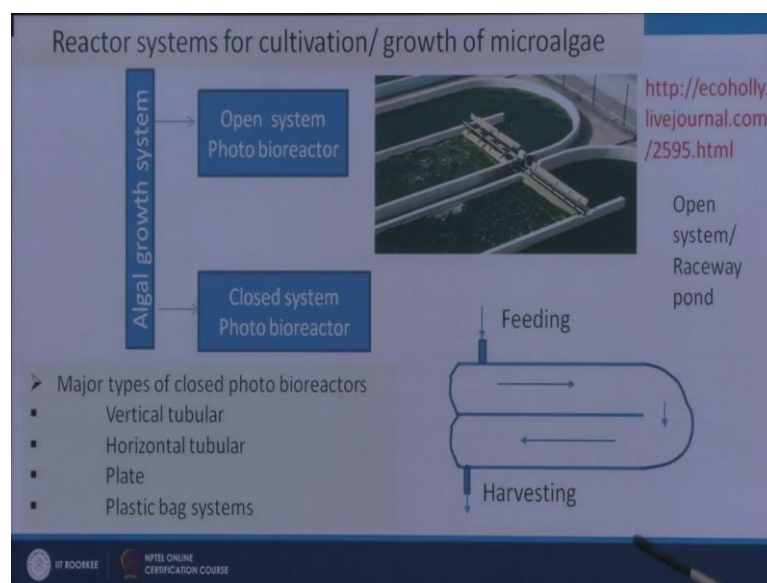


Contents
• Why algal cultivation?
• Algal metabolism and synthesis of fat and protein
• Classification of algae
• Lipid content in algal cell
• Algal lipids and bio oil quality
• Conditions influencing algal lipid quality and quantity
• Enhanced lipid accumulation
• Cultivation/ growth of microalgae as an important step for algae to oil
• Reactor systems for cultivation/ growth of microalgae
• Harvesting of algal biomass
• Treatment of wastewater using microalgae

Hi friends, now we will start discussion on the second part of the module cultivation of algal biomass and treatment of wastewater. In the first part of this module, we have discussed on different types of algal species that can be used for the oil production, and how the lipid content can be increased etcetera.

Now, in this module, we will concentrate on how to grow the microalgae biomass then what will be the reactor systems for cultivation and growing of the microalgae, and what will be the harvesting process how can you harvest the separate the algal biomass from the media, and then treatment of waste water using microalgae.

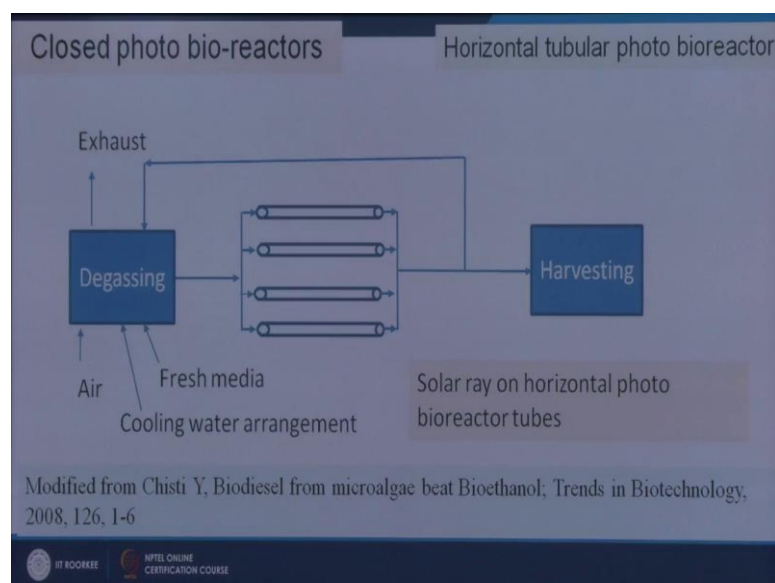
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So, at first we will see the different reactor systems, which can be used for the production and growth of the microalgae biomass. So, the most conventional one is the open bond we can use some microalgae seeds and microalgae open in open pond and those will be growing and then we can collect it as shown here. So, here this is the very open pond systems that is open the just like a pond and that is microalgae is grown here. And another is that is closed photo bioreactor closed systems. So, this is called photo bioreactor, open pond open, and closed systems, photo sunlight is required, biological that is microalgae is required for the conversion of carbohydrates in this reactor and lipids, proteins etcetera in the algae.

So, there are two open systems and closed system photo bioreactor. This is one example is open system. Here the feed gets entry, this is open to atmosphere, open system means open to atmosphere. So, feed is getting entry, the water waste water is coming here. So, this is a pond, and in this pond, it is microalgae is growing. So, after certain time, we are getting out the microalgae. So, we are separating the biomass, and then it is recycled again, this will go to this and will be coming with the feed. So, this is the flow sheet for the open system photo bioreactor, but closed system photo bioreactor this will be closed and the base we can we can use different vessels, tubes may be or columns may be or plat plate may be. So, plate, horizontal tube, vertical tubular, so all those types of photo bioreactors have been used under closed category closed system category. So, plastic bags have also been used in some cases.

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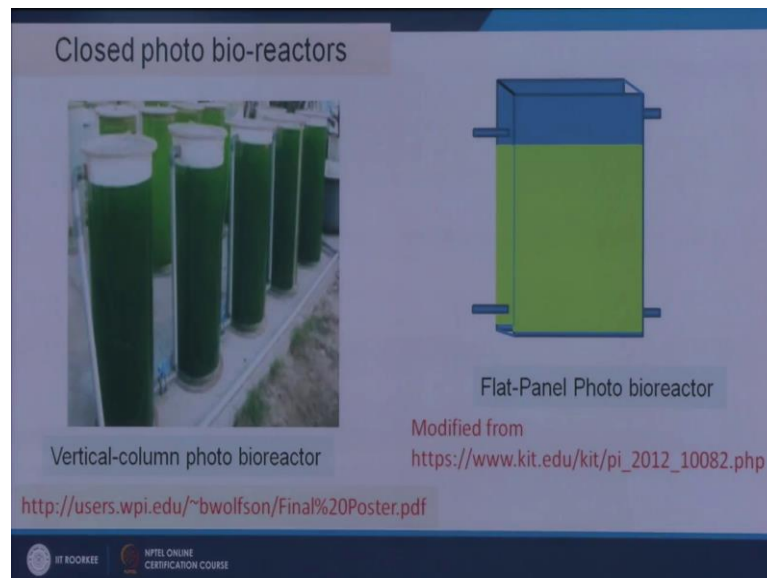


We will see here the closed system photo bioreactor that is horizontal tubes. So, this is our media, we are taking in case of waste water say, this is our waste water and we add some growth nutrients for the microalgae, and this media is pumped through this tubes. So, these are our photo bioreactors. So, these are closed, and there is no direct connection with the environment. Sunlight is falling on this, and they will the sunlight will raise the temperature of this tubes as well as the inside water or the media which is passing through it. After that when it will coming out from the system, after certain time and sufficient growth when we will get, then some part of this biomass will be separated from the water stream and that is called harvesting. So, harvesting will takes place and the rest water some part with microalgae will be recycled to this your media.

But what is happening in these case when we are using sunlight, there are some photosynthesis reactions. So, oxygen will be produced. So, once oxygen will be produced, so that oxygen has to be removed that is why degassing is needed here in case of closed system degassing is required. So, air is sent and exhaust it is going out oxygen is going out, oxygen is not desirable because photosynthesis required CO₂. So, and cooling and heating can also be required depending upon the atmospheric condition because during say summer season, say temperature is around say 40, 45 degree centigrade. So, here the water will also be around that 40 degree centigrade that has to be the temperature has to be reduced. But if it is a cold country or very cold situation, so at that time heating may also be required. So, this is the flow sheet or the concept of the

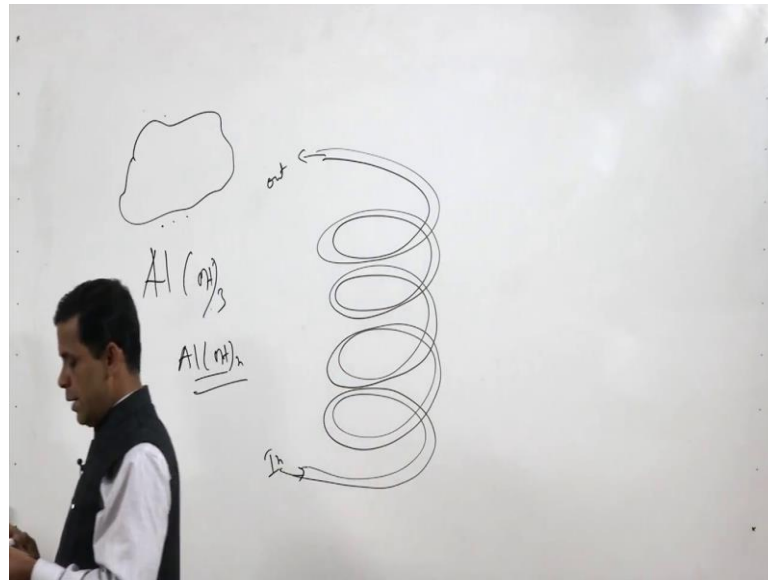
horizontal tubular photo bioreactor. And here we get solar ray on horizontal photo bioreactors. You see here these are the tubes; this can have some spiral shapes also.

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And the column photo bioreactor, this is one vertical column photo bioreactor, these are number of columns. So, from one side, the feed is getting entry it is going out from the paschal on the top again it is getting entry the bottom and from the top, so that way it is going through one after another column. So, it is getting sufficient time for the growth of the microalgae mass; and after certain times, we will collect from one end of this, and we will we will separate the microalgae biomass from it, and we will recycle the water. So, this is the operation of the closed photo bioreactors. Here the closed photo bioreactors may be of helical types also, the spiral types also.

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This type of systems are also available. So, in this in-out, so in yours spiral, but it is in vertical positions, so that way also some arrangements have been tested, the people have tested on this. So, two types of one is a vertical column and vertical spiral vertical column photo bioreactor. And flat panel photo bioreactor is also another example people have tried with these, this is a flat panels when the media escaped, and algae is grown after certain time is separated and harvesting takes place.

Now, we if we compare the open and closed system photo bioreactors then we will see that are very different in nature. See, if we fix some parameters say important parameters for comparison are say required space, space requirement that is very, very important weather water loss, carbon dioxide loss, oxygen concentration and then temperature whether there is any shear.

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Comparison of open and closed photo bioreactor		
Parameter(s)	Open ponds and raceways	Closed Photo bioreactors (PBR)
Required space	High	For PBR itself low
Water loss	Very high, may also cause salt precipitation	Low
CO ₂ loss	High, depends on pond depth	Low
Oxygen concentration	Usually low enough because of continuous spontaneous outgas.	Requires gas exchange devices (O ₂ must be removed to prevent inhibition of photosynthesis or photo oxidative damage)
Temperature	Highly variable, some control possible by pond depth	Cooling often required (immersing tubes in cooling baths)
Shear	Low (gentle mixing)	High (fast and turbulent flows required for good mixing, pumping through gas exchange devices)

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Comparison of open and closed photo bioreactor		
Parameter(s)	Open ponds and raceways	Closed Photo bioreactors (PBR)
Cleaning	No issue	Required (wall-growth and dirt reduce light intensity)
Contamination Risk	High (limiting the number of species that can be grown)	Low
Biomass quality	Variable	Reproducible
Biomass conc.	Low, between 0.1 and 0.5 g/l	High, between 2 and 8 g/l
Production flexibility	Only few species possible, difficult to switch	High, switching possible
Process control and reproducibility	Limited (flow speed, mixing etc.)	Possible within certain tolerances

So, shear and then cleaning and then contamination risk whether any risk of contamination biomass quality and biomass concentration. And then we will see production flexibility, process control and reproducibility. So, if we think about these parameters, then we can get some clear distinction between these two types. All these types have some advantage and disadvantage and those will be discussed now. So, obviously, the space requirement is very for open ponds, but for this photo bioreactor continuous photo bioreactors or closed photo bioreactors this is low. Then water loss is

also very high in case open pond during rainy seasons it will be vaporized. So, water loss will be. Carbon dioxide loss it will also be high in case of open pond.

So, oxygen concentration, in case of closed photo bioreactor we need to degas it, but here we do not need to degas for oxygen separation. And the temperature is highly variable some control possible by pond depth that the temperature control is not very easily attained here. But here cooling often required, we need to cool and we can maintain the temperature and see here and then we are coming to cleaning. Cleaning requirement is also higher in case of closed photo bioreactor, because open bond we do not need to clean the system, but closed system requires cleaning. And contamination risk is also low in case of closed photo bioreactor which is high in case of open photo bioreactor.

Biomass quality; obviously, we will be getting high quality biomass in case of closed biomass photo bioreactor with more control parameter, but here open ponds will not be having that much of quality and most important that that may not be repeatable also. One time we may get certain biomass, the same conditions we may put or try to put and we not get the same amount of biomass. And biomass concentration obviously, the open ponds will be having less that is 0.1 to 2.5 gram per liter, but if it is a closed system then we can get say 2 to 8 gram per liter. So, these are the difference between the open ponds and closed ponds systems; and both the systems are having some difficulty when people are investigating further to improve the performance of these processes.

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Comparison of various types of closed photo bioreactor			
Characteristic	Tubular PBR	Column PBR	Flat panel PBR
Biomass yield	↑↑↑	↑	↑↑
Exposed surface area for light illumination	↑↑↑	↑	↑↑
Manufacturing cost	↑	↑↑	↑↑↑
Problem due to O ₂ & CO ₂ accumulation	↑↑↑	↑	↑↑
Scale-up possibility	↑↑↑	↑↑	↑
Maintenance cost	↑↑	↑	↑

↑ - Low, ↑↑ - Medium, ↑↑↑ - High

Mondal and Soni 2012

Now, we will compare different types of closed system photo bioreactor that is tubular column flat panel. So, this three types if you compare and basically the biomass yield if we expose surface area for light illumination that is very important because that will be directly related with the cost of the process and so cost manufacturing cost. And then we are getting problem due to O₂ and CO₂ accumulation, then scale up possibility and maintenance cost. So, these are the different parameters or characteristics if we consider.

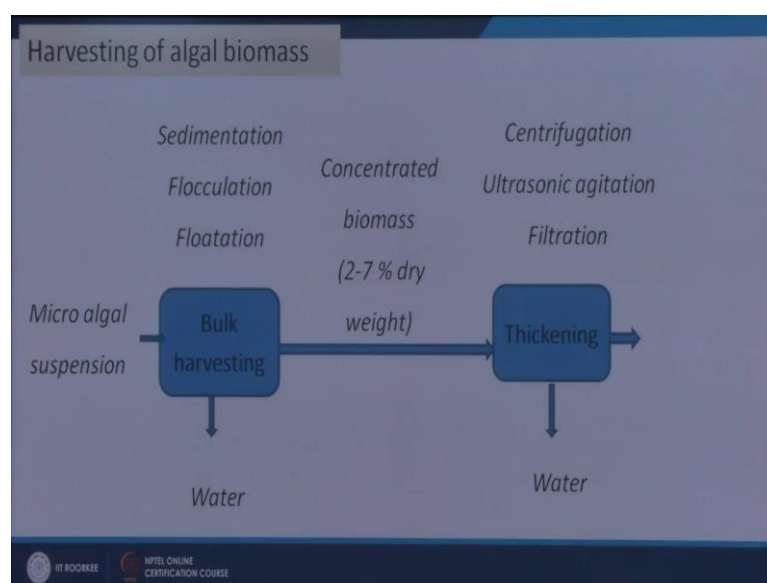
Then we see the tubular is maximum biomass yield, maximum exposed area for sunlight illumination, manufacturing cost is low and problem due to O₂ and CO₂ is also very high because it is tabulate is closed. And n this case, it is having the maximum the o two and c o two accumulation and scale up possibilities is also high in this case tubular one and maintenance cost is also medium, but it is more than other column and flat panel. So, all those three types has their own characteristics and all are tested, but which one is the best one so far it is not yet understood more investigations are going on this.

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Dimension of various types of photo bioreactor (research scale) Soni, 2012	
Photo bioreactor type	Typical dimension
Open Pond	Depth: 1-15 cm
Horizontal tubular photo bioreactor	Tube internal dia. : 2.5 cm
Vertical Column photo bioreactor	Column internal dia. : 2.6 cm
Flat Panel photo bioreactor	Width: 3.2 cm

Now, this slide gives us some dimensions for research scale investigations or open pond horizontal tubular photo bioreactor, vertical column photo bioreactor, and flat panel photo bioreactor. So, these are the typical dimensions. So, is the people have considered. So, that is depth 1 to 15 centimeter for open pond for horizontal tubular photo bioreactor around 2.5 centimeter diameter, and this vertical column photo bioreactor 2.6 centimeter, and flat panel that is 3.2 centimeter.

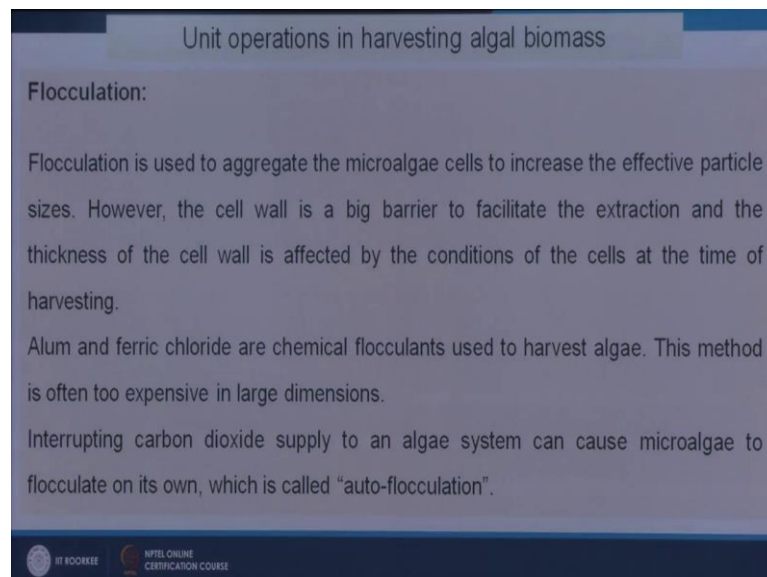
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Now, we are coming to harvesting part. The photo bioreactors will help to produce the algal biomass and then harvesting is coming and important for the separation of the algal biomass from the media. So, there are two steps; the first step, in the first step that is called bulk harvesting our target is to get more biomass along with water. So, here 2 to 7 percent dry weight we are getting. Once we are getting the 2 to 7 percent dry weight biomass, further we will go for filtration. So, we will remove all the water present in it and this will be water will goes up and will get dry biomass actually.

So, this is the flow sheet for the harvesting, and two steps methods this is and first is bulk harvesting and then followed by thickening. And this bulk harvesting, there are different techniques the people have tested that is sedimentation, flocculation and floatation; and for thickening centrifugation, ultrasonic agitations and filtrations. So, these are the different techniques have been reported in literature for the harvesting of algal biomass, but still we will see now all the processes having their own limitations and advantage and some characteristics and requires further investigation and improvement.

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Unit operations in harvesting algal biomass

Flocculation:

Flocculation is used to aggregate the microalgae cells to increase the effective particle sizes. However, the cell wall is a big barrier to facilitate the extraction and the thickness of the cell wall is affected by the conditions of the cells at the time of harvesting.

Alum and ferric chloride are chemical flocculants used to harvest algae. This method is often too expensive in large dimensions.

Interrupting carbon dioxide supply to an algae system can cause microalgae to flocculate on its own, which is called "auto-flocculation".

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Now, we will see what is flocculation and how it is works. The flocculation is used to aggregate microalgae cells which are dispersed in the media, and then this flocculation helps to increase the effective particle size. However, the difficulties that for engaging effective particle size, we need some flocculants; and these flocculants alters the nature

of the cell walls so that is the main drawback of this process. And the wall is affected by the conditions of the cells at the time of harvesting.

The normally alum and ferric chloride are added as the flocculants, and this method is often too expensive also. Apart from these two, auto flocculation is also possible particularly when metals are present. And we are disrupting carbon dioxide supply, then the pH of the media changes and the metals are positively gets positively charged and they capture the negatively charged algae biomass, so that is called auto flocculation.

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Mechanism of flocculation

Flocculation is a process wherein colloids come out of suspension in the form of floc or flake; either spontaneously or due to the addition of a clarifying agent.

Perikinetic flocculation refers to flocculation (contact or collisions of colloidal particles) due to Brownian motion of colloidal particles. Orthokinetic flocculation refers to contacts or collisions of colloidal particles resulting from bulk fluid motion, such as stirring.

Flocculants promote flocculation by causing colloids and other suspended particles in liquids to aggregate, forming a floc.

Many flocculants are multivalent cations such as aluminium, iron, calcium or magnesium. These positively charged molecules interact with negatively charged particles and molecules to reduce the barriers to aggregation. In addition, many of these chemicals, under appropriate pH and other conditions such as temperature and salinity, react with water to form insoluble hydroxides which, upon precipitating, link together to form long chains or meshes, physically trapping small particles into the larger floc.

Long-chain polymers such as modified polyacrylamides also act as flocculants

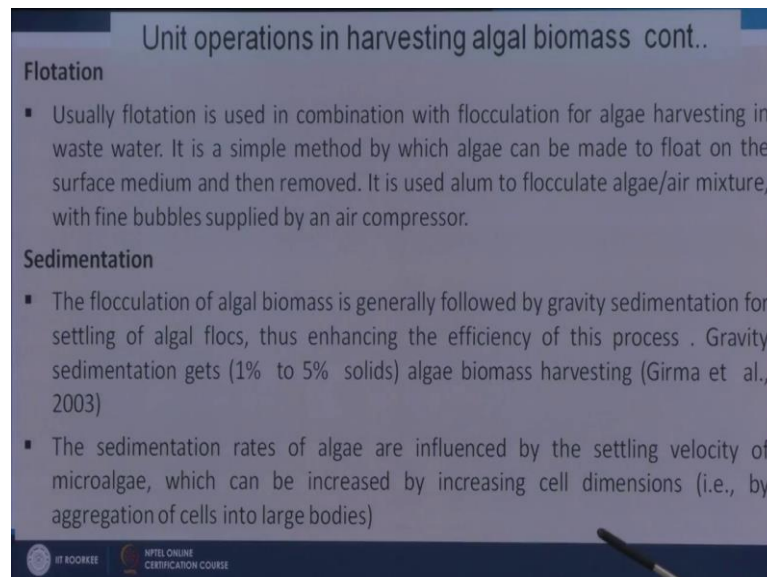
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Now, we will some mechanistic path of the flocculation. So, flocculation is the process as I have discussed that helps to reach the colloidal particles they the particles come closer and mix floc or flake and then either spontaneously or due to addition of clarifying agent. So, we may add some clarifying agent or we may need to add some clarifying agents. So, there are two types of flocculation that is one is perikinetic, another is orthokinetic. So, perikinetic flocculations is refers to the flocculations due to Brownian motion of the colloidal particles. The colloidal particles will be moving and will be colliding each other and then they will be aggregation will take place, and orthokinetic means some slight agitation is required. So, this apart from this we need to add some chemicals in some cases those are called flocculations. So, this is practically used for the separation of the algal biomass.

And what happens alum, iron or calcium or magnesium salt, if we use depending upon the pH, this salts forms hydroxides. So, aluminum hydroxide Al OH_3 , it may be Al OH_n a number of gigantic molecules is formed. So, bigger agglomeration is formed here of this aluminum salt means hydroxide. So, this aluminum hydroxides captures, if there are small particles here, so though captures it. So, this is the mechanism of the flocculation. So, apart from this, some organic molecules are also added, and those organic molecules like say modified polyacrylamides, they are also having a bigger size and they also captures the small particles and separates it. So, this is the mechanism for the separations of the flocculation.

Now, flocculations and sedimentation these are two also important process for the harvesting of algal biomass. So, what will be happening when we will add some flocculants then small algal cells will be coming together very close and will create agglomeration and bigger size particles. Now, if we pass some air in the media then this particles may be in floating form and this will be coming at the top of the media and that can be separated, so that is called floatation and sometimes if the size of the particles after agglomerations is bigger. So, those can be settled also.

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Unit operations in harvesting algal biomass cont..

Flotation

- Usually flotation is used in combination with flocculation for algae harvesting in waste water. It is a simple method by which algae can be made to float on the surface medium and then removed. It is used alum to flocculate algae/air mixture, with fine bubbles supplied by an air compressor.

Sedimentation

- The flocculation of algal biomass is generally followed by gravity sedimentation for settling of algal flocs, thus enhancing the efficiency of this process. Gravity sedimentation gets (1% to 5% solids) algae biomass harvesting (Girma et al., 2003)
- The sedimentation rates of algae are influenced by the settling velocity of microalgae, which can be increased by increasing cell dimensions (i.e., by aggregation of cells into large bodies)

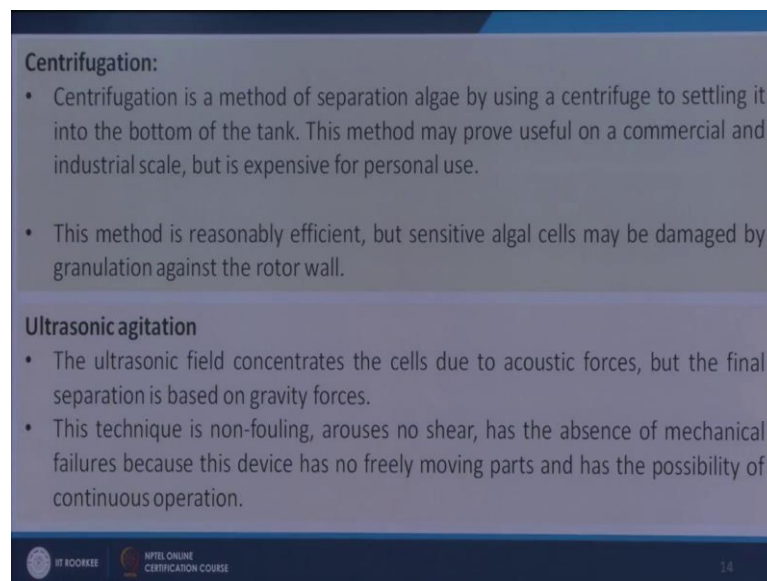
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See in that can we will we will get the sedimentation. So, sedimentations enhances the efficiency of the process, but this through this gravity sedimentation we can get 1 to 5 percents of the solids in the settled part of the settled biomass and the sedimentation rate

depends upon the size of the particles which are generated after the agglomeration. Now, we will see the centrifugation and ultrasonic agitation.

As I have just discussed that sedimentation rate depends upon the size of the microalgae agglomeration. Now, if the microalgae cells are very small, the agglomerated particle size is not big, the sedimentation is not able to settle it; at that time centrifuge will work. Because in centrifuge the acceleration which is generated that is more than 1000 times than the g values or the gravitational acceleration, so that is why smaller particles can also be separated using centrifuge. But this method is very effective, the efficiency is high, but it has some disadvantage, it requires higher energy as well as when the microalgae is separated if high rpm is used then those microalgae cells may be damaged. So, these are the disadvantage of the centrifugations.

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Centrifugation:

- Centrifugation is a method of separation algae by using a centrifuge to settling it into the bottom of the tank. This method may prove useful on a commercial and industrial scale, but is expensive for personal use.
- This method is reasonably efficient, but sensitive algal cells may be damaged by granulation against the rotor wall.

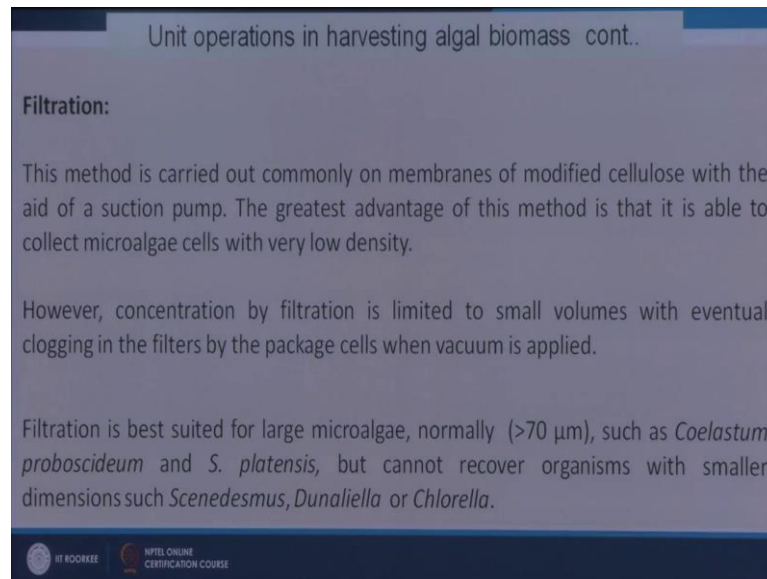
Ultrasonic agitation

- The ultrasonic field concentrates the cells due to acoustic forces, but the final separation is based on gravity forces.
- This technique is non-fouling, arouses no shear, has the absence of mechanical failures because this device has no freely moving parts and has the possibility of continuous operation.

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And ultrasonic agitations nowadays some researchers have reported that ultrasonic field if we apply in the growing media then microalgae will be agglomerated, it will be under the acoustic forces. And this technique when the particles are agglomerated and those will be separated through gravitation gravity sedimentation. So, this is the mechanism through the ultrasonic agitations the microalgae cells can be separated from the media.

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The slide is titled "Unit operations in harvesting algal biomass cont..". It discusses the process of Filtration. The text states that this method is commonly used on modified cellulose membranes with a suction pump, allowing for the collection of microalgae cells with very low density. However, it notes a limitation: concentration by filtration is restricted to small volumes because filters eventually clog with cells when vacuum is applied. It also specifies that filtration is most effective for large microalgae, typically greater than 70 micrometers, such as *Coelastum proboscideum* and *S. platensis*, and is not suitable for smaller organisms like *Scenedesmus*, *Dunaliella*, or *Chlorella*. The slide footer includes the IIT ROORKEE logo and the text "NPTEL ONLINE CERTIFICATION COURSE".

Unit operations in harvesting algal biomass cont..

Filtration:

This method is carried out commonly on membranes of modified cellulose with the aid of a suction pump. The greatest advantage of this method is that it is able to collect microalgae cells with very low density.

However, concentration by filtration is limited to small volumes with eventual clogging in the filters by the package cells when vacuum is applied.

Filtration is best suited for large microalgae, normally ($>70\text{ }\mu\text{m}$), such as *Coelastum proboscideum* and *S. platensis*, but cannot recover organisms with smaller dimensions such *Scenedesmus*, *Dunaliella* or *Chlorella*.

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Next is filtration another method which is used for the separation is filtrations. This methods uses some membranes of modified cellulose. And the advantage of this process is this that this method that is able to collect microalgae cells with very low density. But what is the disadvantage, this membranes can be clogged and for a longer time, this may not be able to operate. So, the limitation is that the small volumes of the media, they can handle. And the filtrations is best suited for large microalgae normally greater than 70 micrometer of the smaller microalgae may be passing through the pores or of the membrane.

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Microalgae species	Method	Effectiveness	Condition
<i>Spirulina Platensis</i>	Vacuum Filtration	-	Vacuum filtration equipped with regenerated cellulose membrane with a pore size of 0.45µm
<i>Stephanodiscus hantzschii</i> , <i>S. astraea</i> and <i>Cyclotella Sp</i>	Tangential flow filtration	70-89% recovery	Cross filtration with a membrane pore size of 0.45 µm is used
<i>Tetraselmis</i> spp. And <i>Chaetoceros calcitrans</i>	Centrifugation	Greater than 95% recovery	-
<i>Chlorella + Oocytis</i>	Centrifugation	90% recovery	-

Now, we will see some techniques the people have used for the harvesting of microalgae. So, here vacuum filtration, tangential flow filtrations, centrifugations and centrifugations have been reported and where we see the vacuum filtrations and tangential flow filtrations the membrane pore size was used as 0.45 micrometer and 70 to 89 percent recovery of the biomass was possible. And by this process, centrifugations around 90 percent to 95 percent recovery was possible.

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Microalgae sp.	Method	Effectiveness	Condition
<i>B. braunii</i>	Dispersed flotation	air 93.6% recovery	-
<i>Chlorella minutissima</i>	Flocculation followed by sedimentation	60% Recovery by efficiency	1g/L of $Al_2(SO_4)_3$ and $ZnCl_2$ and took 1.5 h and 6 h, respectively
<i>Dunaliella salina</i>	Micro bubble flotation	99.2% recovery	Using microbubble generation at a pH of 5 with the aid of ferric chloride coagulant (150 mg/L)

Here dispersed flotation flocculation followed sedimentation and micro flotation the information are provided here. Here which is 93.6 percent in case of dispersed flotation and micro bubbles it is 99.2 percent biomass recovery. And flocculation it is only giving us 60 percent recovery. And the flocculants added here is aluminum sulfate and zinc chloride and in this micro flotation, the pH was maintained 5. In ferric chloride coagulant was added also in this case. Now, other some examples gravity sedimentations and flocculations, gravity sedimentations was used that was able to 60 percent biomass recovery; and flocculations 85 to 90 percent biomass recovery. Then here 25 milli mole per liter aluminum sulfate was used for this another flocculants was also used that is sodium hydroxide at the pH of 11 to 12. So, these are some comparison and some information on some process or the harvesting of algal biomass from the media.

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Algae Harvest Method	Relative Cost	Yield TSS %	Energy Usage kWh/m ³	Remarks
Gravity sedimentation	Low	1.5	0.1	Not very efficient for rapid biomass recovery from high-rate algal ponds.
Inorganic chemical flocculation	High	90	0.33	The efficiency of the method is affected by media pH.
Polyelectrolyte flocculation	High	15	14.81	
Centrifugation	Very high	22	8	Cost and energy intensive, suitable for high value product.
Filtration(natural/ pressurized)	High	6/27	0.4/0.86	Efficiency depends on hydrodynamics, concentration, and properties of microalgae.
Floatation	High	1-6	10-20	
Auto flocculation	NA	NA	NA	Happens in absence of CO ₂ .

Now, we will compare the different methods. So, gravity sedimentations, inorganic chemical flocculations, and polyelectrolyte flocculations, and centrifuge, filtrations, natural and pressurized, and then floatation, auto flocculation, so these are different methods we can use for the harvesting purpose. And the relative cost are different, the very high cost is with centrifugation and very high TSS yield with inorganic chemical flocculation, but this method has some difficulty that we are talking as the MEM the cell wall will be changed and that may create difficulty for oil extractions in later stage. And the efficiency of the method is affected by the media pH. Then gravity sedimentations is not very efficient for rapid biomass recovery from high rate algal ponds.

And the centrifugation we have already discussed the high cost and filtrations and floatation also depends on hydro dynamics of the systems, concentration and properties of microalgae and no information on available on this relative cost yield and energy usage on auto flocculations. So, from this table it seems that all the methods are having some limitations. So, more investigations are going on to develop some new methods with less limitations and more economic feasibility this is the major part which contributes for the cost to the whole process.

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Waste Water	Algal Strain	Nutrient Removal (%)	Process condition	Ref.
Municipal wastewater (Autoclaved)	Chlorella sp.	N-89.9, P-80.9, COD-90.3	Initial N, P and COD : 132.3, 215 and 2390 mg/l respectively; Inoculation-10%, Temp-25±2°C, Light intensity-50µmol/m²s ; Batch ,14 days	Li et al; 2011
Municipal wastewater (Raw)	Chlorella sp.	N-89.1 P-80.9 COD-90.3	Initial N, P and COD : 116.1, 212 and 2304 mg/l respectively; Inoculation-10%, Temp-25±2°C, Light intensity-50µmol/m²s ; Batch ,14 days	Li et al; 2011

Now, at last we will see some example of wastewater treatment using algae. So, algae how to grow we have discussed, how to harvest we have also discussed. Now, we will see some example through which the wastewater treatment have also been achieved. So, both in this slide we will see that municipal waste water both Autoclaved and Raw form has been used and reported by Li et al in 2011, and they have considered three that is nitrogen, phosphorous and COD present in the waste water that are 132.3, 215 and 2390 mg per liter respectively. So, they have perform the (Refer Time: 25:39) in batch reactor for 14 days and 50 micro mole per meter square second light density was used at 25 plus minus 2 degree centigrade temperature. And they are got say nitrogen removal of around say 90 percent, phosphorous around say 81 percent, and COD around 90 percent. So, the same removal was also achieved with Raw wastewater.

So, not only municipal wastewater, industrial wastewater have also been tested by some as reported Su et al 2011 and Su et al 2013. So, here Soyabean processing wastewater and here they have used piggery wastewater. So, *chlorella pyrenoidosa* was the first case and in this case *chlorella zofiengensis* was used and say initial nitrogen, phosphorous and COD concentration was 190, 46 and 8087 milligram per liter respectively, where the nitrogen, phosphorous and COD removal say 90 percent, 75 percent around and 84 percent. So, similarly the information for this are also given here. So, from this slides, it seems that the microalgae are also able to remove nitrogen, phosphorous and COD from the wastewater. So, up to this in this module.

Thank you very much for your patience.