Course on Industrial Biotechnology
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Lecture No 60
Summary and Conclusion

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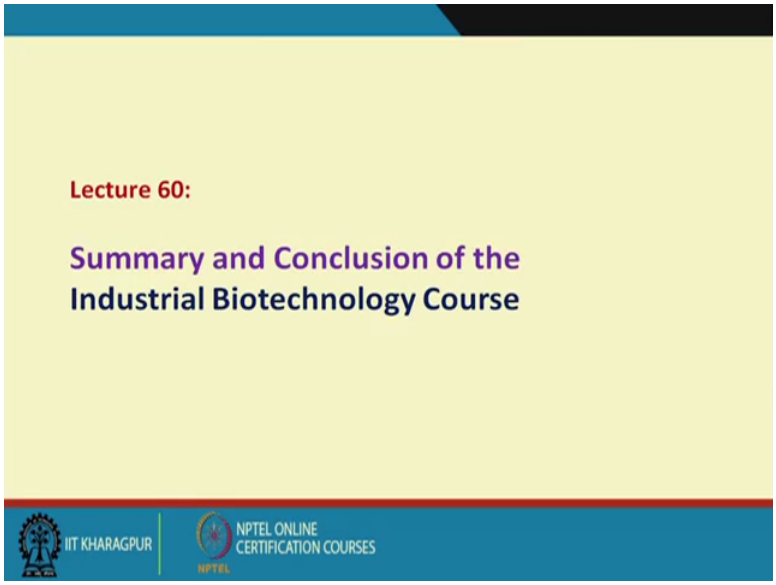

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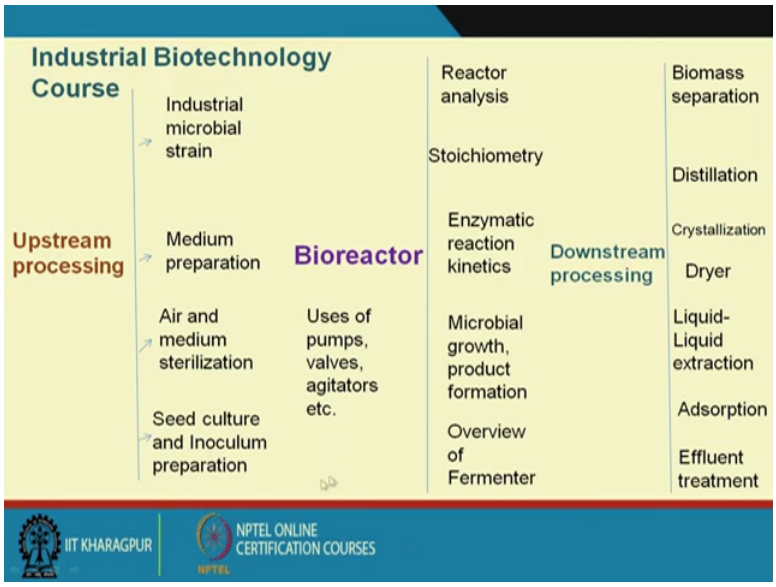

Welcome back to my last lecture on the Industrial Biotechnology course that is on summary and conclusion of the Industrial biotechnology. Let me explain how I organized this course if you look at the any kind of the industry biochemical industry it has three different parts, one is upstream processing, another is bioreactor, another is downstream processing. Now if you

look at the upstream processing that involves the development of the industrial microbial strength because the organism that we use that should be genetically stable not only this should be genetically stable but they should have higher productivity, higher osmo tolerance higher productivity all this characteristics they should use the cheaper raw materials.

So this is very important then media preparation also very important in the upstream processing, the media is a vital thing that is I told you media comprises of carbon source, nitrogen source and also for product formation nitrogen required only for cell growth and metals and vitamins they mostly required to carry out the metabolic reactions in the metabolic pathway this use as a cofactors.

So this media preparation is very important then these reactions usually carried out under aseptic conditions because we want to grow your desired organisms in the reactor. So that you can get your desired product because at the initial stage I tried to make a clear cut definition, clear cut differentiation between the chemical and biochemical process let me repeat it again. The difference between the chemical and biochemical process is that, in the chemical process as your product changes your raw material changes

But in the biochemical process same raw materials can produce n number of products, only what we change whether we change the microorganism, if we change the microorganism we can get the different types of products. So I can give the examples if we use saccharomyces cerevisiae you will get under anaerobic condition it produce ethanol if you use from the glucose by the glucose if you use aspergillus nigar it produce citric acid.

If you use the lactobacillus dellbrueckii or then the glucose will be converted to lactic acid, so you have different types of, only what you do? You change the microorganism and another advantage of this process is that most of the biochemical process they operated at ambient temperature and atmospheric pressure and whereas the chemical process mostly operated at high temperature and high pressure, this is how they differ from each other and here only the different is that we shall have to allow growth of a particular organism because we know that air comprises of, our environment comprise of so many microorganism there available throughout the air and water.

So any kind of contamination problem will not allow your desired organism to grow. So you have to make the condition totally aseptic that is why air and medium sterilization is very important and then the seed culture preparation and the inoculum preparation here and this is

very important because I told you the volume of the inoculum that is used for the production fermenter is about 5 to 10 per cent of the production fermenter.

So suppose I walk with citric acid industry we have 200 cubic meter fermenter and one 225 cubic meter fermenter. So our volume of the inoculum was about 14 cubic meter, 14 cubic meter means 14 thousand liters that much of culture we cannot prepare in the lab. So you have to prepare in the plant itself. So in the lab what you have to prepare? You have to prepare seed culture, now I told you in case of unicellular cell it is not a problem because if the number is proportional to mass, so you can through your RND you determine that what number will be more suitable for the inoculum preparation but in case of fungal cell it is the filamentous growth, so we cannot use the vegetative cell as a seed culture for the inoculum vessel.

We prepared seed culture with respect to spore and spore formation take place under stress conditions and then we can count the spores. If you count the spores we can quantify that how much spores will be suitable for the inoculum production that is how it is done. So that is here I have mentioned that seed culture and inoculum preparation that is very important. So upstream processing deals with all these things, this we try to incorporate in the course then as per bioreactor is a particular place where that is the heart of the biochemical industry because where the reaction take place.

So question come how the reaction takes place? How we can monitor the reaction? How rate of reaction can be calculated? How stoichiometry of bioprocess can be expressed? So all these if you look at the reactor analysis what are the different types of reactors are available? We should know the stoichiometry of the bioprocess I told you gives us three different information, it intermolecular relationship of the different components present in the reaction mixture also it gives the information that how much heat is evolved in this process to a particular aerobic fermentation process?

And thirdly the validity of the experimental results, all the three information we can generate from this stoichiometry of bioprocess. The enzymatic reaction I told you that bioreactors is a vessel in which the reaction take place in presence of some kind of component comes from the biological system. If not necessarily it should be living cells it may be the nonliving because enzymes is nonliving material. So it comes from the biological system, so if any reaction takes place in presence of the enzymes we call it Bioreactions, so you know biochemical reactions.

So this is like this, so we should know the enzymatic reaction kinetics then microbial growth and product formation and overview of the fermenter which is very important because we should know what are different components present in the industrial fermenter? What are the different controls that we had we require in the industrial fermenter? So details of the fermenter is very, I tried to explain the batch control fermentation process then I have given the kind of picture of the industrial fermentation process so that you can have better look on the process

And then this process without the pump and valve and agitator we cannot operate different types of pumps we use, I can give the example of centrifugal pump that is largely used then in the biological system we use the peristaltic pump and also we use different types of valves I told you that we have gate valve, we use the ball valve for the sampling purpose because it should be instantaneously opened and instantaneous closed because if we use the globe valve it will not work because if your globe valve is slowly open and slowly closed if you do that your material may spread here and there that is undesirable.

So we have given the information of different types of valves and agitators also I have given the, what are the different type of agitator used? So these are the information we tried to incorporate in the course and then finally we have downstream processing. In the downstream processing it is nothing but the purification of the product it comprises of first step that we have when the reaction take place your microbial cells plays very important role for getting the product but if the product is other than microbial cells then microbial cells we have to separate for, this is the impurity present in the reaction mixture.

So this should be biomass separation we have to do, I told you that different type of cell mass separation techniques we have depending on the size of the biomass. In case of bacterial cell we might have to use the centrifuge or you have to use some kind of flocculator but in case of yeast since the size is little bit bigger the bacterial cells size is 0.5 to 2 microns and yeast is 3 to 7 microns.

So you can use the plate and flange filter press for separate the yeast particles. Now if in case of fungal cell it is couple of millimeters, so it can be separated by using rotary vacuum filter so different type of filter is used for the separation of the biomass. So this I tried to explain then distillation process there are different processes of purification of the product as for example for ethanol that separation we use the distillation process, the reason is that the

ethanol has boiling point is 78 degree centigrade, so we can easily through the process of fractional distillation we can easily separate out.

Crystallization another very important process that we have in the biochemical industry I showed you how citric acid can be separated with the help of that crystallization? And dryer is a very important thing because whenever you market the product it should be easily drier. I tried to explain the different type of dryer, one dryer commonly used that is the rotary drum dryer. We have other dryer also there, spray dryer and other dryer also there.

Liquid-liquid extraction process also very much used in the biochemical industry I told you this is largely used in the penicillin production process where penicillin and acidic pH is more soluble in amyl acetate or butyl acetate or penicillin comes from the aqueous layer to the solvent layer this is how the purification takes place. We have adsorption process, streptomycin it is absorb in the absorption column and we can separate the impurities that from this streptomycin.

When you get the final product every industry, every chemical and biochemical industry they produce some kind of waste water what you call effluent and this effluent you cannot dispose in the water stream because it contains lot of organic matter. If you dispose this organic matter then the bacteria that present in the atmosphere they grow and multiply that will pollute our water stream to a great extent, not only that they will reduce the dissolved oxygen concentration of the water stream that will affect the growth and metabolism of the aquatic plant and animals.

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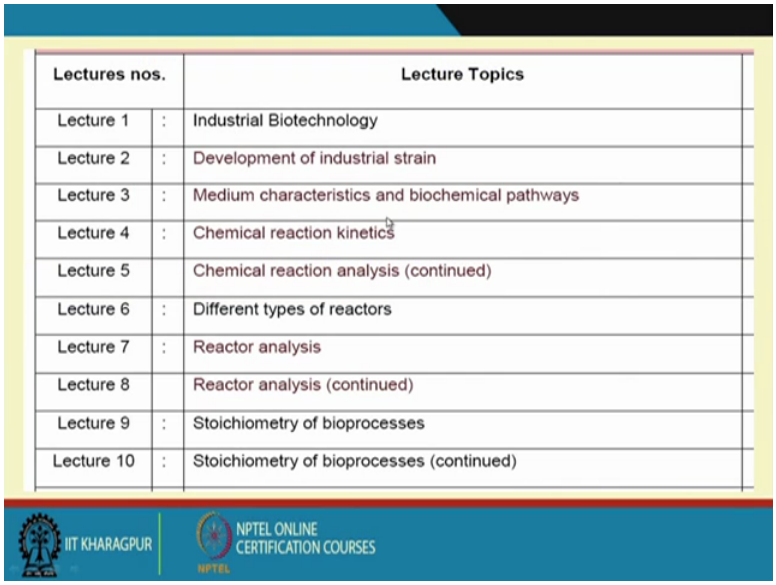

So these are the things how industrial biotechnology course is organized and we have given different examples of the different industrial fermentation process and let me explain the course one after another. The first lecture I tried to discuss the industrial biotechnology what is our objective and what are the different byproducts are available with us? I told you the byproducts may be of three different things we have a high value low volume products, medium value medium volume products and low value and high volume products

So with respect to that, we can classify the products and then I discussed how to develop the industrial strength, which is the very important in the biochemical industry. The medium characteristics and the biochemical pathway that is also very important for the growth of the microorganism. Now question comes, to understand the chemical biochemical reaction taking place in this system we should first understand the chemical reaction kinetics.

I tried to explain the different type of chemical reactions how it can be analyzed? How we can determine the constant of the different kinetics constant of the chemical reaction then I mainly emphasis on two types of reactions, one is called reversible reaction and the chain reaction which mostly in operation in biochemical processes, how this can be analyzed? And try to find out how you can develop the equation for individual reaction components present in the reaction mixture?

So this I tried to find out then I tried to discuss different types of reactors that are used in the biochemical industry then how this reactor can be analysis? The reactor analysis is very much required to design any kind of fermenters because suppose you want to produce one tone of

Baker’s yeast, question comes produce one tone of Baker’s yeast what should be the size of the reactor?

In a batch process continuous process if all the information are available then how we can find out the volume of the reactor? That can only be found out if you can do the reactor analysis. We have the stoichiometry of bioprocesses through which I told you it gives a three different information, intermolecular relationship of the different component present in the reaction mixture. How much moles of A reactor is how much mole of B to convert how much mole of C and D? Because this you can only find out through with the help of stoichiometry and also it gives other two added information additional information.

One is how much heat is evolved? because we know the biochemical reaction mostly take place at ambient temperature and atmospheric pressure and seems it is ambient temperature so if the temperature rises, so you have to have some cooling arrangement and we are in tropical country, particularly in summer our ambient temperature increases as high as 45 degrees centigrade.

So you require more cooling arrangement during summer to control the temperature of the reactor because microorganism I told you they are very sensitive to the environment as if until and unless we give the proper environment for the microorganism they cannot grow and multiply then another thing I told you that this gives the information on the validity of the experimental results that you can find out.

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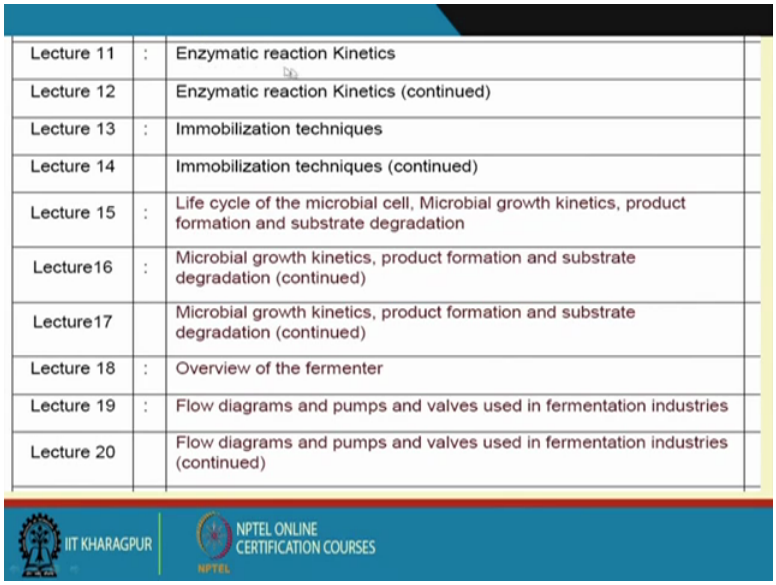

Then I tried to explain the enzymatic reactions kinetics because if you look at the microbial system we have multiple enzyme system because we have metabolic pathways where multiple steps are involved and every step required one enzyme so to understand those processes first you should understand the enzymatic reaction kinetics. We try to explain the enzymatic reaction kinetics and then immobilize techniques because when you use the enzymes the problem is that enzymes flee after the reaction is over it remains an impurity in the reactions mixture.

But if you immobilize the enzyme on the solid matric then you pass the substrate and get the product. You can reuse the enzyme again and again but when you use the free enzymes then after the reaction you have to throw it out not only that it remains as the impurity in the reaction mixture. So you have to spend some money to separate the enzyme from the product. So this is the problem that we have with the free enzyme system. So immobilized system largely use in the industry.

I have given the example of high fructose corn syrup then because life cycle of the microbial cell plays very important role in the microbial fermentation process I tried to explain the different phase of growth we have log phase, we have lag phase, log phase, stationary phase and death phase.

The log phase it appears to be what you call active phase, where the organisms are very active. In all the inoculation of the organism should be done in between the mid log phase to

late log phase because during these phases the organisms will be mostly active then and only then you can get your product in a proper way.

So this is very important and also I told you stationery phase during the stationary phase secondary metabolize formation takes place this is called starvation phase and lag phase is considered as a acclimatization phase, so that should be as minimum as possible. So we try to explain the life cycle of the microbial cell, microbial growth kinetics, product formation and substrate degradation.

After that I gave the overview of the fermenters, how the different control system we have in the fermenters? Then we give the information of flow diagram, here the flow diagram plays very important role in the biochemical industry with the help of flow diagram we can understand what is the different units present in the fermentation industry? Because fermentation biochemical industry does not mean only it has the fermenter, it has upstream processing we have downstream processing.

So several upstream processing units are there, several units of downstream processing is there. All these things in combination we can build up the flow diagram and flow diagram may be of two types, one is called block flow diagram another is process flow diagram. Block flow diagram means we represent every unit as a block but in a process flow diagram we try to give the sketch of the particular process. Seeing the sketch symbol of the particular process we can understand this is pump, this is heat exchanger and this is reactor like this.

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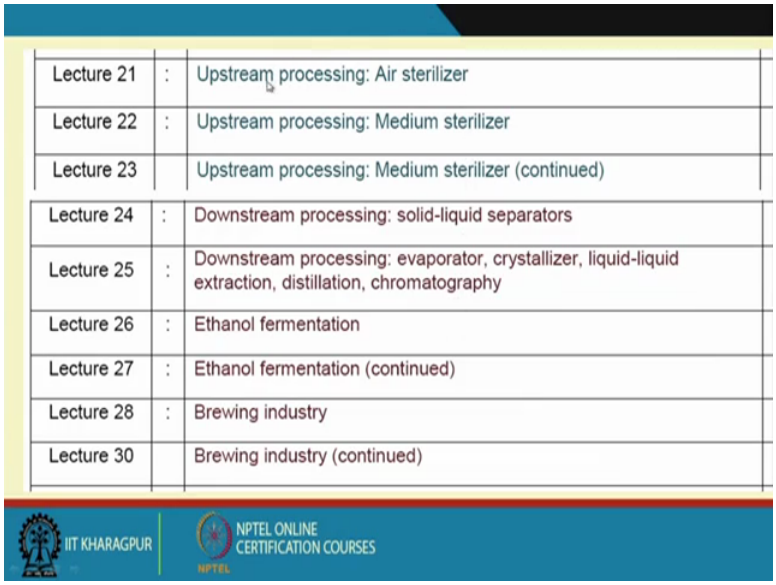

Now we have upstream processing we have explain the air sterilization, another is medium sterilization we explain and during our exercise we discussed different numerical problems, we have given the solution of that. So I request all of you please go through very nicely.

If you have any question please let us know we try to solve that, then we have in the downstream processing we have up to this upstream processing then we come to the downstream processing this deals with the purification of product. First step that we have that is the how you can separate the cell mass, what you call solid-liquid separation then in the downstream processing we have several units we have evaporator, crystallizer, liquid-liquid extraction, distillation, chromatography etc.

Then after giving all the information then we switch over to the individual fermentation process. First we take an example on the ethanol fermentation process then brewing industry. Ethanol fermentation process I told you two type of ethanol available in the market, one is tax alcohol and another non tax alcohol, tax alcohol is considered as a pure alcohol which is used for the human consumption as well as in the pharmaceutical industry also perfume industry and nontax alcohol mostly used as a chemical feed stock for the chemical industry.

So this production process I tried to explain then brewing industry the beer making industry two type of beer largely in the market one is lager beer and the ale beer, it produced by using top fermenting yeast another is bottom fermenting yeast. So if you consider the lager beer, this is usually produced by the bottom fermenting yeast, saccharomyces carlsbergensis and the top fermenting yeast is produced ale. One is stronger another is little bit lighter.

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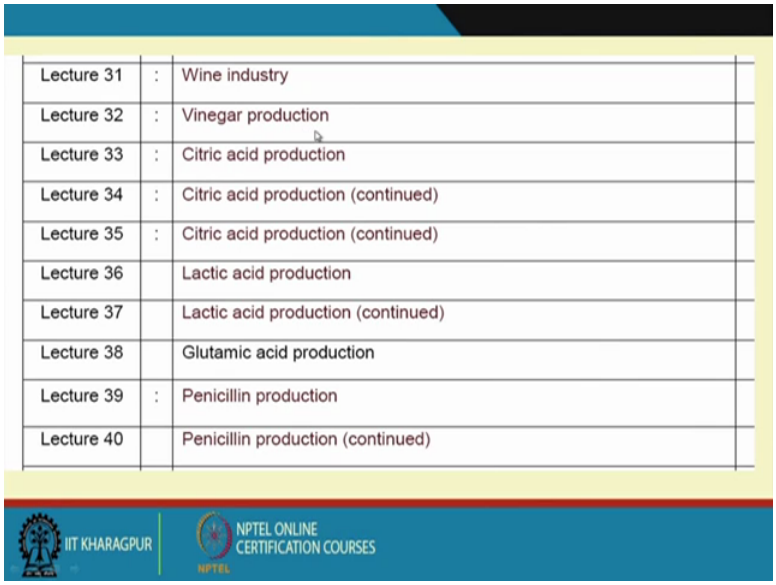

So then it is followed by the wine making industry different types of wine, particularly we have same sparkling wine and still wine, sparkling wine where we have carbon-di-oxide bubbling through this wine and still wine there is no carbon-di-oxide. Another wine we have what you call dry wine it does not have any kind of sugar in the wine then vinegar production this is one of the important product which nothing but acidic acid largely used in the domestic purpose.

Then we have citric acid which has lot of applications in the industrial sector as well as, particularly I can tell you this is used for cleaning the boiler tubes the scale formation of the boiler can be removed with the help of citric acid because this is weak acid then we have lactic acid largely used by the industries acts as a preservative and poly lactic acid has greater application in the pharmaceutical industry.

Then Glutamic acid kind of amino acid which is largely used in the different food industry and particularly also it is used for the preparation of different medicinal tonic, then we came to the different antibiotics production process we have we consider first the penicillin because this is the first antibiotic that has been identified and this is used when we get any kind of wounds during our playing or you know injury we get we have some pus formation this can be recovered by using this penicillin, it is largely applied industrially how the development has been take place how is it produce this I explained.

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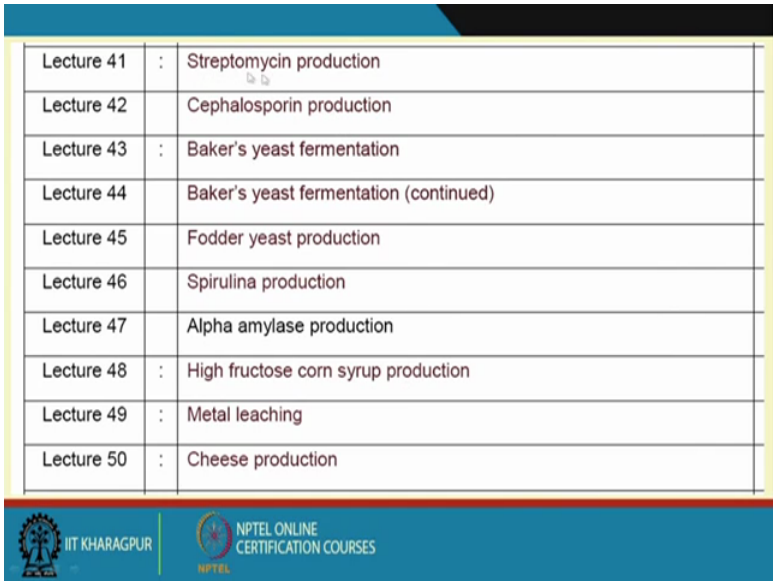

Then I discussed the streptomycin production, cephalosporin production this is also kind of what you call that penicillin, only the orientation will be little bit different maybe I consider 41 as cephalosporin and streptomycin we can change it then Baker's yeast fermentation process. The Baker's yeast largely used in the bread making industry it is used for the leavening one important characteristics of the Baker's yeast it should be dispersible disperse in the liquid otherwise your bread you will have different types of holes inside the bread, this is undesirable.

And also it increases the nutritional quality of the bread to a great extent. We have fodder yeast the basic difference between fodder yeast and the Baker's yeast is that fodder yeast can utilize both hexose and pentose sugar whereas the saccharomyces cerevisiae can use only the hexose sugar then we come to the another single cell protein what you call spirulina which largely marketed in the form of powder, in the form of tablet, in a form of candies in a different way.

Then I discuss this alpha amylase production this has also tremendous potential in the market then high fructose corn syrup I told you this is the convection used in the confectionary in the western country, metal leaching process this is at least 40 different industries is available in the world which successfully used this technology for the recovery of the metal because when the ore contains very less amount of metal this process is quite effective.

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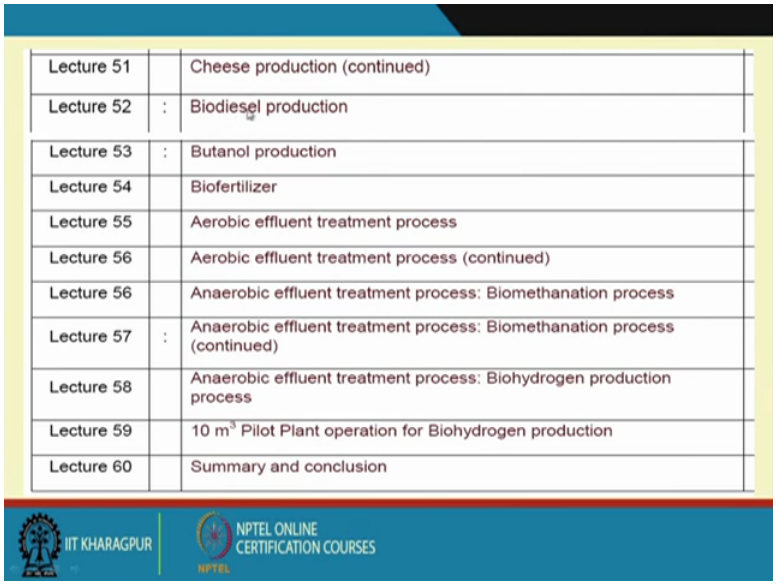

Cheese production is very important because cheese is a product through which we can preserve the milk, protein and fat for longer period of time after that we discuss the biodiesel and Butanol which is considered as a Biofuel for the future and biodiesel can be produced from the vegetative oil from the animal fats, also it can be produced from the microalgae and Butanol also is produced through the fermentation process from the different organic material.

After that I discussed the importance of the Biofertilizer I indicated due to use of chemical fertilizer we lost the fertility of the soil to a great extent that is why it was largely recommended that we should replace this by the Bioferilizers so that the fertility of the soil, one of the important characteristics of the fertility is the water retention property of the soil because plant take their nutrient through the process of diffusion and this is very much required and by using organic fertilizer that is kind of Biofertilizer, the humus contain in the soil that increases that increases the water retention properties of the soil.

Then I discussed the effluent treatment process, aerobic and anaerobic effluent treatment process particularly I want to mention this anaerobic due to the development of anaerobic effluent treatment process now it is possible to get revenue from the effluent treatment process previously we industry does not have any kind of revenue from this process here we can get the revenue in the form of methane we can use that as a energy source and finally I tried to discuss our 10 cubic meter pilot plant that we operated successfully at IIT Kharagpur and we are looking for the consularization of the process.

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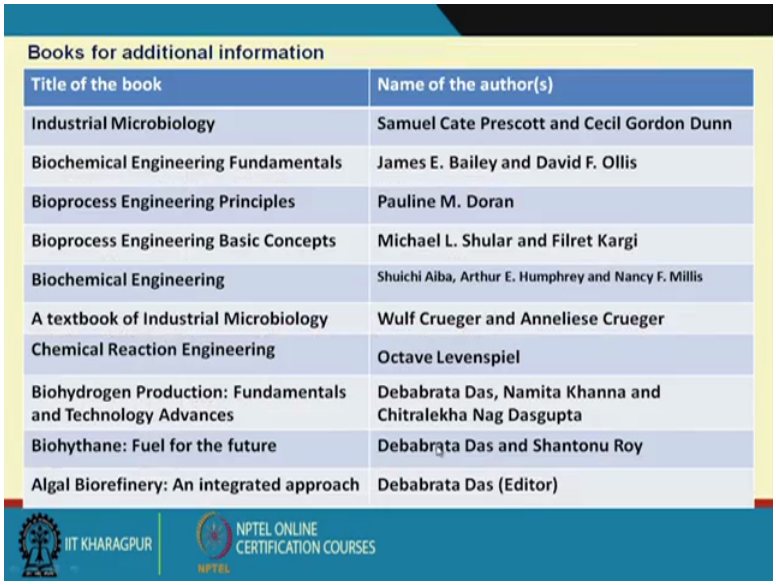

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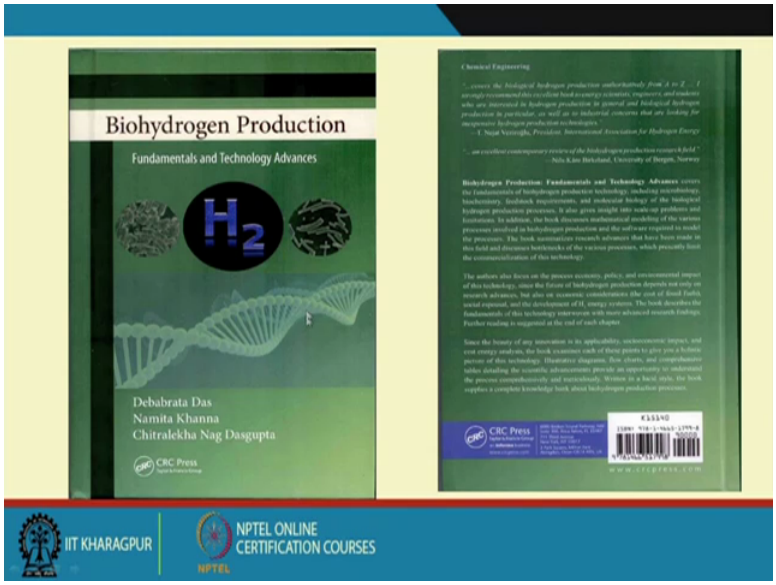

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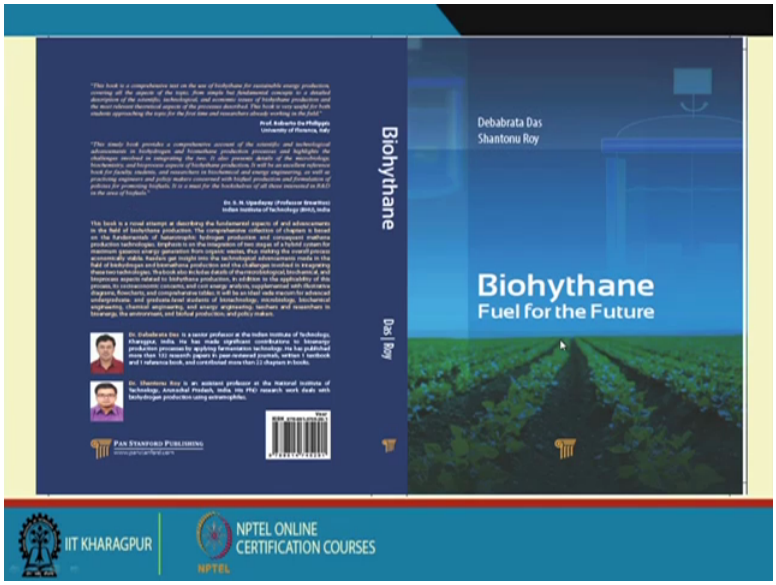

Now this are the different books please refer that industrial microbiology by Prescott Dunn and biochemical engineering by Bailey Ollis different books is listed here please go through that and this is the book our book that is the Biohydrogen production process, you please go through that this is Biohydrogen again you go that you will get lot of information

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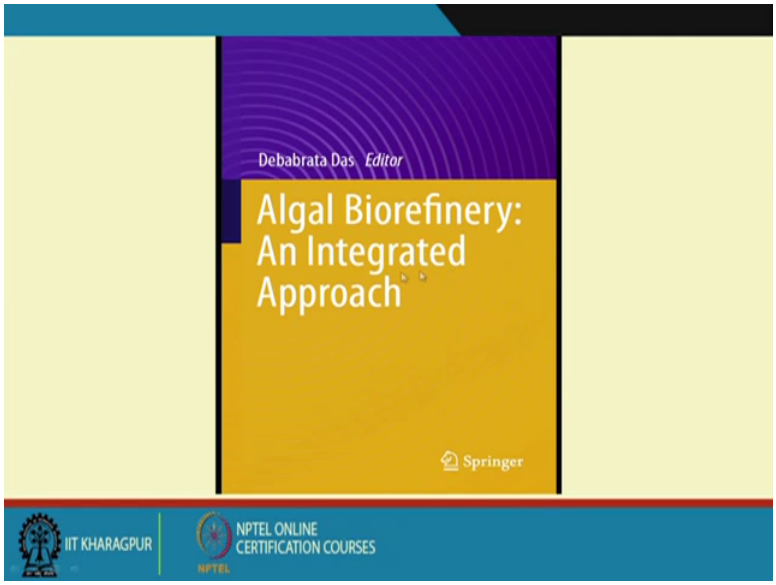

Another book I forget to mention this is algal Biorefinery and integrated approach this is also our book, this published by Springer you can get lot of information of algal growth.

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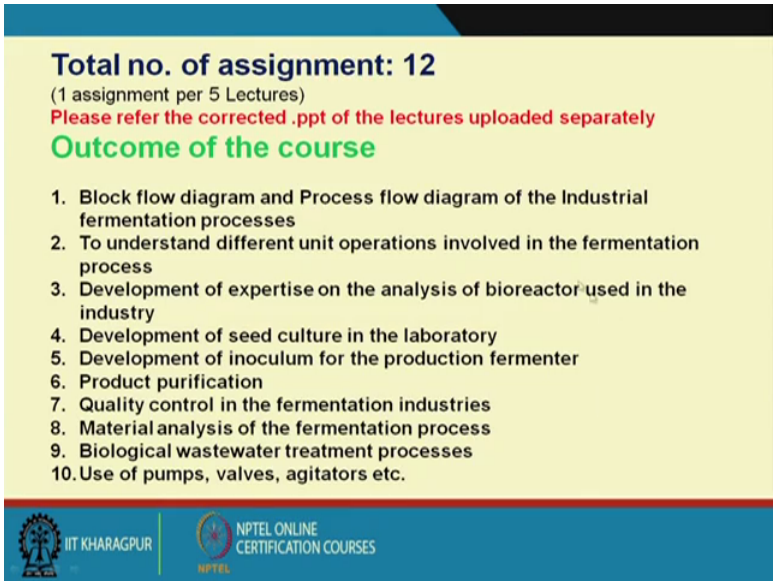

Now regarding the course let me tell you the total number of assignment that will be given to you 12 and 1 assignment per 5 lectures, so you have to solve that and please refer our corrected ppt of the lecture uploaded separately because here I want to stress here that whatever ppt I showed during the lecture it required some minor corrections that we will be showing in the separate ppt, so please refer the corrected ppt.

Now outcome of the course is like this, so you develop the expertise on the block flow diagram and process flow diagram of the industrial fermentation process you will get the information how the different process, they are in operation then to understand the different unit operation involved in the fermentation process, what are the name of the different unit operation that you can come across? Development of expertise on the analysis of bioreactors used in the industry, how the reactor can be analyzed? Because I can tell you when you walk in the industry main purpose of the engineers is they have to do the right the information in the log book what the operator has to do?

So until and unless you have the material analysis of the process you cannot right that, so that kind of expertise you can develop. Development of the seed culture in the laboratory, how we can develop the seed culture? I tried to explain. Development of the inoculum for the production fermenter that also tried to explain, product purification I also explained. Quality control of the fermentation industry I told you there are three different quality controls of three different sectors for the raw materials, for the process, for the product. So three different quality control measures you have to take into account in the biochemical industry.

Then material analysis of the fermentation process that is very important that also we try to cover then biological wastewater treatment process how it operated because more than 70 per cent wastewater treatment process are controlled by the biological means that also we try to explain and also you will get the information of pump, valves and agitator during this fermentation process.

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So I have two teaching assistant who help me to give this presentation to share our ideas with you and if you have any question you can write to them they will be with you they will help you to clear your doubts, thank you very much. I hope this course will be very much useful for all of you and I shall look forward for your comments, thank you very much.