Course on Industrial Biotechnology
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Mod01 Lecture03
Medium Characteristics and Biochemical Pathways

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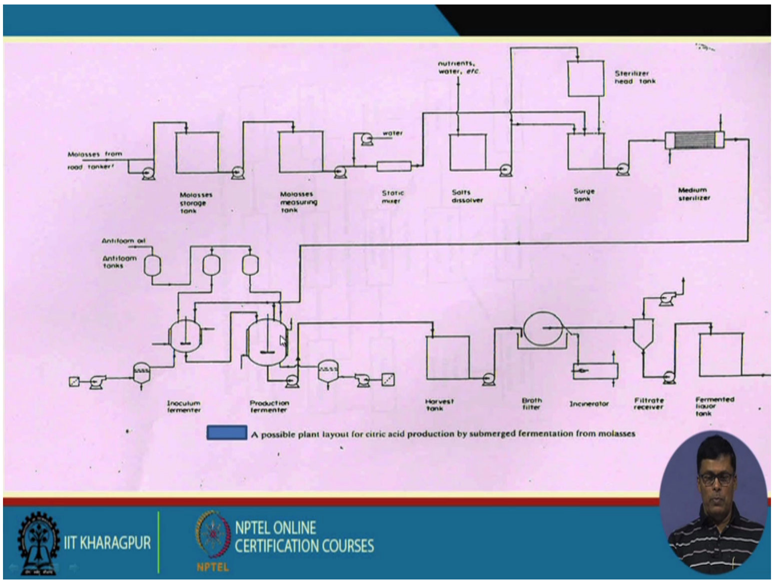

Today I am going to discuss medium characteristics and the biochemical pathways, as you know that media plays very important role in the fermentation industries and I have taken the specific example here, the citric acid production by submerged fermentation from cane molasses and if you look at here, the cane molasses usually procure from the sugar manufacturing companies, because molasses is the byproduct of the sugar production unit and this comes to the factory and this usually kept in the molasses storage tank and which is located to close to the main gate and molasses measuring tank. This is usually located in the inside the fermentation plant and then this is mixed with water the reason is that cane molasses contains about the 50 percent of sugar, so we dilute it with water, so that is, it will be suitable for the growth of the organism and then we we mix with desirable mutant (()) (1:30) than water and then we mix it thoroughly and pass through the sterilization unit and then it comes to the we have inoculum fermented another yeast production fermented.

So the media that is used for inoculum fermented and production fermented usually different, because the purpose of the media in the inoculum vessel is to produce the same mass and the purpose to the use of media in the production fermenter to get the desired product. So naturally that the requirement as difference, since the requirement is different, the composition of media also will be different. So we today I am going to discuss this in details.

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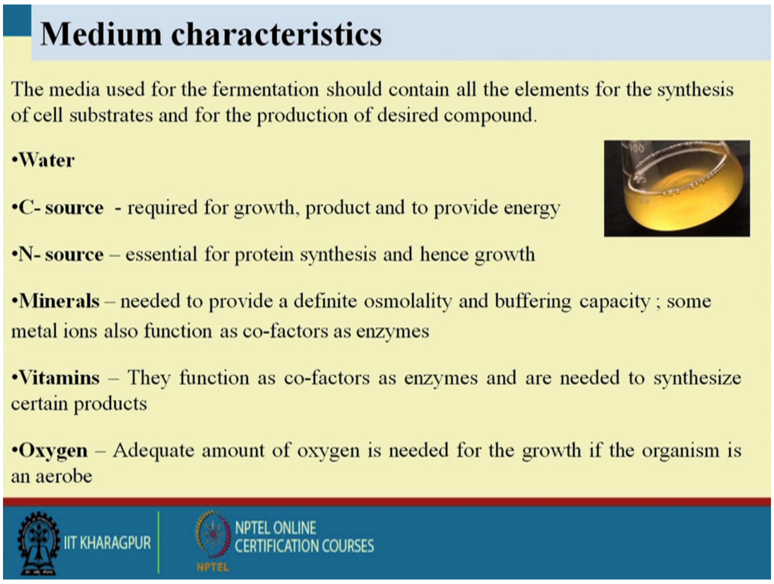

Now if you look at the media characterization, water plays very important role, because question comes what should be the quality of water obviously, it should be free from the contaminance, because it should be as the microbial load should be as low as possible, so that sterilization process will be little bit easier, but at the same time question comes whether the water should be the tap water or the soft water, because tap water will be cheaper where if you consider the soft water it would be little bit costlier.

So now we shall have when we use the tap water we usually it determine the characteristics of water, characteristics of the water is determine on the basis of the mineral contents of the particular water. If the minerals, those mineral does not have any influence on the fermentation process then we can reuse directly the tap water for the fermentation purpose.

Next is your carbon source which is very important, because carbon source contribute for three different purpose you see that, one is for the growth, another for the product and it also provide the energy, because it purpose three different purpose that one is growth, because the cell mass (())(3:42) which is very much required and product which you are looking for the run this process and another is that, it gives the energy to the organism for grow and multiply. A nitrogen source is essential for the growth of the cells and minerals that usually give the balance of the osmotic balance of the media, because you know that osmotic pressure also plays very role and also it helps to develop some kind of buffering capacity of the media, because most of the biological system when the organism undergo the fermentation process, which produce the organic acid and if this acid formation is there then pH will go down and this will affects the fermentation process.

The buffering capacity of the media that plays very important role and also that if this metal ion also has some role to play to find out the as (())(4:42) co-factor for that different enzymes that is they act in the metabolic pathway of different microorganism. Now vitamins that is usually the acts as a co-factor for the enzymes and are needed for the synthesis of certain product and oxygen here, it should be required adequately adequate amount and here I to point out that we all human beings we take oxygen from the air, but microorganism when they grow and multiply, they use the oxygen which is dissolve in the fermentation media.

So naturally the dissolved oxygen concentration is a very plays very important role, because we know the oxygen is (())(5:25) soluble in water. So if the if we do not supply the oxygen here sufficiently there you growth of the organism will be ampule if the growth of the organism with ampule (())(5:35) then our whole fermentation process will be affected.

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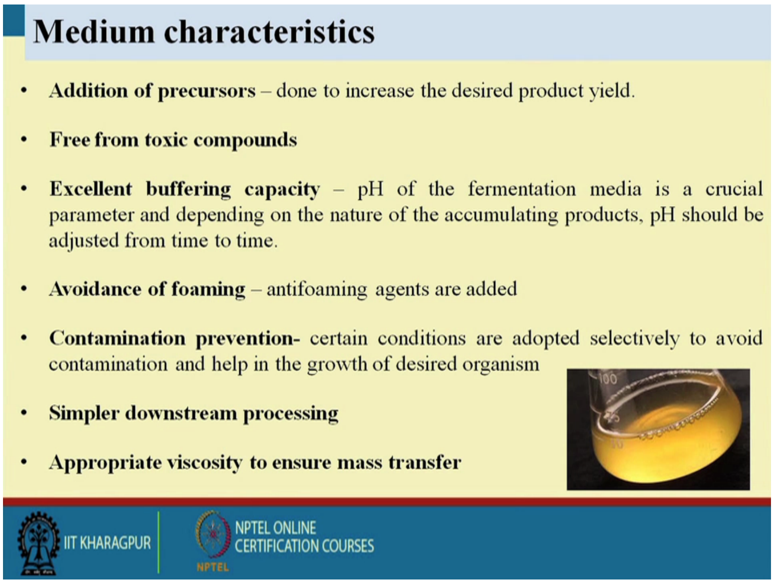

Now next is that addition of precursor, because (I have) I can give a very simple example of penicillin fermentation process if you look at the penicillin G and penicillin V, penicillin V usually used for the capsule and penicillin G in the as a injection fluid and we use for the production of penicillin G we use the assyle (())(6:04) phenyl acetic acid penicillin V we use the phenoxy acetic acid the free and also the media should free from the toxic compound, which is very important and I already mention that you (())(6:18) should have the excellent buffering capacity.

And another very important aspect is the foaming formation to avoid the foam, because in the fermentation industry we required the eiditator (())(6:31) and in the eiditator (())6:33) at the top we have the mechanical seal (())(6:35) and mechanical seal that actually protect the entrance of the air from outside, because if any air enter into the system this will make the contamination of the system whole fermentation process will be affected. So to avoid and if we allow the foam formation then what will happen, foam will rise in the fermentation block (())(6:57) and it will enter into the mechanical seal and rupture the mechanical seal as soon as the mechanical seal is ruptured then the contamination of the system will take place. So is this is undesirable to the same, in this we use some kind of the antifoam oil, so subside (()) (7:13) the foam.

Then contamination prevention that is very important, because some cases we change the composition of the media so that that is the contamination problem in the fermentation will be (())(7:25) can be avoid it and simply downstream processing. Downstream processing is very important aspects, because whenever you do the marketing of the product it should be in the purified form. Now question comes how easily you can do that and appropriate viscosity to ensure the mass transfer that is mass transfer plays very important role particularly that when we talk about the dissolved oxygen concentration, the oxygen transfer that depends on the viscosity of the particular media.

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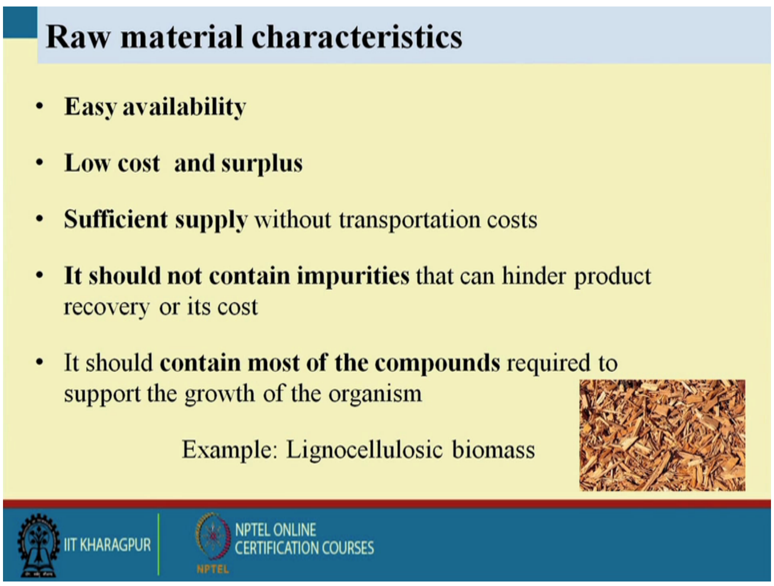

Now other different quality that we have that is the easily availability of the raw materials, because whatever raw materials that we use in the media that should be available not only available particular season (())(8:08), but it will should be available throughout the year. Low cost and surplus that is also very important aspect, sufficient supply without transportation cost, transportation cost we will see that as minimum as possible, then it should not contain any impurities which hinder the product recovery and its cost. More impurities that our production that recovery cost will be more, so it is directly affects the cost of production and it should contain most of the compounds required to support the growth of the organism, example is the lignocellulosic material.

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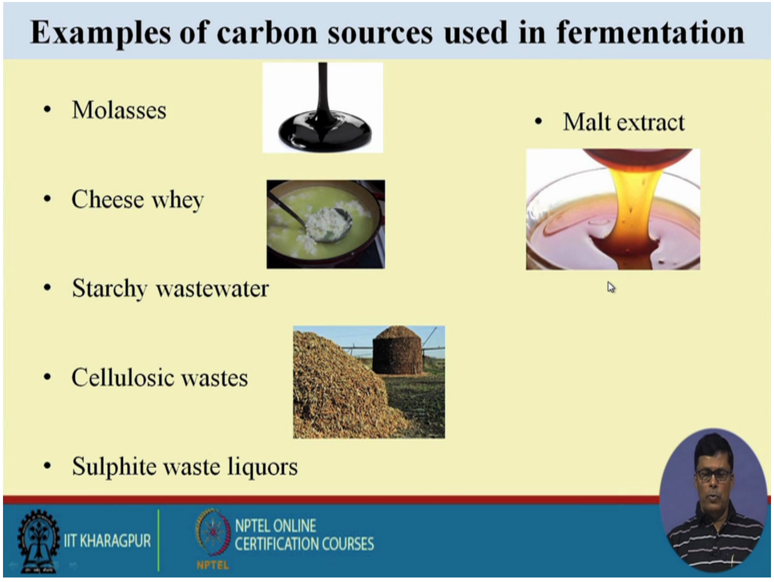

Now let me give you some specific example of the carbon sources, we have first I already discuss that is the in case of citric acid fermentation process we use the cane molasses. Molasses usually marketed in two different forms, one is call cane molasses and another is beet (())(9:01) molasses. Cane molasses is available in India, mostly it is available in India and also in brazil and beet (())(9:09) molasses is mostly available in the western country and this molasses contain about the 50 percent of sugar and sugar is the basic raw materials and one of the beauty of these biochemical for processes is that from sugar we can produce the different type of compounds. So this is the very good raw materials for different fermentation process particularly, it is very god raw material for yeast fermentation process for the citric acid fermentation process.

Then comes the whey the cheese whey which is the type (())(9:48) byproduct of the sugar industry the we the byproduct of the milk industry and that you know after separating the milk, protein and fat, we get particular liquid we get particularly in the cheese making industry, we get the after salting process we get the whey and this whey can be used the carbon source particularly it is very good raw material for the production of lactic acid.

Then (())(10:13) starchy raw material that is also very important , which is usually produce from the rice processing industry particularly in India, but in west Bengal if you consider as another India that we have our is rice the (())(10:28) food and we have lot of starchy material desire we get from the rice processing industry and also we can get this starchy raw material from 42 manufacturing industry. The cellulosic waste we have lot of cellulosic wastes, like we have rice straw, wheat straw that corn (())(10:48) different type of cellulosic waste we have and say we have the sulphate waste liquor that is the waste the effluent from the pulp and paper industry that can be used as a in the carbon source, also we can molt extract also can be used as a carbon source. The molt extract contains different minerals which also helps in the fermentation process.

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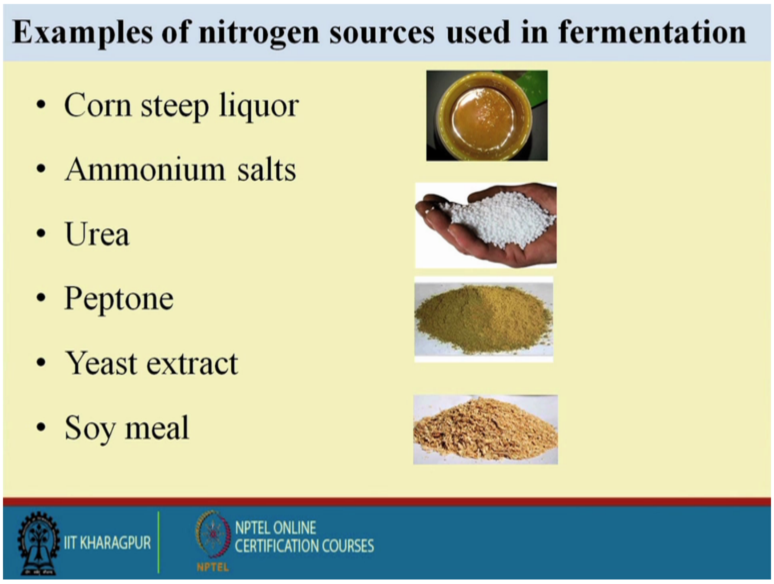

Now if you look at the nitrogen source we have corn steep liquor, then this is the used in the particularly this is used in the penicillin industry, then ammonium salt, this is used in the bakers yeast production process, because here I want to mention that in the bakers yeast fermentation process, the PH of the media is there be usually acidic and to maintain the pH of the media acidic we do not have to as acid separately the simple if we use the ammonium sulphate. Ammonium sulphate when we dissolve in the water your pH will be acidic that is good enough to maintain the acidity in the media as a acidic pH in the media, then we have the urea that is used as a good nitrogen source, peptone is good nitrogen source, yeast extract and soy meal this is as a good nitrogen source that is use and I told you that nowadays we use also some the (())(12:16) oil cake as a nitrogen source and we find it has very good results and some cases we use the water (())12:22) as a nitrogen source, there also we get good result some cases we use the algae as a nitrogen source we got the very good results in some cases.

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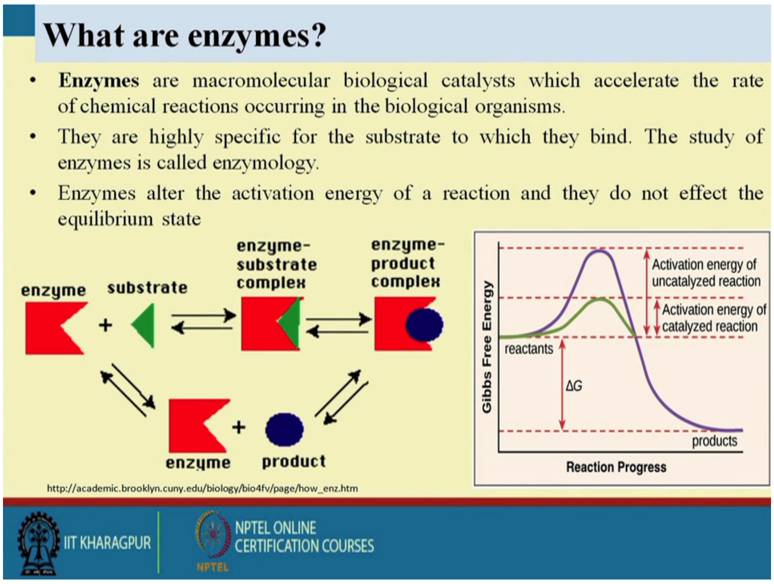

Now question comes what are enzymes. So enzymes are the macromolecular the biological catalysts which accelerate the rate of chemical reaction occurring in the biological organism. Now you can see here that activation energy is this, this is a activation energy. Now purpose of this cata enzyme is that reduce the activation energy so the rate or reaction is faster and if you look at here, enzyme and substrate complex, they form enzyme substrate complex and then it gives the product. Now after the product formation is thereby over the enzyme comes in the original form that is the that is why this is very specific with respect to substrate, this is I can give the examples that glucose isomerase enzyme than acts on glucose and convert glucose to fructose. It will not act any other any other substrate.

If We consider the (())(13:30) it acts on sugar and it hydrolyze the sugar to glucose and fructose. They are very specific as per substrate is concern. The enzymes the main purpose of the enzyme as I pointed out that it reduce the activation energy, so the rate of reaction will be faster.

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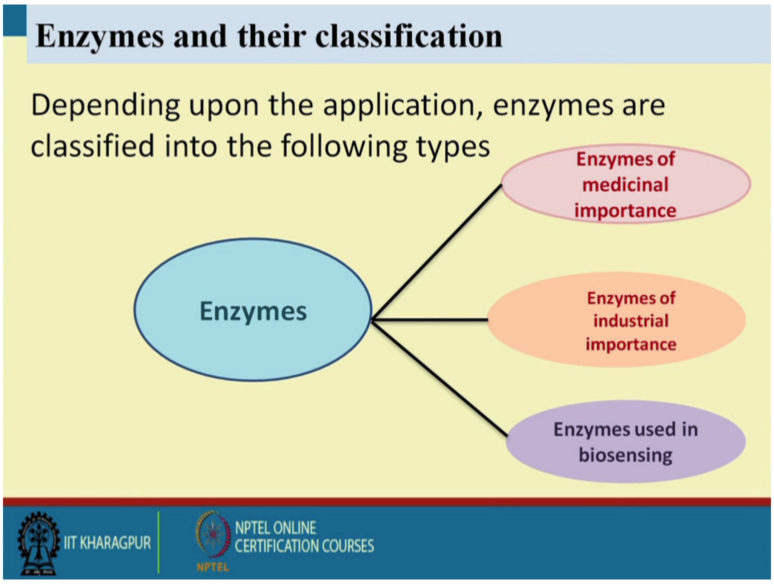

Now enzymes may be of 3 types. Enzymes of medicinal importance, enzymes of industrial importance, enzyme used in bio sensing.

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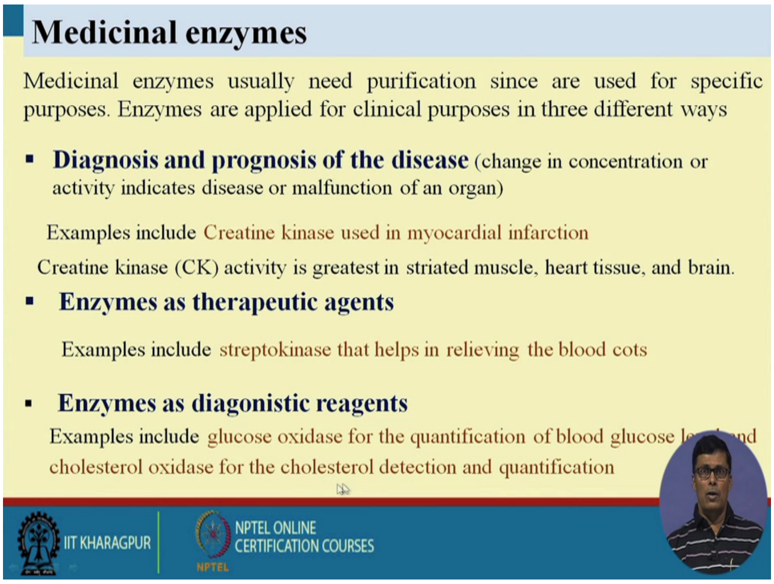

Now if you look at the enzymes used in the for the medicinal purpose with a three different purpose, one is call diagnostic or prognosis of the disease as for example of creatine kinase used for myocardial infarction. An enzyme therapeutic agents we know the streptokinase which help the relieving the blood clots, particularly the for you know for heart disease or you know (())(14:27) disease lifesaving drug that is used in the pharmaceutical in the pharmacy, it is largely available and we have enzymes can be used for the diagnostic purpose as for example glucose oxidase can be used for quantification of the blood that glucose present in the blood, also cholesterol oxidase used for the detection of cholesterol present in the blood and the and the quantification.

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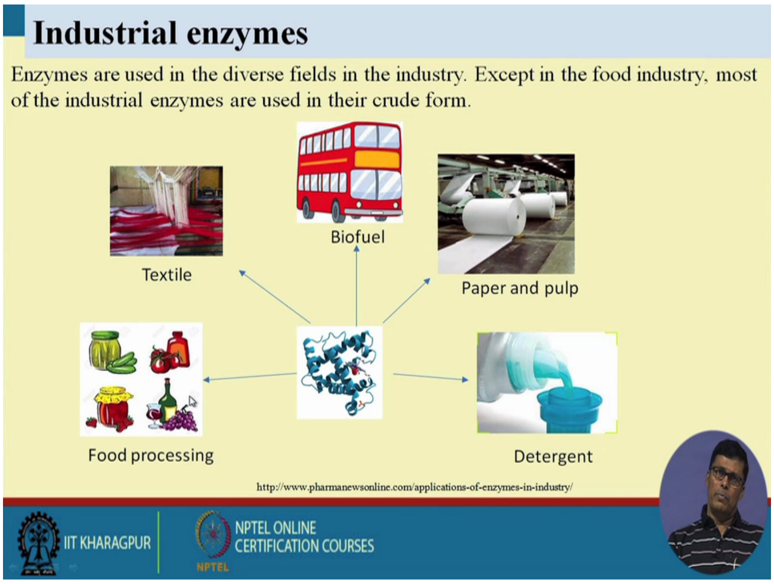

Now the enzymes also used for the industrial purpose. It is used for the textile industry and it is used for the as a by fuel you know that biodiesel I can I can tell you the biodiesel that is produce from different biological sources and nowadays it is operated by for different buses different the vehicles we operate I can tell you lot of biogas buses are available in the water country.

Nowadays in India also some buses are operated with the help of biogas with the help of biodiesel some train are in operation then it is used in the pulp and paper industry. Paper is the nothing but, it is the kind of your cellulose and you know that for the separation we use some kind of enzyme so that you can degrade different type of material so that the cellulose can be separated from this. Now it is used in the detergent industry, like you know I in the last lecture I was talking about the strain formation in the in our (())(16:07) and that can be removed with the help of the enzymes, like protease and ligase and the enzyme also used in the food processing industry I can give the example of rennin that is used in the cheese making industry for the precipitation of protein.

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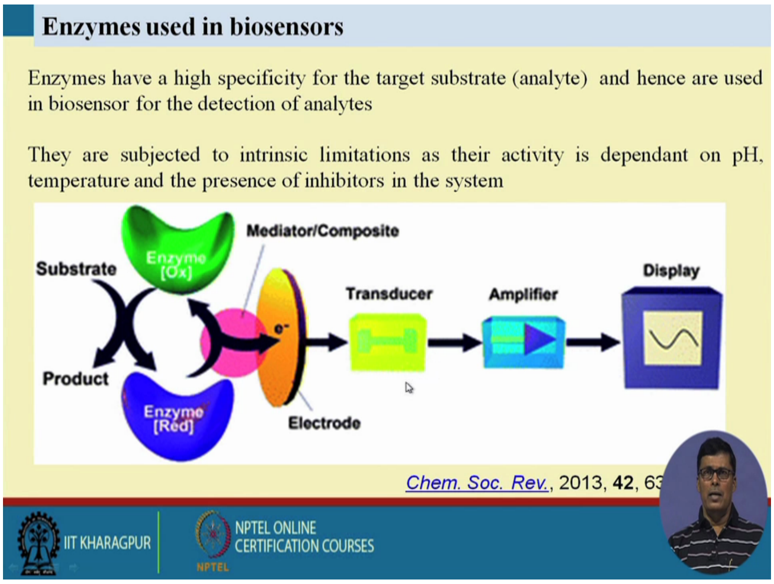

Now the enzymes that we can be used as the biosensor is very very important, because you know this is the in the biosensor we have two units, one is call molecular recognition unit and the we call another call transducer. The molecular recognition unit is comprises of for the enzymes, this is the immobilized enzyme which recognize the substrate suppose you have I I was talking about the glucose oxidase. Now if the glucose oxidase present here than in the in presence of glucose it reacts with glucose and from the gluco-lactone and this change this is recorded with the help of some kind of electronic device what you call transducer and this is the mainly of two types, one is amperometric another is potentiometric. Amperometric (()) (17:13) means the we measure the change of current and potentiometric we measure the voltage then we amplifier which amplify that and here we record.

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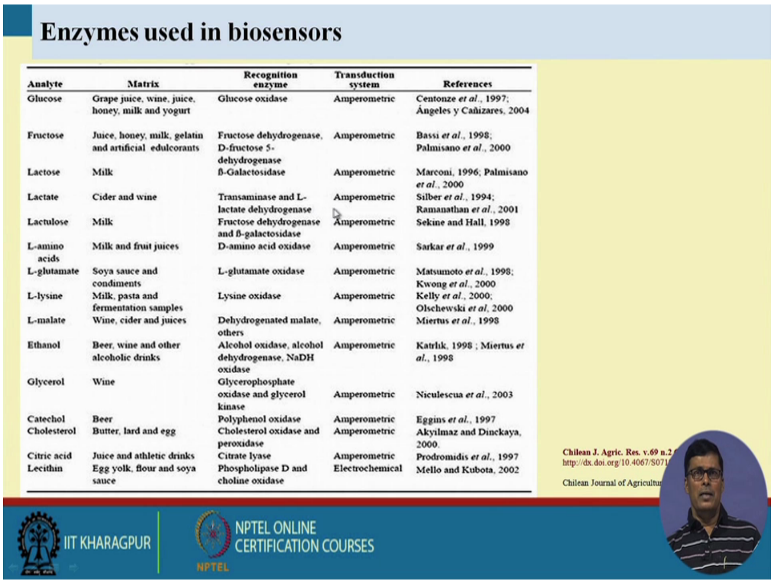

Analyte	Matrix	Recognition enzyme	Transduction system	References
Glucose	Grape juice, wine, juice, honey, milk and yogurt	Glucose oxidase	Amperometric	Centonze *et al*., 1997; Ángeles y Cañizares, 2004
Fructose	Juice, honey, milk, gelatin and artificial edulcorants	Fructose dehydrogenase, D-fructose 5-dehydrogenase	Amperometric	Bassi *et al*., 1998; Palmisano *et al*., 2000
Lactose	Milk	ß-Galactosidase	Amperometric	Marconi, 1996; Palmisano *et al*., 2000
Lactate	Cider and wine	Transaminase and L-lactate dehydrogenase	Amperometric	Silber *et al*., 1994; Ramanathan *et al*., 2001
Lactulose	Milk	Fructose dehydrogenase and ß-galactosidase	Amperometric	Sekine and Hall, 1998
L-amino acids	Milk and fruit juices	D-amino acid oxidase	Amperometric	Sarkar *et al*., 1999
L-glutamate	Soya sauce and condiments	L-glutamate oxidase	Amperometric	Matsumoto *et al*., 1998; Kwong *et al*., 2000
L-lysine	Milk, pasta and fermentation samples	Lysine oxidase	Amperometric	Kelly *et al*., 2000; Olschewski *et al*, 2000
L-malate	Wine, cider and juices	Dehydrogenated malate, others	Amperometric	Miertus *et al*., 1998
Ethanol	Beer, wine and other alcoholic drinks	Alcohol oxidase, alcohol dehydrogenase, NaDH oxidase	Amperometric	Katrlık, 1998 ; Miertus *et al*., 1998
Glycerol	Wine	Glycerophosphate oxidase and glycerol kinase	Amperometric	Niculescua *et al*., 2003
Catechol	Beer	Polyphenol oxidase	Amperometric	Eggins *et al*., 1997
Cholesterol	Butter, lard and egg	Cholesterol oxidase and peroxidase	Amperometric	Akyilmaz and Dinckaya, 2000.
Citric acid	Juice and athletic drinks	Citrate lyase	Amperometric	Prodromidis *et al*., 1997
Lecithin	Egg yolk, flour and soya sauce	Phospholipase D and choline oxidase	Electrochemical	Mello and Kubota, 2002

And these are the different examples we have. the reasons why biosensors are largely in the market that we can analyze the sample very very short time and number of sample can be analyse also very high that is why is largely this is available in the market.

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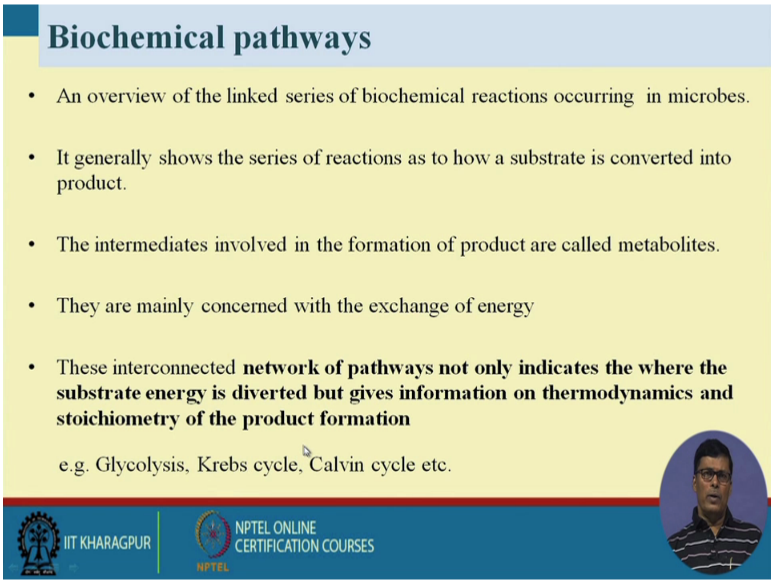

Now if you look at the biochemical pathways now every microorganism, they have metabolic pathway and in the metabolic pathway you will find the different steps are involved and every steps you have one enzymes and this in it is shows that series of reactions as the how a substrate is converted into product. The intermediate involved in the formation of product is call the metabolites and they are mainly concerned with the exchange of energy. The interconnection of the network of pathways not only indicate the where the substrate energy is diverted, but gives the information on thermodynamics and stoichiometry of the product formation.

The example of the glycolysis pathway and Krebs cycle, kelvin cycle, this is the very popular cycle that is popular metabolic pathway very largely operated in the biological system particularly glycolysis cycle is available the most of the organism, because it when glucose undergoes glycolytic pathways it produce the pyruvic acid and when it is pass through the kreb cycle is converted into the carbon dioxide and water.

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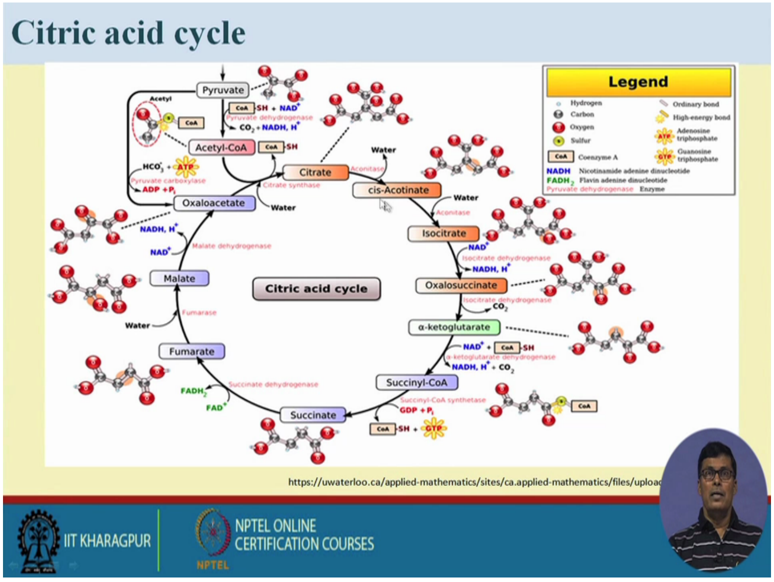

So now this is the citric acid TCA cycle we call a TCA cycle. Now here we can see the pyruvic pyruvate is converted from the glucose it converted to pyruvate with the help of glycolytic pathway, then this pyruvate with the help of the enzymes we acetyl we for the acetyl-coA and this acetyl-coA and the axalecetate with the help of the convention (())(19:41) enzyme what synthesis, this produce citric acid.

Now after citric acid citric acid we have since aconitase, isocitric acid, oxalosuccinate acid, alpha ketoglutaric acid, succinate acid, fumarate acid mainly this is TCA (())(20:02) this is how it operates in our system. Now when here I want to point out that how the citric acid formation takes place. Here you see that in the TCA cycle if you allow the TCA cycle in the operation then what will happen this will not allow the citric acid to accumulate here, it will (())20:23) converted to other metabolites.

So what we basically do during the fermentation process if we can inhibit (())(20:30) the acidity of the (())20:32) the enzyme like aconitase enzyme, here there is enzyme called aconitase enzyme. Here we have (())(20:39) enzyme if this enzymes to enzymes are inhibited and then and only then citric acid accumulation in the system will take place.

Now it has been observed that particularly when we use the (())(20:55) then in a by using the cane molasses then it has been observe that if you do not control the pH automatically it will it will inhibit the these two enzymes and as the time passes on these activity of enzyme totally inhibited, but it depends on the that metal ion concentration we find I do not have manganese (())(21:20) that plays very important role in the citric acid fermentation process.

Now if in case of citric acid fermentation process we remove that you know I do not and manganese then and only then you will find the this kind of you know (())(21:36) cited accumulation in the system, but if significant amount of the iron and manganese present in the fermentation blog then we find that this enzyme will be active and it will be subsequent with the degraded to the respected metabolites (we have) we will get very less amount of citric acid production.

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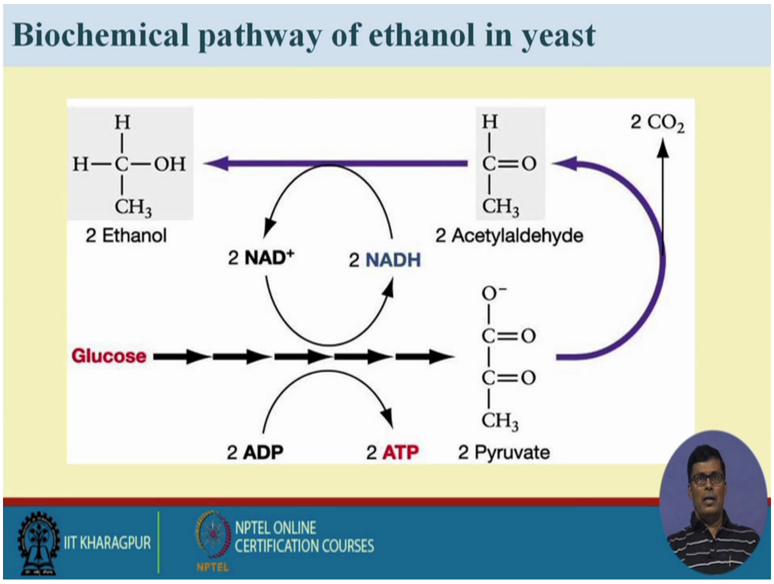

So like this every organism they have they follow the different metabolic pathway to get the different type of products I can give the example of this biochemical pathway how the ethanol formation take place we I told you the glucose after the stable states of operation, it produce the pyruvic acid and this pyruvic acid when undergo the decarboxylation reaction. It so it forms the acetaldehyde and acetaldehyde when it undergo the reduction reaction it produce the ethanol. So one mole of glucose the stoichiometry is very important one mole of glucose can produce 2 moles of ethanol the 2 moles of ethanol, so if you in the last class I was discussing bout the most of the alcohol producing industry, initially that old days we have seen that the fermentation industry, they have the alcohol production, it was 7 to 8 percent. So 7 to 8 percent means the glucose that or sugar concentration will be along 14 to 16 percent, but nowadays due to the development of the some special type of microbial strain, it is possible to increase the alcohol concentration to a great extent up to 14 percent 14 to 15 percent alcohol when you the concentration of sugar will be about 30 percent which is very high.

So (())(23:29) I told you that as you increase the sugar concentration then the problem is that some osmotic pressure that also develop in the fermentation media, due to develop or when the osmotic pressure the activity of the organism would be affected and this is the when constant of these process, so we try to develop the genetically modified the organism some kind of mutant organism so that we can have the high productivity and industrially why it is important, because as the product concentration increases the our recovery cost will be minimize and if the recovery cost is minimize then our cost of production also affect to a great extent.

Now when we use different type of raw materials for the fermentation process we take into account I told you that not only for one particular carbon source, but also we should look into the different 2, 3 different type of carbon source so that with the abilities of the raw material that will be more in there market and we do not have the any kind of scarcity of the raw material. So to get the desired product.

Now I was talking about that different fermentation process, like citric acid, penicillin and when you really we talk about this fermentation process we have seen that they there media composition they are really different as for example I told you that in case of citric acid fermentation process we do not required any precursor, because precursor some cases it required without the precursor we cannot get the desired amount of product formation, but the if you look at this penicillin fermentation process we required the precursor without precursor we cannot get the penicillin.

So what I want to point out that that composition of the media playas very important role in the fermentation process and I can give a very typical example to the beer making industry. The beer making industry that one important steps is the massing process. In the massing process, the hydrolytic enzymes like (())(25:56) they act on the starchy or protein and to degraded there is to solubilize this this insoluble material.

So we find particularly alpha amylase it if the calcium ion is present then it thermo stability of the enzyme will increases. The usually the saccherification that usually prefer the high temperature, because the gelatinization is a very important steps for the saccherification process. So that is why that if the water contains some calcium ion obviously it will (()) (26:34) the massing process.

So you know that the water composition as I mention that you know whatever we use in the fermentation process, it may vary from process to process one industry the composition will be different as compared to other industry, thank you.