Course on Industrial Biotechnology
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Module 08
Lecture No 36
Lactic Acid Production

Welcome back to my course industrial biotechnology. Now in the last lecture I discussed that citric acid fermentation process and I told you the citric acid is the secondary metabolites and it produce by using aspergillus Niger and two type of fermentation process we use for citric acid production one is surface fermentation and other is submerged fermentation, but in the industry mostly they use submerged fermentation process and we have we observed that media that is used for the cell growth and media that we used for the citric acid fermentation process is different, because in the in case of inoculum vessel we look for we produce the cell mass, in case of production fermenter we go for this product formation that is citric acid.

Now we observe that two metal plays very important role, most important significant role as for example iron and manganese, because iron is the co-factor for the enzyme accumeters, because if you allow the accumeters enzyme to be active, then you will get very less amount of citric acid in the fermentation media. So that is to be removed from the production media and also manganese iron that antagonized that effect of phosphofructokinase. The phosphofructokinase has been inimited by as the citric acid formation takes places, so that inimition effect can be antagonized due to the formation of ammonia which is takes place when manganese and iron is presence in the fermentation media is quite less.

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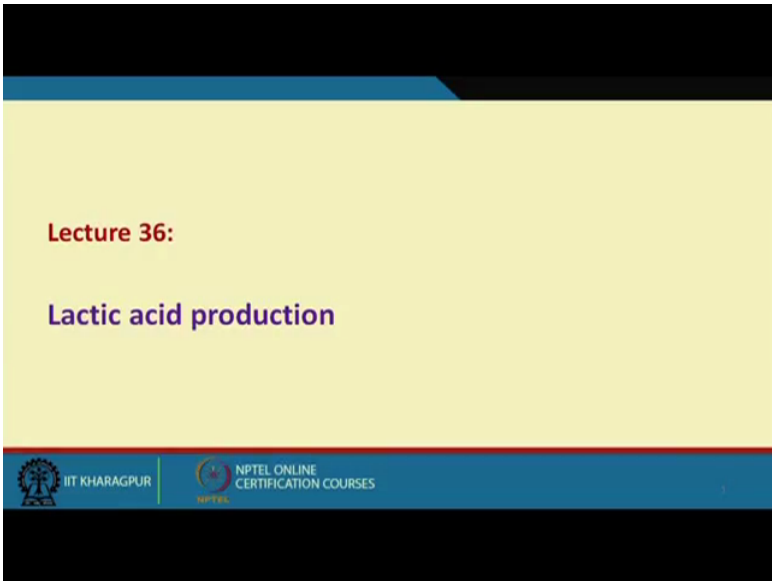

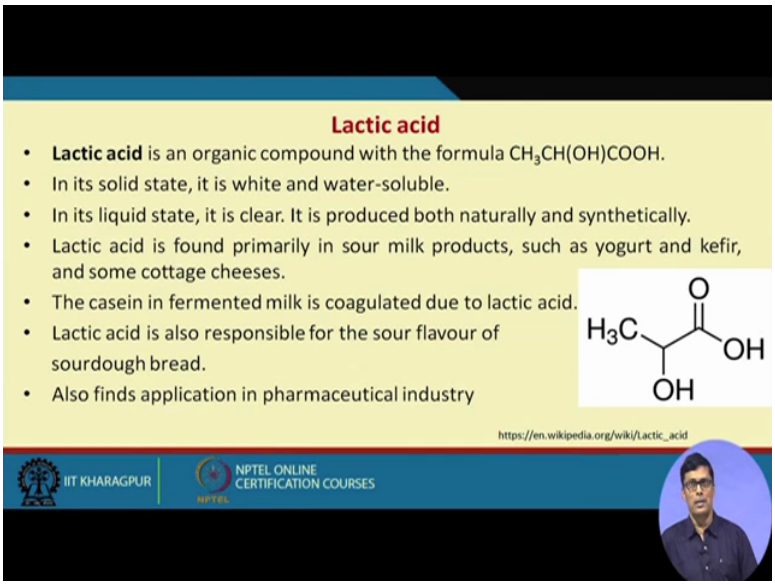

So then we have discussed about the different uses of citric acid and details of the downstream processing. Now today we want to discuss one very interesting fermentation process that is lactic acid, largely used by the biochemical industries and lactic acid does not required any kind of introduction, because lactic acid is largely used as a food preservative. We might be have it that I can give a typical example, that milk is an essential food for our survival, because this content most of the essential amino acids or and good lipids that is called the (())(03:09) and all this that is good for the human health. But problem is that we cannot preserve the milk for longer period of time, even if you pasteurized after 3-4 days it will be you have to use it.

So milk, protein and fat which is very good for the human consumption that is usually preserve in the form of cheese, because how cheese is produce? Cheese is produced by using the curd, actually curd making process and during curd making process we produce basically the lactic acid and lactic acid act as a preservative. That is how your milk, protein and fat because it has low moisture content, milk has very high moisture content, so all most lot of pathogenic organism can grow very easily, but since the moisture content in the cheese is very less, so your contaminants that you know that pathogenic growth will be very less also salt concentration in the cheese is also quite less.

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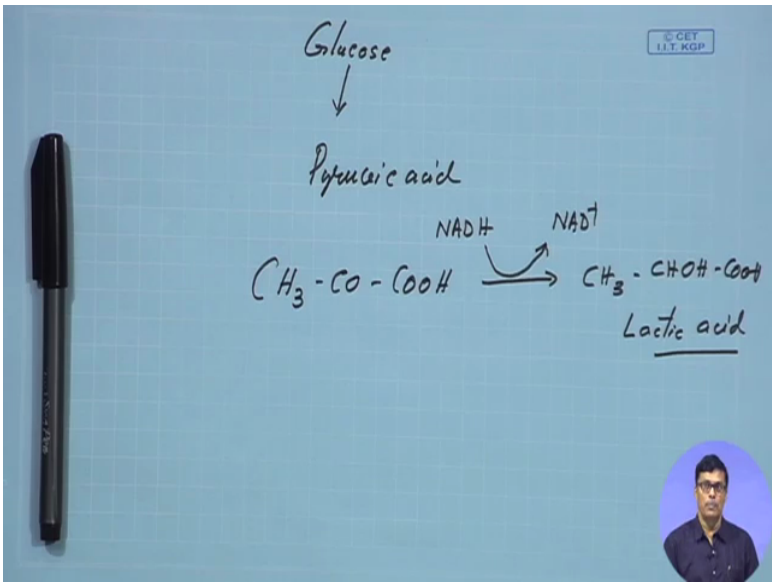

So lactic acid is very important fermentation process and it is lot of use in for different purpose. So this is this is it is solid state is white and water soluble usually it is because we know the Indole Miro Pathway, in the Indole Miro Pathway glucose is converted to pyrobic acid. Now this pyrobic acid has the formula CH3-CO-COOH. Now this in presence of NADH, so hydrogen that it is reduces and it forms CH3-CHOH and C double O H.

This is what is written here CHOH and C double O H that is what we return here, this is called lactic acid this is we have this is lactic acid. So mostly the lactic acid fermentation process we produce lactic acid in the form of calcium lactate, because we use the calcium hydroxide as a for controlling the pH in the fermentation media and lactic acid is usually is converted to calcium lactate. And lactic acid found primarily in the sour milk products, such as the yogurt, kefir, and some cottage cheese.

The casein in fermented milk is coagulated due to lactic acid and lactic acid is also responsible for sour the flavor of sourdough bread. Also find application in the pharmaceutical industry. Particularly, I want to point out here the poly-lactic acid has tremendous potential in pharmaceutical industry. Particularly, if we look at the during operation, particularly we have when we have internal that operation in the early days we usually use the nylon thread for any kind of stitching purpose, now we use some kind of poly lactic acid material with clips and other things and with the help of that we avoid kind of using the nylon thread.

So if we use nylon thread again you have to open the system and take the out take it out but if we use this lactic acid poly lactic acid that will be absorb in the system, so you do not have to take it out that has tremendous potential in the Pharmaceutical market. So you have two forms of lactic acid, we L-lactic acid, we have D- lactic acid. So mostly we produce the L-form of lactic acid.

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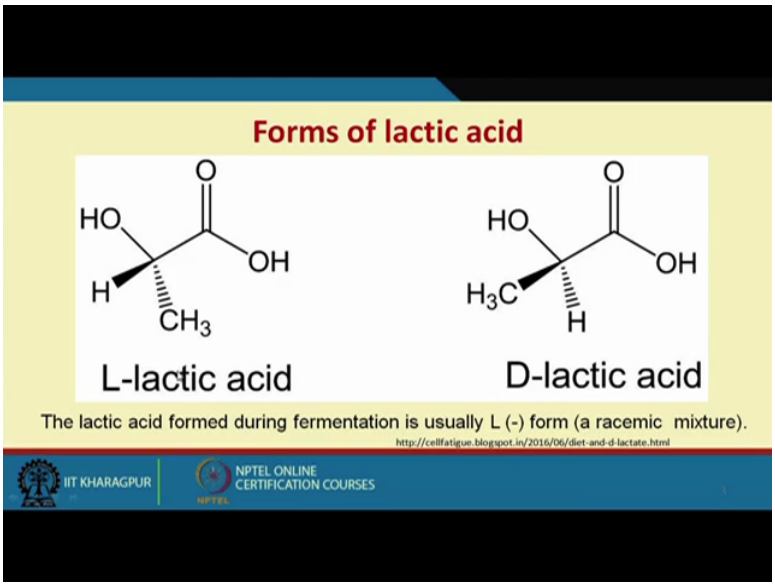

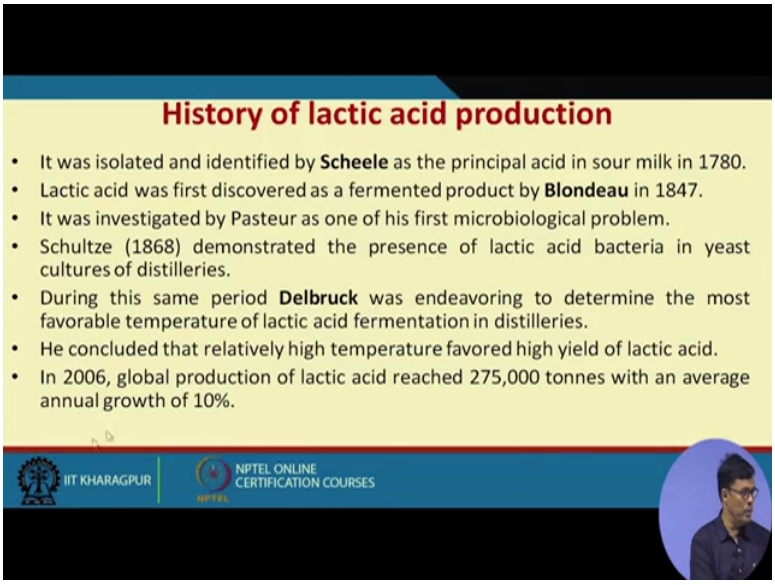

Now if you look at the history of the lactic acid it is very interesting. It was isolated and identified by Scheele that as the principal acid of sour milk in 1780. Then lactic acid was first discovered as a fermented product by Blondeau in1847. Then it was investigated by Pasteur as one of the first microbial problems and Schultze that demonstrated the presence of lactic acid bacteria in the yeast culture particularly distilleries.

Here I want to point out another few interesting thing, that I shall discuss after the discuss the bakers fermentation process then I shall discuss it there. Then in the bakers fermentation process purposefully they add lactic acid bacteria in the fermentation broth, because why we add lactic acid in the fermentation broth, it produced because this lactic acid act as a preservative for this for storing this yeast cells.

During the same period the Delbruck, this is another scientist was endeavoring to determine the most favorable temperature of lactic acid fermentation in distilleries. Then he concluded that relatively high temperature about 45 degree centigrade, 44-45 degree centigrade there is high yield of lactic acid. Then 2006 global production of lactic acid reached about 275000 tons with an average growth of 10 percent.

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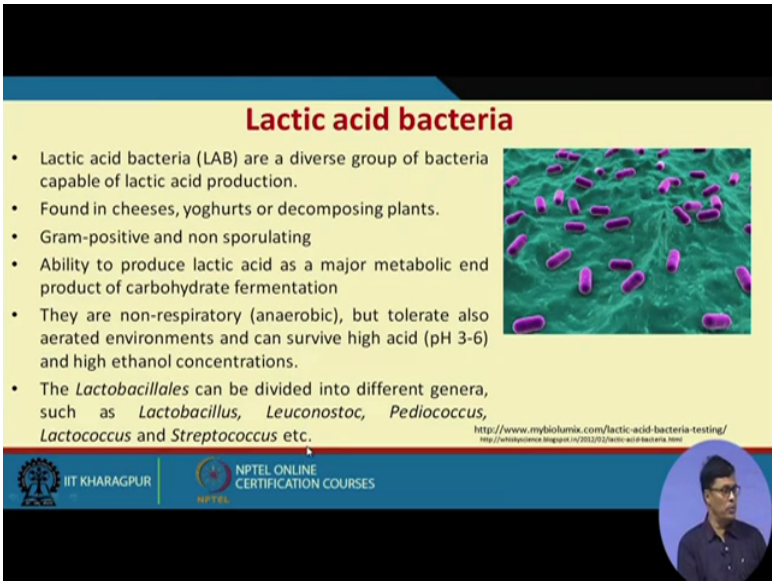

So this indicates the importance of lactic acid has the industrial sector. Now lactic acid bacteria are diverse group of bacteria capable of producing lactic acid, because in the day to day life we take we use the curd and curd contains also lot of lactic acid bacteria. Here we have mentioned Lactobacillus, Streptococcus lactates is the largely present in our curd. And found in cheese, yoghurts and decomposing plant. It is gram- positive and non sporulating bacteria, the ability to produce lactic acid as the major metabolic end product of carbohydrate fermentation. They are non-respiratory that anaerobic but tolerate also aerated environment and can survive pH 3 to 6 and high ethanol concentration.

So here I just want to emphasis that this is a micro aerophllic fermentation process, because due to air is required for this fermentation process also but it is also anaerobically it can also grow. The Lactobacilli can be divided into different genera, such as the Lactobacillus, Leuconostoc, Pediococcus, Lactococcus and Streptococcuss.

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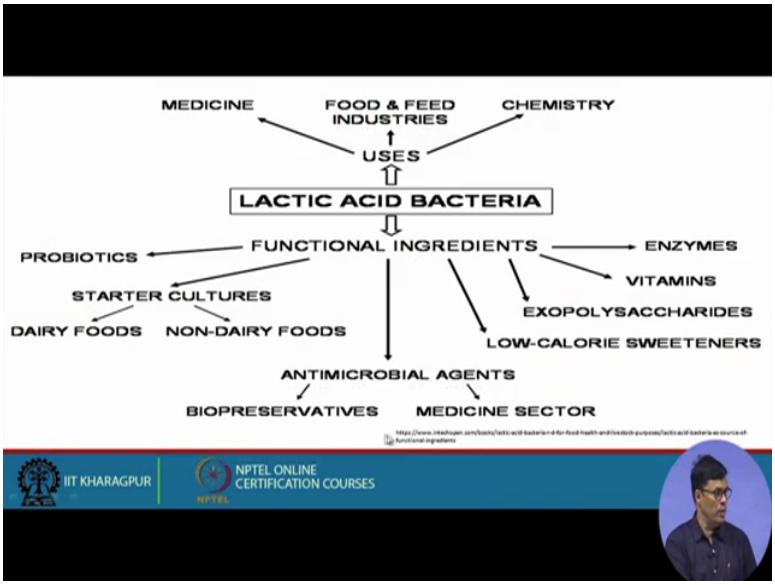

Now I was talking about the potentiality of lactic acid in the different sectors. First I told you already that in the medicine we have lot of use lactic acid. Then the food we have the Food and Feed industries we have lot of use of this lactic acid. I can give the example of preservation of vegetables, as for example that cucumber and cabbage you know that if we can store if you dry it and put it in a polythene pouch, you can store it for longer period of time. But problem is that when you dry this cabbage and cucumber, due to Brownian reaction this color will brownie because the color the yellow color will be lost.

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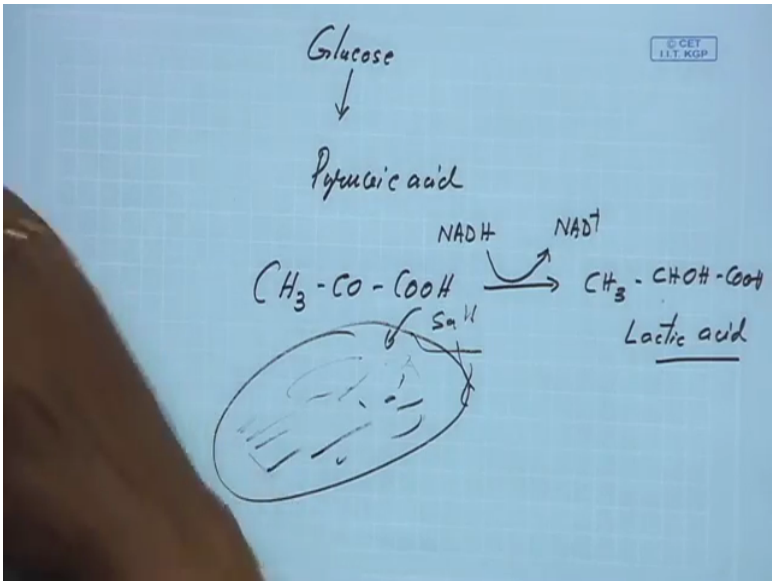

So you can preserve but you know the color will be lost, the natural that is a green color will be lost. Now question comes, how you can preserve this vegetable in the natural form for a

longer period of time? And this can be done by using these lactic acid bacteria, how? Just in a polythene pouch I can tell you, this is a polythene pouch, inside the polythene pouch you can keep the shredded vegetables or shredded cabbages and then use some salt and then you close it you can close it here with the help of rubber band or something like this. It will as soon as you use some salts, some water will be secret out from the vegetable, and this water lactic acid bacteria will grow, and due to the production of lactic acid this lactic acid bacteria automatically will preserve the vegetable for longer period of time.

This largely used in the food and the feed industries and chemistry also it is quite importance. It is used as the probiotics, nowadays lot of pharmaceutical industry they market it probiotic as a medicinal aid, because this is the good bacteria that is required for our digestive track. So you know that they produce this bacteria and use as a in the form of market it in the form tablet.

And starter culture for the dairy food and non-dairy food is used as the starter culture. Then we have antimicrobial agent, your bio-preservatives I have already talk about. Then medicinal sector I told you, told you that pharmaceutical sector that poly-lactic acid have tremendous potentiality. Then it can be used as a ingredients that is, enzymes, vitamins, exopolysaccharides and low calorie sweetener.

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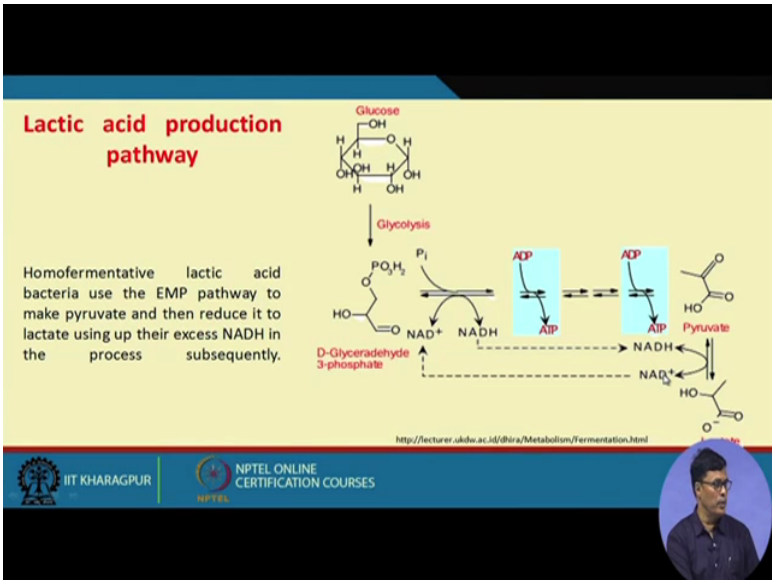

Now if you look at the lactic acid production is two type of fermentation usually carried out for lactic acid fermentation process? One is called homo-fermentative process and hetro-fermentative process. Now question comes what is homo-fermentative process? Homo-

fermentative process usually that we follows the Indole Miro Pathway for mostly we get the lactic acid as the product. But when we use the hetro-fermentative process that it follows the pentose it follows the Pentose Pathway it follows the Pentose Phosphate Pathway.

And so let me talk about the and what the in the hetro-fermentive process the problem is that the product not only that lactic acid with lactic acid will get some other additional product, as for example ethanol and other things carbon-dioxide it will form in the fermentation broth. So homo- fermentative process how it forms I told you this it follows the EMP pathways. In the EMP pathways we get the pyruvate acid and I showed you here the pyruvate acid when you NADH that is it pyruvate that NADH is converted to lactate this is how it is produced.

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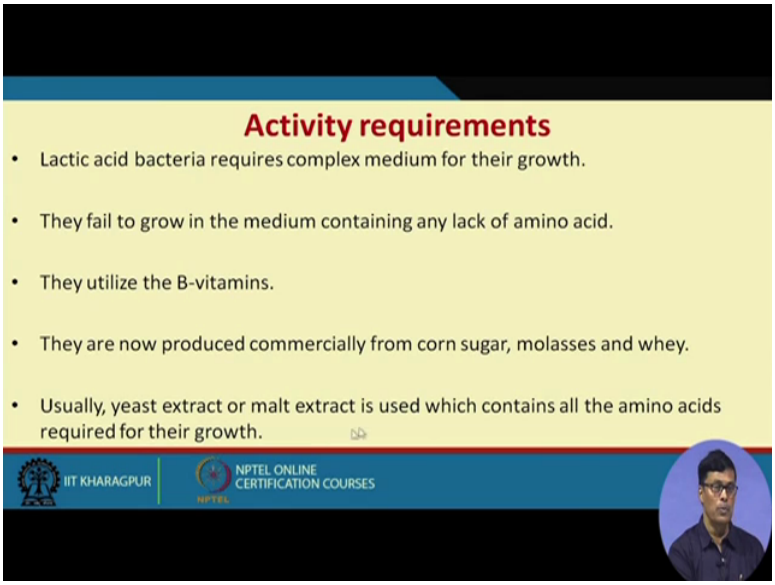

Activity required the lactic acid bacteria requires the complex media for their growth. Now here I want to point out that, what do you mean by complex media and synthetic media? Complex media means that when if the composition of the media is not well defined. As for example when you use in the media like yeast extract any kind of natural product it is very difficult to define in the composition of the media. But when you use any kind of pure chemical it is possible to define the exact composition of the media.

Now if the composition of the media is well defined we will call it a synthetic media and when it is undefined we call it as a complex media. So lactic acid requires complex media for their growth. Then they fail to grow in the media containing lack of amino acid. Then they utilize the B-vitamins and they are now produced commercially from corn sugar, molasses and whey. Whey is the very good raw material which contains very good amount of lactose

and it is very good raw material for the lactic acid production. And usually the yeast extract or malt extract is used which contains all the amino acids required for the growth of lactic acid bacteria.

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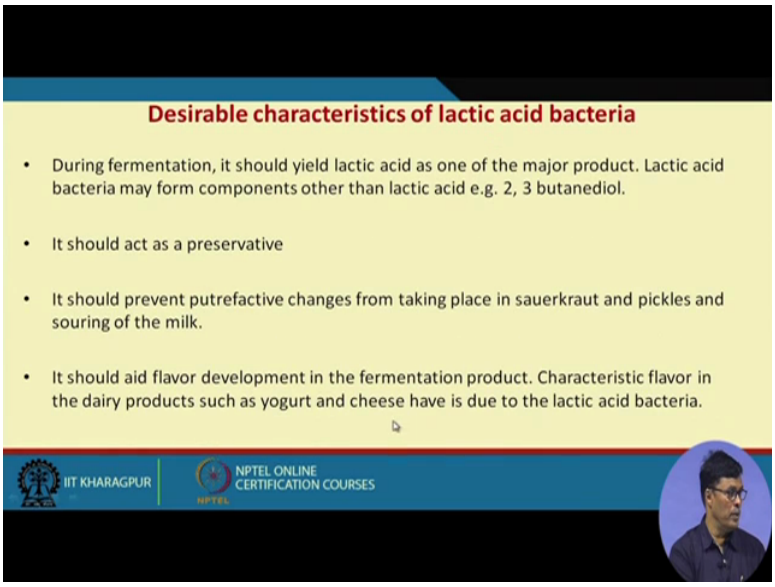

Now desirable characteristic of the lactic acid during the fermentation it should require the lactic acid one of the major products. Lactic acid bacteria may form components other than lactic acid as for example 2, 3 butanediol. It should acts as a preservative; it should prevent the putrefaction changes that taking place in sauerkraut. I told you sauerkraut with largely (()) (17:08) it prevents the putrefaction. Putrefaction means degradation of the proteins and pickles and souring of the milk. It is it should aid flavor development in the fermented fermentation product, characteristics flavor of the dairy products such as yoghurt and cheese have is due to the lactic acid bacteria.

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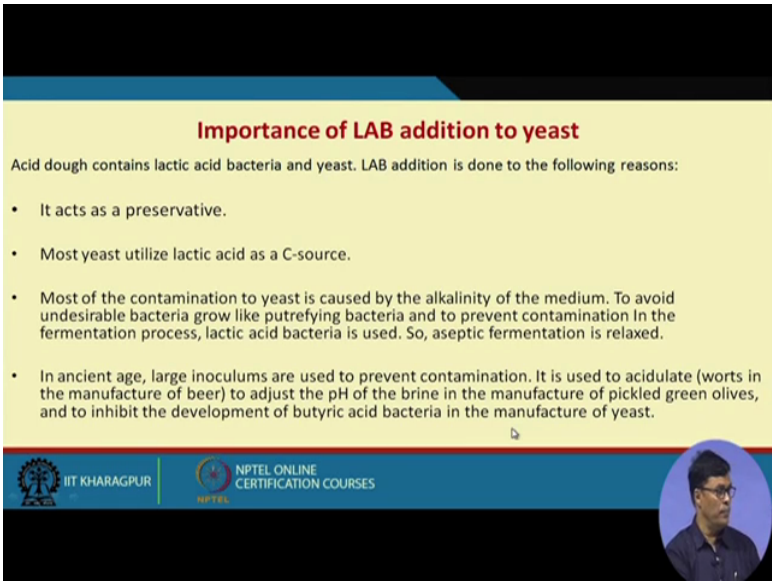

The importance of lactic acid bacteria addition yeast cell, I already discuss I also told you that a purposefully we add the lactic acid bacteria in the yeast fermentation process, because it acts as a preservative, you see that it acts as a preservative. Most yeast utilizes lactic acid as source of carbon, most of the contamination to yeast is caused by the alkalinity of the media. To avoid undesirable bacteria grow like putrefying bacteria. To prevent contamination in the fermentation process, lactic acid bacteria is used, so aseptic fermentation is relaxed. In ancient age large inoculums are used to prevent the contamination, it is used as acidulate to adjust the pH of the brine in the manufacture of the pickled green olives and to inhibit the development of butyric acid bacteria in the manufacture of yeast.

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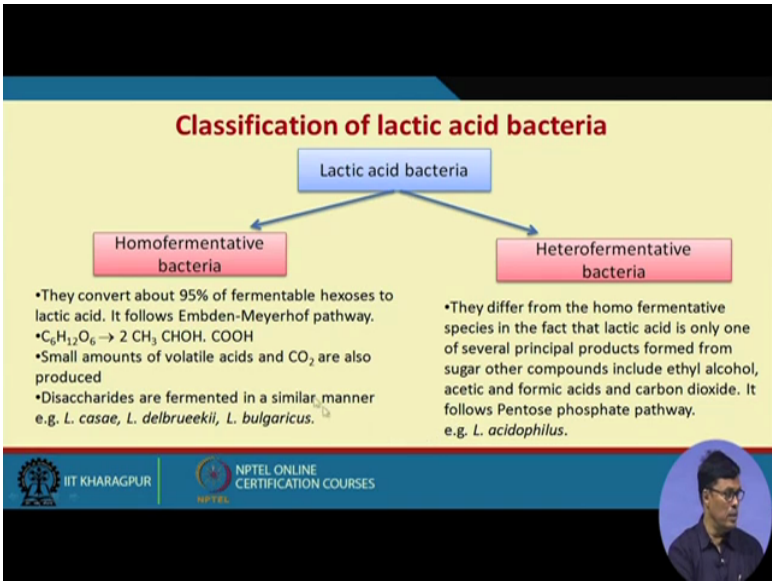

Now this is the again this is the differentiation between the homo-fermentation the homo-fermentative bacteria and the hetro-fermentative bacteria. As I pointed out that here mostly the product we have the lactic acid and carbon-dioxide and may be small amount of volatile (())(18:58) acid is produced and most the bacteria is used this fermentative process that is lactobacillus-casea, lactobacillus-delbrueekeii, lacto-bulgaricus.

Now I want to point out here in the large scale application mostly we use either lactobacilli-delbrueekii or lactobacillus-bulgaricus. And homo-fermentative process they differ from homo-fermentative process species in the fact that it produces the ethanol includes ethanol, acidic acid and formic acid and carbon-dioxide. It follows the pentose phosphate pathway and organism that is lactobacillus-acidophilus that is used.

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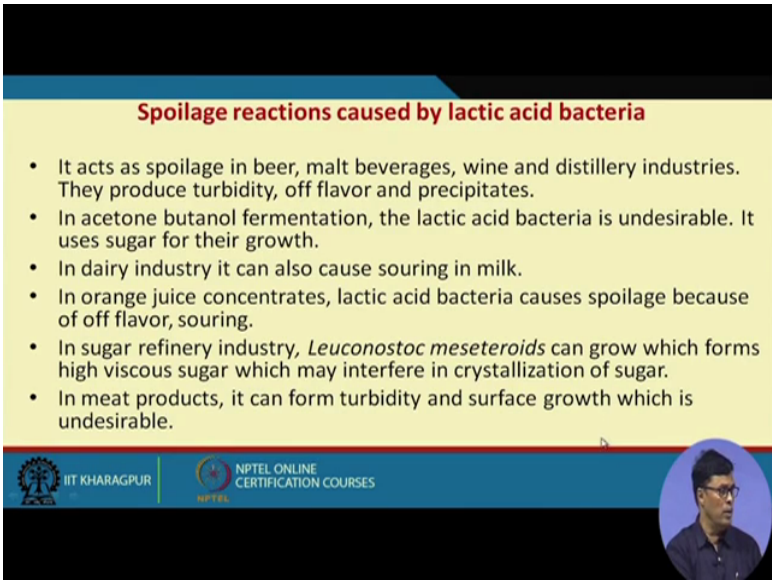

Now spoilage reaction this lactic acid has some causes some kind of that problem with this industry, as for example that it acts as spoilage in the beer, malt beverages, wine and distillery industries. They produce turbidity, off flavor and precipitation. In acetone butanol fermentation, lactic acid bacteria is undesirable, it uses sugar for their growth. In dairy industry it can use it can cause the souring of in milk.

In orange juice lactic acid bacteria causes spoilage because of the off flavor and souring. In sugar refinery the leuconostoc meseteriods can grow which forms a viscous sugar which may interfere the crystallization of sugar, this is also very important. In meat product it can form turbidity and surface growth which is undesirable, so lactic acid bacteria not only used for has all the advantages but it has also several disadvantages also.

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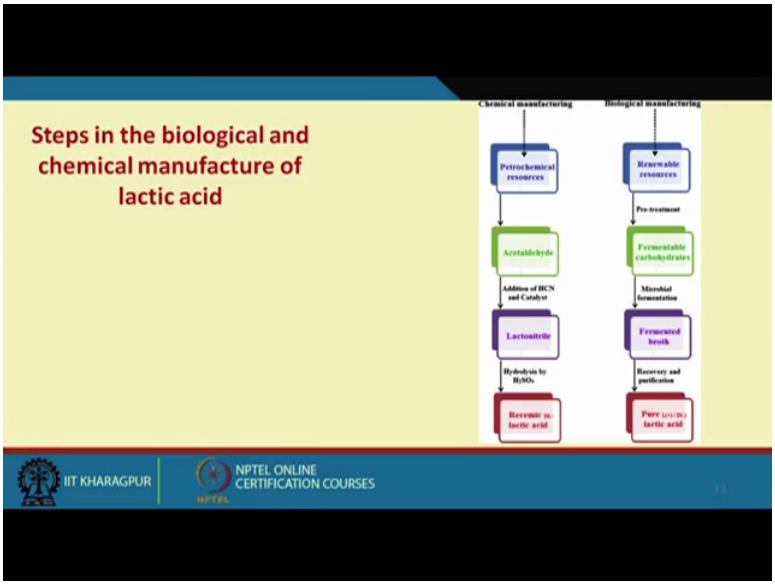

Now if we will compare that chemical and biological manufacturing of this lactic acid production it is like this, chemically we produce from petrochemical resources, it reproduces acetaldehyde the addition of hydrocyanine, then catalyst it produced the lactonytryl, then hydrolysis with H2SO4 it pose the recievic mixture of (())(21:13) de-lactic acid.

Now biological process is very simple, we have renewable resources I hope all of you understand what is called renewable resources? Renew means which is available again and again. As for example I want to tell you that any kind of agricultural product that is renewable, because we can reproduce again and again. So as for example if we talk about starch, if we talk about glucose, if we talk about (())(21:47) material all are renewable resources, because you can produce again and again. So this renewable resources we can do the pre-treatment then we produce the carbohydrates, that is all soluble carbohydrate. This undergoes the fermentation process then we get in the fermentation broth then through the purification process we get the pure hail and de-lactic acid.

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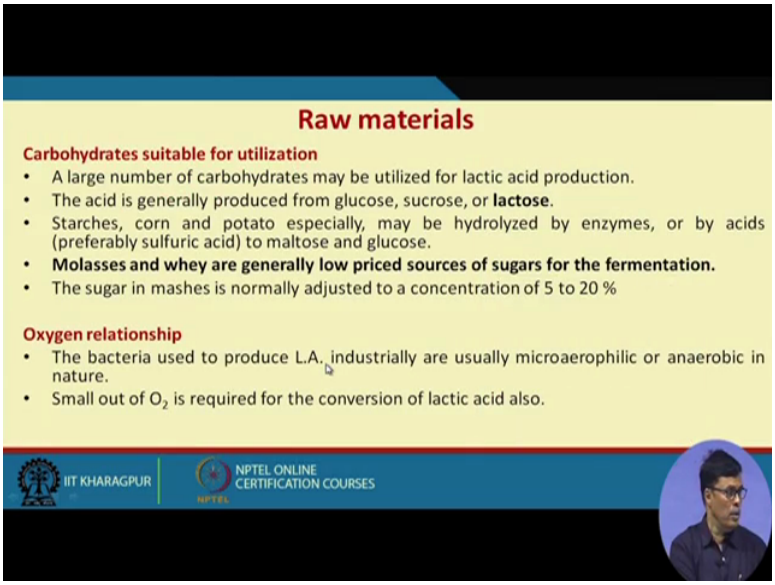

Now carbohydrates suitable for the utilization, large number of carbohydrate may be utilized for lactic acid production. The acid is generally produced glucose from glucose, sucrose and lactose. The starch, corn and potatoes specially, may be hydrolyzed by enzyme or by acid preferably sulfuric acid to maltose and glucose. Molasses and whey are generally low priced sources of sugar for the process of fermentation, because we have already seen that cane molasses is used as a raw material for citric acid industry.

Then sugar in mash is normally adjusted to a concentration of 5 to 20 percent. The oxygen relationship, that bacteria as I pointed out is used to produce lactic acid, industrially are usually micro-aerophllic or anaerobic in nature. Micro-aerophllic means it in it requires little bit of oxygen for their growth and metabolism also mostly grows on the anaerobic conditions. Small amount of oxygen is required for the conversion of lactic acid also.

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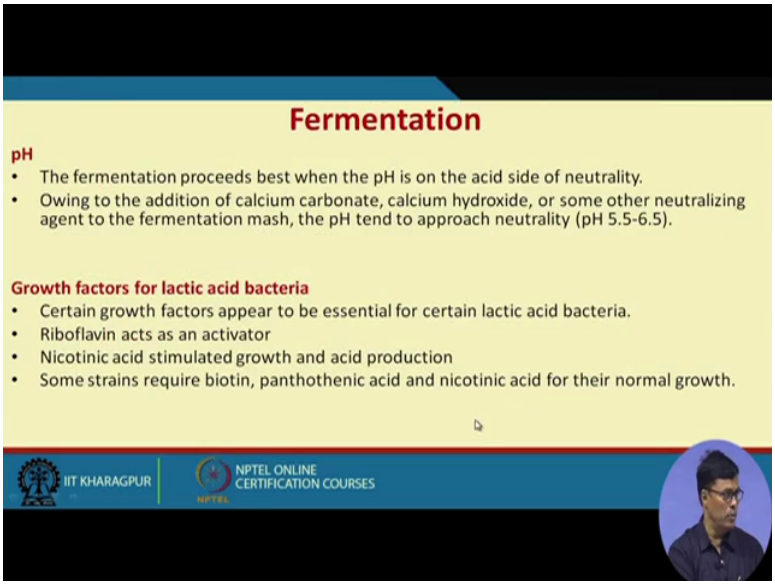

Now pH that is very important as per lactic acid fermentation is concerned. Here I want to tell you that we have seen that in case of citric acid fermentation process we allow the pH to drop down as low as possible that favor the citric acid fermentation process otherwise we will get the gluconic acid or oxalic acid in the fermentation broth. Now here the pH control is required, the fermentation proceeds between best when the pH on the acid side of neutrality. Owing to the addition of calcium carbonate or calcium hydroxide of some other neutralizing agent in the fermentation mesh, the pH tends to approach to the neutrality that is 5.5 and 6.5 is usually find most suitable for lactic acid fermentation process.

And since we are adding the calcium hydroxide, calcium carbonate, so mostly in the fermentation broth we get the calcium lactate as a product. Now then then we do the hydrolysis, acid hydrolysis to get the pure lactic acid. Now growth of growth factor of lactic acid certain growth factor appears to be essential for certain lactic acid bacteria. Riboflavin acts as a activator, nicotinic acid stimulated growth and acid formations. Some strain requires biotin, pantothenic acid and nicotinic acid for their normal growth.

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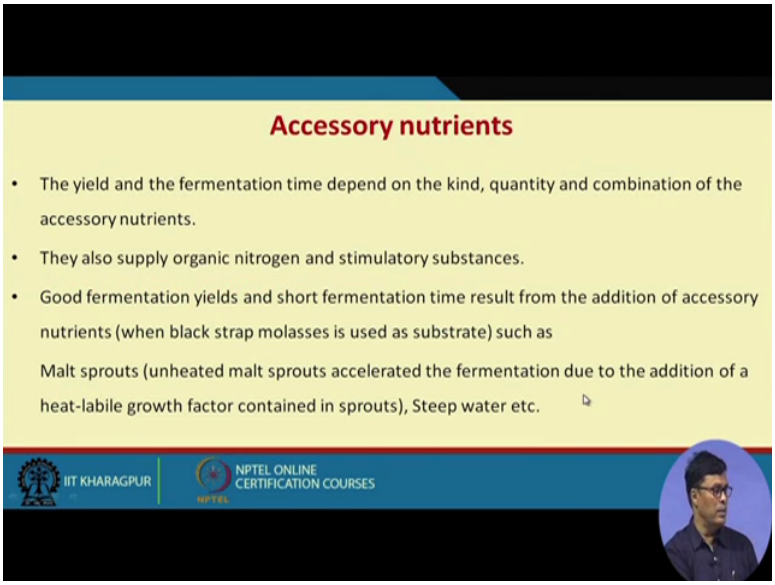

Now accessory nutrient, the yield and fermentation time depends on the kind and quantity and combination of the accessory nutrients. They also supply the organic nitrogen and stimulatory substances. Good fermentation yield, short fermentation time results from the addition of accessory nutrients, that is such as malt sprouts and steep water that is used for this as a accessory nutrients in this fermentation process.

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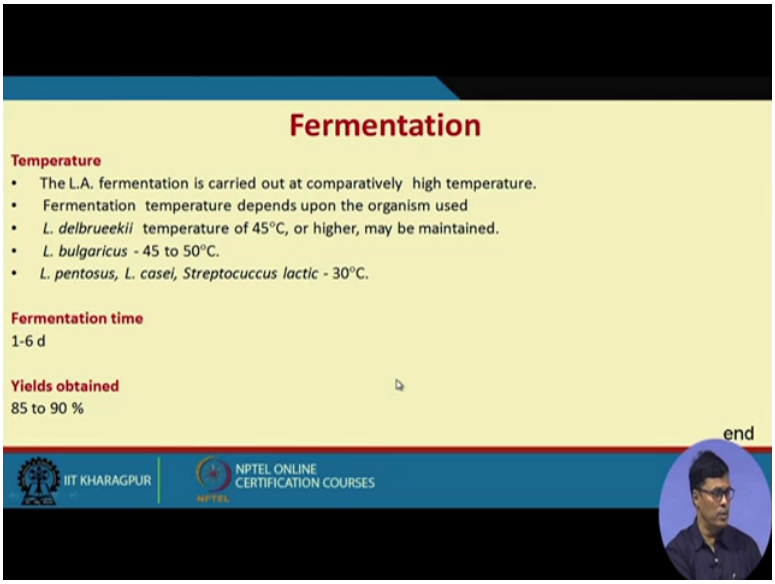

Now this lactic acid fermentation that usually as I told you that usually take place very high temperature, is usually 40 to 50 degree centigrade temperature. And it can use different carbohydrate, it can base, it can use lactose, it can use sugar, it can us glucose as a carbon source and also the starch. If you want to use the starchy raw material you will have to

hydrolyze to glucose or maltose. This can be used as a as substance for lactic acid fermentation process.

Now lactic acid is carried out comparatively high temperature, fermentation temperature depends on the organism used, as for example in case I told you that this 2 bacteria largely used by the industry, lactobacilli-delbrueekii and lactobacillus-bulgaricus. Lactobacilli delbrueekii requires the temperature of 45 degrees centigrade or higher, and may be that may be maintained and lactobacilli-delbrueekii comparatively little bit higher, because 45 to 50 degree. So lactobacillus-casei, lactobacillus-pentose and streptococcus lactic they require relatively low temperature.

And so this temperature usually favor for our homemade curd production. We know that in home we produce lot of curd and during curd making also this particular lactobacillus-casei, streptococcus largely grow in the curd. So this requires comparatively low temperature. But fermentation time is quite high, 1 to 6 days that is required, and yield is about 85 to 90 percent of the that sugar that is used in the fermentation media.

So lactic acid so in conclusion I want to tell that lactic acid is considered very useful raw useful product in the industrial sector, because it has several applications and particularly for food preservation it is largely used. I told you poly-lactic acid has large application in the pharmaceutical industries plus it has other several applications and this is usually produced by the lactic acid bacteria.

And 2 bacteria plays very important role what is called lactobacillus-delbrueekii and lactobacillus-bulgaricus and they are mostly they are homo-fermentative. The hetro-fermentative organism is also used for the lactic acid production, but homo-fermentative mostly we get the lactic acid as the major product and carbon dioxide besides little organic acids. So I think in this in the next presentation I shall show you the down-stream and up-stream processing of the lactic acid fermentation process. Thank you very much.