Course on Industrial Biotechnology
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Module 08
Lecture No 38
Glutamic Acid Production

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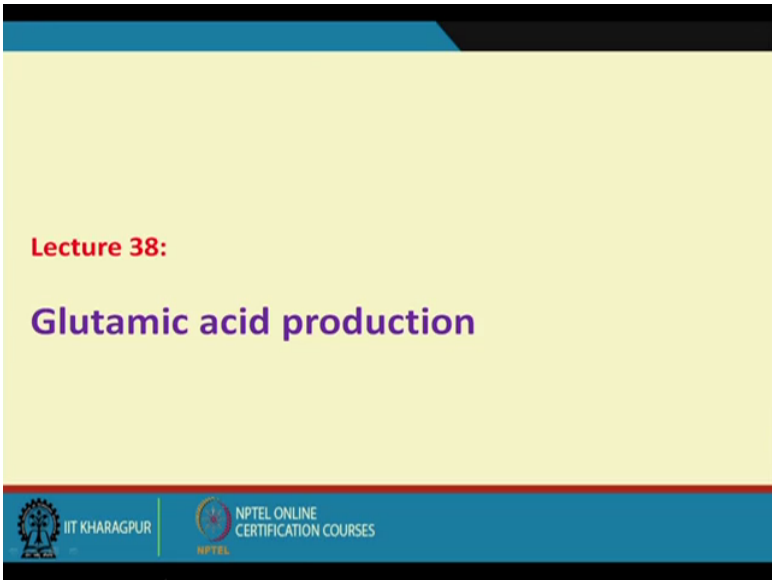

Welcome back to my lecture industrial biotechnology. Now today I want to discuss another new fermentation process that is the glutamic acid fermentation process. Now we know that that there are 20 essential amino acids that is very much required for us, because essential amino and there are 20 different amino acids that we have in nature, that is permutation combination they form the different type of protein molecules.

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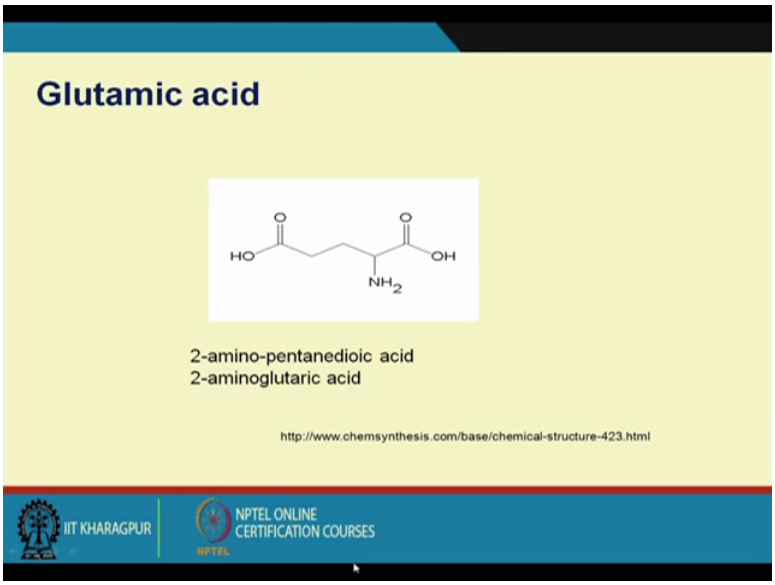

So in one protein molecule that is protein that one amino acid part of the protein molecule that is that plays very important role that that is the glutamic acid. Now let me tell you that little bit introduction to this glutamic acid, because glutamic acid is considered as 2 amino pentanedioic acids or 2 aminoglutaric acids. It can be either of this way we can so we can see that it has amino group, we know that all the amino acids they have the amino group and the carboxylic acid group plus we have CH2 and C00H that we have.

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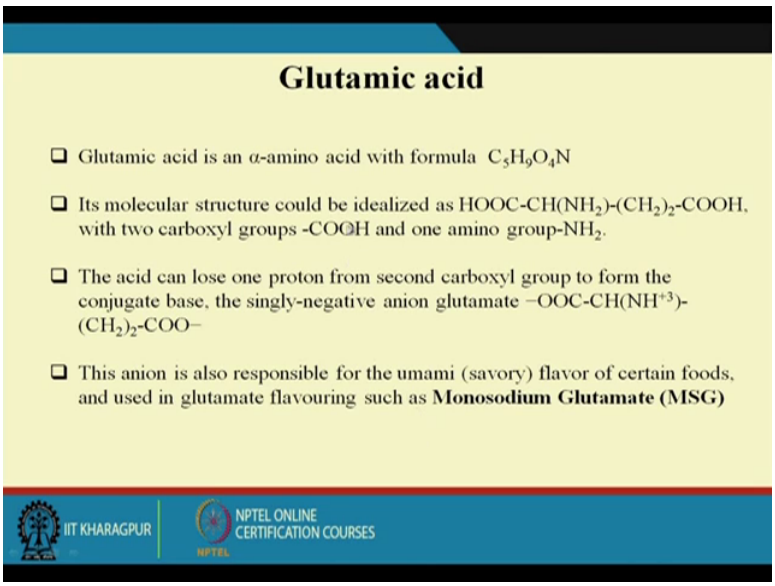

So if you l if you look at the glutamic acid is the alfa-amino acid with the formula of this. Now one thing I want to point out what is the importance of the amino acid in our day day-to-day life? Because it has been observed that utilisation efficiency of any protein depends on

the amino acid composition, because if the amino acid they are not present in the proper particularly essential amino acid they are not present in the proper way then the utilisation efficiency of that particular amino acid reduced to a great extent.

The now glutamic acid we use largely in the food item, because it has it gives some kind of typical flavour to the food that is. So it is chemically empirically it says that C5H9O4N this is the empirical formula that we have, and as I told you that it has 2 carboxylic acid group and CH2 2 NH2 and CH that is a basically for molecular structure is idealized in this form with 2 carboxylic group and 1 amino acid group.

Now the acid can lose 1 proton from 2nd carboxylic acid group to form a conjugate base and singly-negative anion glutamate. So this is how it forms the monosodium glutamate, because which is largely marketed that is this anion is also responsible for the umami flavour of certain foods and used for glutamic flavouring that is monosodium glutamate, in abbreviation form we call it MSG.

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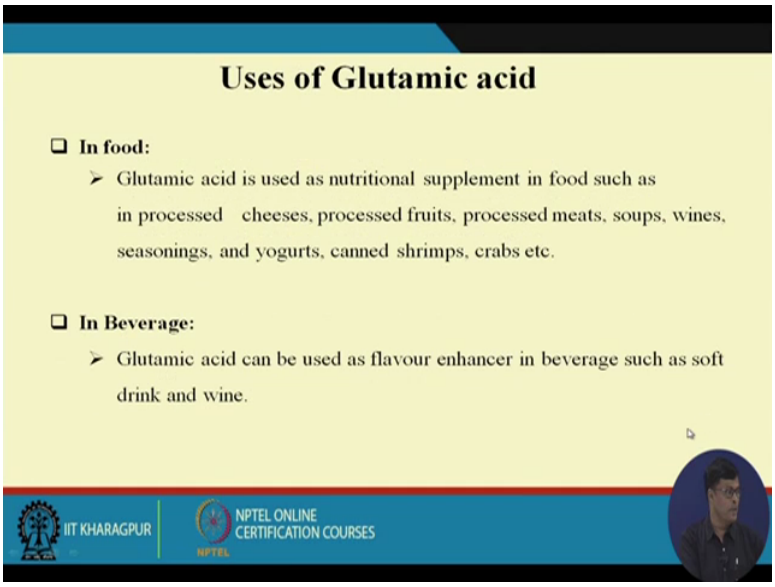

Now that glutamic acid is used as a nutritional supplement for food because as I told you if a food has shortage of kind of amino acid then we find that if you have weight balance than neutralisation efficiency increases to a great extent. Such as processed food like cheese, I have already explained what is cheese? Cheese is a kind of fermented food that is used for the storing the milk, protein and fat. And processed food, we know the processed food particularly we have several processed food now we have in our market because whatever food that is available in a particular season.

So due to the invention of this canned food or processed food it is possible to have the particular food throughout the year, because we can preserve this for throughout the year. We have processed meats like this particularly for military people who walk in a very remote area they used this processed food. And soups then we have wines, seasoning, yoghurt, canned shrimps and crab. These are the different food we use glutamic acid the rate it as a nutritional supplement as well as it gives some kind of flavour to it. Then in beverage glutamic acid is used as a flavour enhancer, in beverage such as soft drinks and wines.

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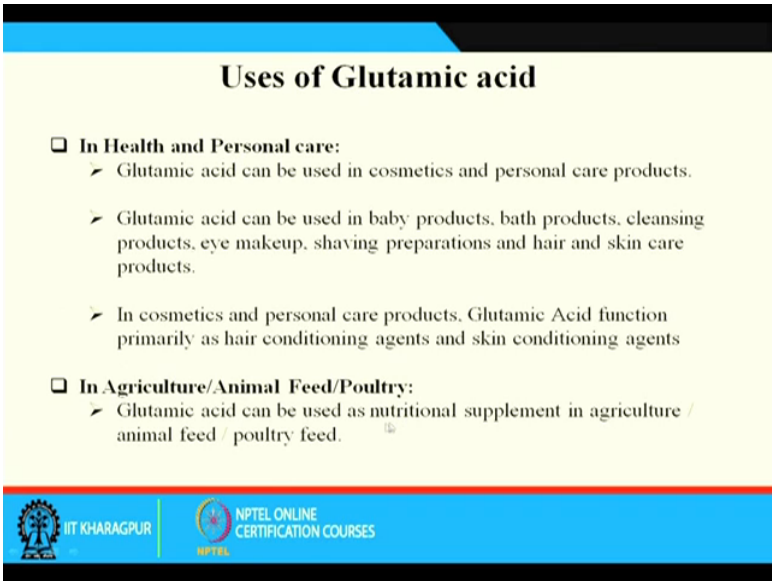

Now use of glutamic acid in health and personal care. Glutamic acid can be used in cosmetics and personal care products, because in day-to-day life there are several cosmetic products, so it used for the cosmetic and the personal care products. Glutamic acid can be used as a baby product, bath product, cleansing hair product, eye makeup, shaving preparation, hair and skin care products. Cosmetic and personal care products, glutamic acid functions primarily as a hair conditioning agent and a skin conditioning agent. This is now a day's lot of us is very careful about our skin as well as about our hair, so glutamic acid plays important role for keeping the quality of that particular material.

And agricultural and animal feed and poultry, glutamic acid can be used as a nutritional supplement in agricultural, animal feed and kind of poultry feed. Because I told you that utilisation efficiency of the protein directly increase if you used in any food product as the deficiency of a particular amino acids if you give it that utilisation efficiency increases to some extent. Particularly if you look at the essential amino acid, that plays very important role.

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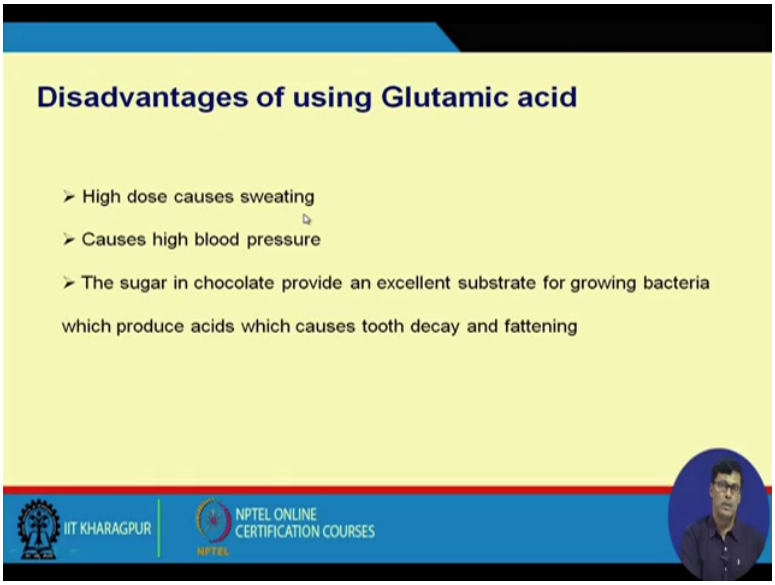

Now disadvantages of glutamic acid, there are several high doses causes the sweating, because this is the one disadvantage we have. It causes the high blood pressure; this is another disadvantage we have. The sugar in chocolate this is particularly for the children they take the chocolate to a great extent and the sugar in chocolate provides an excellent substance for growing the bacteria because this is amino acids, so bacteria can grow very because this is kind considered nitrogen source bacteria can grow very easily which produce acids that causes the tooth decay and fattening. So this is the problem that we have with this this chocolate, because chocolate they have detrimental effect, maybe due to this particular amino acid, because it helps for the growth of several bacteria.

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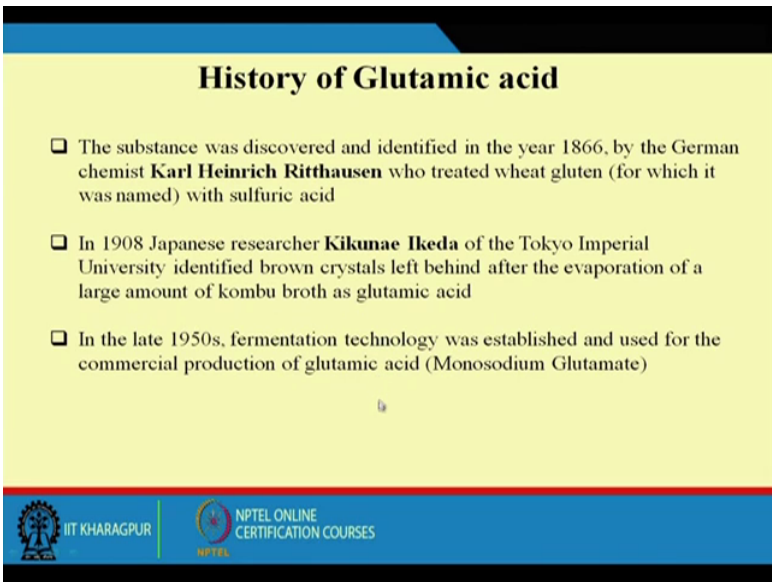

Now if you look at the history of the glutamic acid, the substance was discovered and identified in the year 1866, by German chemist was the Karl Henrich Ritthausen that who treated the wheat gluten for which he it was named with sulphuric acid. We all know that wheat contains lot of gluten, because that is kind of protein that we present in the wheat flour. So you know that so Karl identified that gluten present in the and he treated with this sulphuric acid and from that he recovered this kind of glutamic acid and since it is coming from gluten it is called glutamic acid, because name has come from that.

In 1908 Japanese researcher that is Ikeda of Tokyo Imperial University identified Brown Crystal left behind after the evaporation of large amount of Kombu broth as glutamic acid. The Kombu broth you know kind of fermented product that if you evaporate and after that that some brown crystal remain and they identified this as a glutamic acid. In the late 1950s the fermentation technology established and used for the commercial production of glutamic acid and mostly we target for the production of monosodium glutamate.

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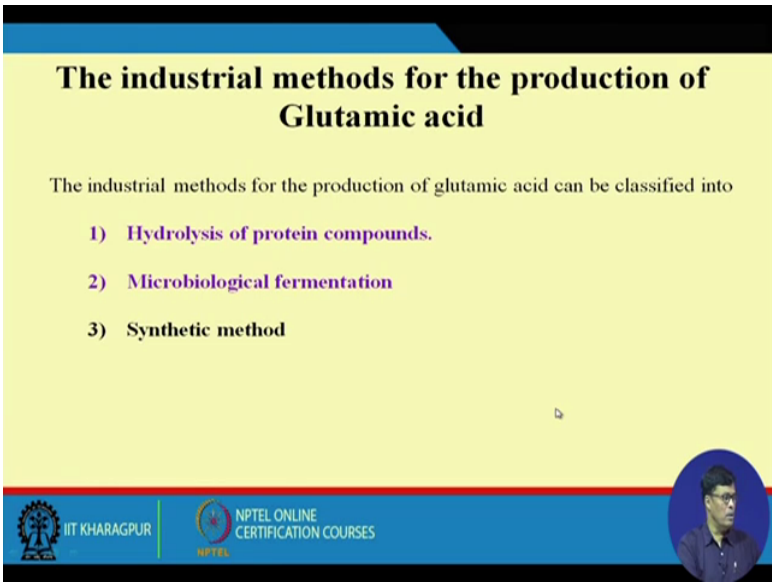

Now industrial method for the glutamic acid, both biochemical as well as chemical methods are available, because since this codes it is with the industrial biotechnology, so I shall try to concentrate on the biochemical processes. The industrial method for the glutamic acid can be classified as, hydrolysis of protein compounds. As I mentioned that first identification that done from gluten molecule by the by the German scientist Karls and then also the it is microbial fermentation process we can produce and besides that chemical process also can be used for the production of glutamate.

Now the first let me discuss how the protein through protein hydrolysis we can produce glutamic acid? Now this is the acid hydrolysis of the protein rich compound, the raw material used in this process are those protein reach in glutamic acid such as gluten, soya bean cakes and casein. So these are the material that usually considered as a raw material for the production of glutamic acid.

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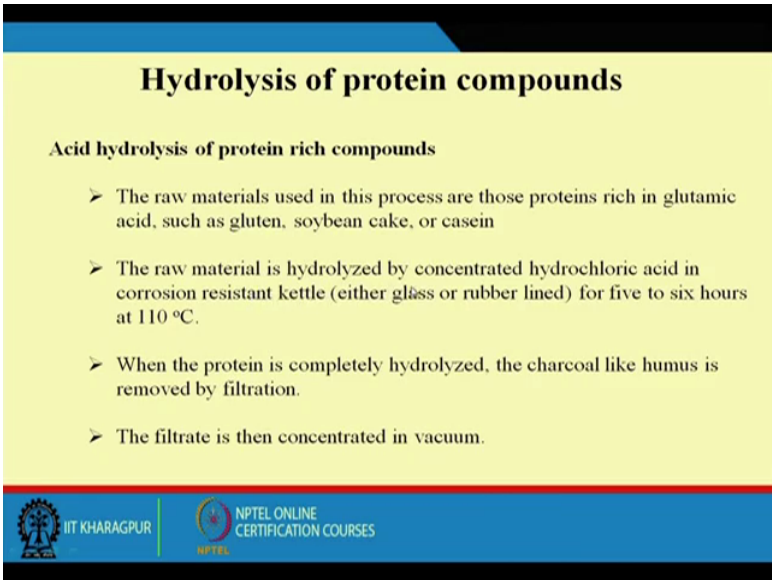

The raw material is hydrolysed by concentrated hydrochloric acid in corrosion resistant kettle, we know kettle is kind of you either made of glass or rubber lined for 5 to 6 hours at 110 degree centigrade. So we heat it in presence of this hydrochloric acid, then the material this material gluten, soya bean cake and casein they get hydrolysed and when the proteins is completely hydrolysed, charcoal like humus is removed by filtration, because and then the filtrate is then concentrated in vacuum that vacuum evaporation we can concentrate the filtrate.

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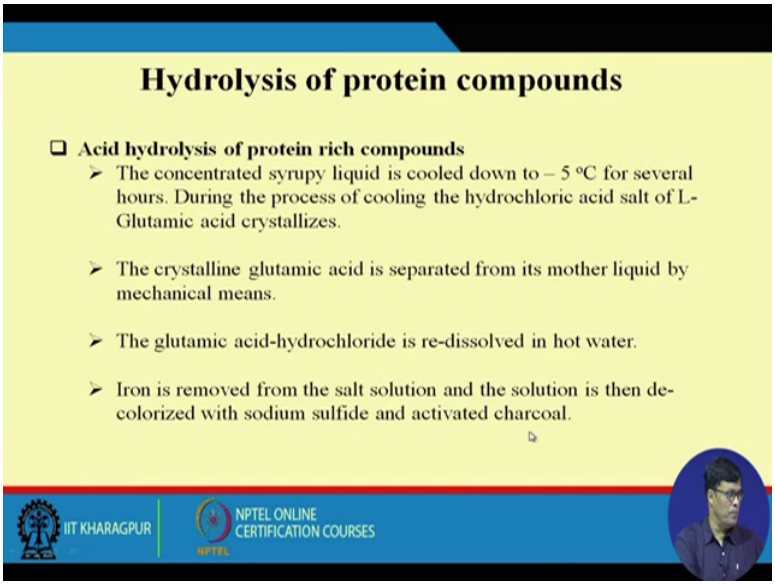

Now acid hydrolysis of proteins rich compounds, the concentrated syrup liquid is cooled down to minus 5 degree centigrade for several reasons several hours and during this process of cooling the hydrochloric acid salt of L-glutamic acid crystallises. So when you cool down then in the solution we have already seen in case of citric acid fermentation process also, as the temperature reduces then the citric acid crystals will we separated out. Here also it is similar to that if you when you make a solution of concentrated solution of this hydrolyse material and cool it down to minus 5 degree centigrade than crystalline of glutamic acid hydrochloride that will be crystallises out.

The Crystal glutamic acid is separated from the mother liquid by mechanical means. Mechanical means by centrifugation or by filtration this is kind of physical separation technique we used for separation of this crystals. The glutamic acid hydrochloride is re-dissolved in hot water and iron is removed from the salt solution and the solution then de-colourised with sodium sulphide and activated charcoal. We have already pointed out activated charcoal largely used in the industry for the decolourisation of the solution which is largely used in chemical and biochemical industries.

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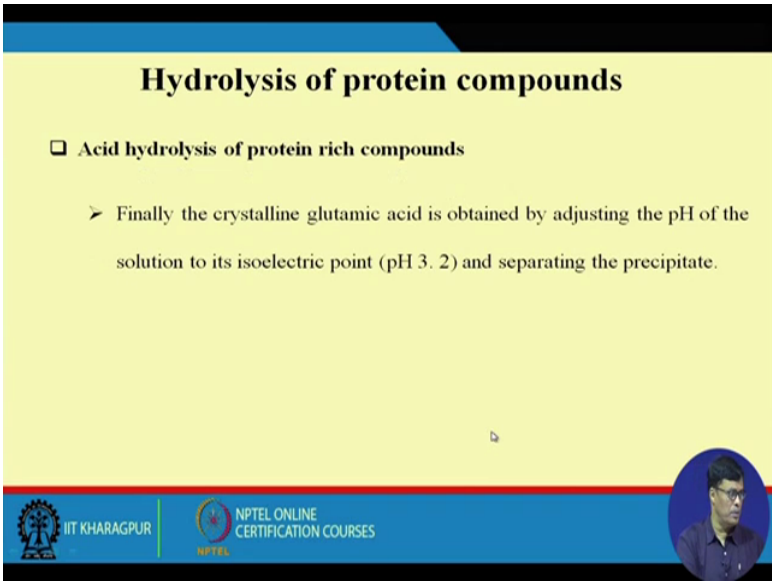

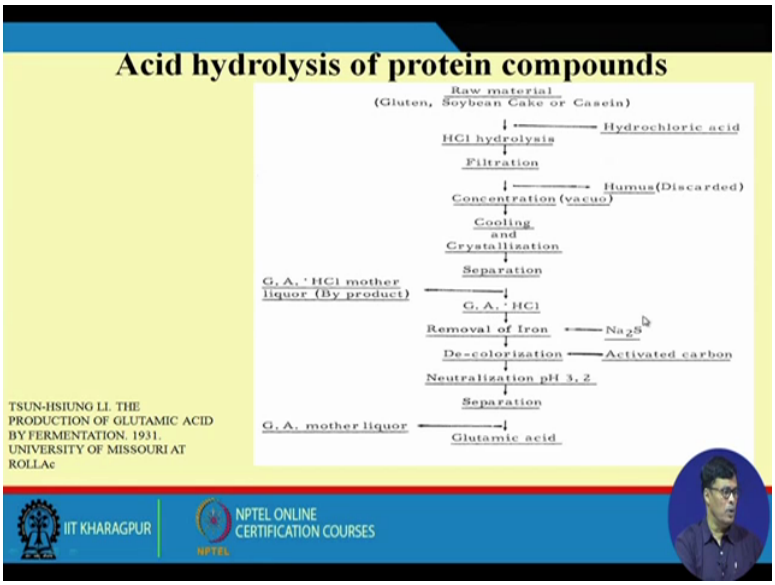

Now, finally the crystalline that glutamic acid obtained by adjusting the pH of the solution with the isoelectric point pH 3.2 and separating the precipitate. This is the finally we can separate out particular this glutamic acid like this. Now if you look at the steps involve in this particular glutamic acid for that process from protein hydrolysis, so we take gluten, soya bean cake and casein.

First we hydrolyse with respect by adding the hydrochloric acid the hydrolysis takes place due to the filtration to separate the humus or kind of organic material that we have and then we concentrated in vacuum, then we cool it down and crystallises and the crystals we separated out we get the glutamic acid hydrochloride crystals in a the yeast cell mother liquor you can make a solution, then add sodium sulphide and then for removing the iron then we

add activated carbon to remove the colour and then we adjust the pH to 3.2 and then we separate the glutamic acid mother liquor, we separate the glutamic acid that is how it is done.

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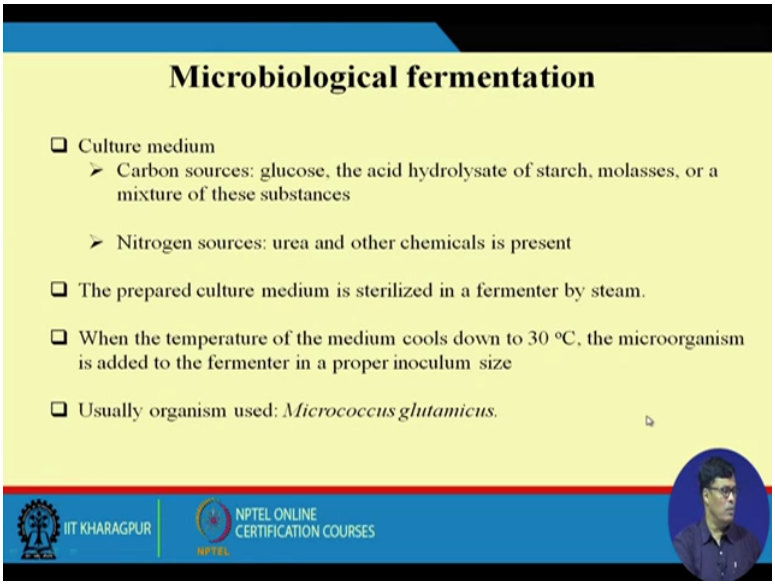

Now let me tell you something about the microbial fermentation process, how it is done? The here is the microorganism that is used is Micrococcus glutamicus this is the kind of microorganism that is used for the glutamic acid production. Now, in the culture media we require different carbon source like glucose or acid hydrolysate of starch, what do you mean by acid hydrolysate of starch? Because when we hydrolyse the starch molecules, so we get oligosaccharides, we not only get only glucose we get monomer, we have dimer, trimer or different type of oligosaccharides. So that considered as a acid hydrolysate, then molasses and a mixture of this substances. Nitrogen sources we use the urea and other chemicals is present.

So as per the requirement we know that for the growth of bacteria we do not carbon source, we do not nitrogen source, minerals and vitamins. The prepared media is sterilised in a fermenter by steam and when the temperature of the media cools down to 30 degree centigrade, microorganism is added to the fermenter in proper inoculum size. I told you that inoculum size of the industrial fermentation process or normal fermentation process is usually vary from 5 to 10 percent volume by volume. Now, in some cases the volume might be more so you know that but usually the volume of the inoculum should be 5 to 10 percent.

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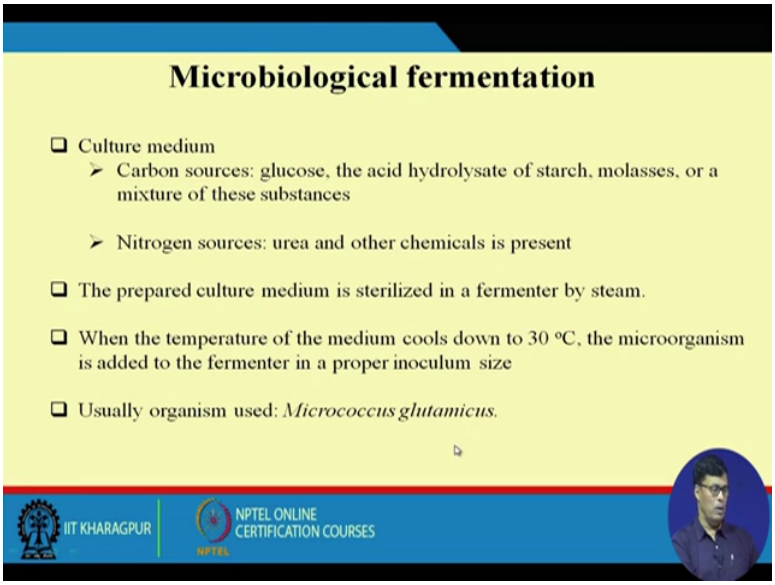

Now microorganism is inoculum incubated 36 to 48 hours during which the time the pH, temperature and aeration rate are carefully controlled. Because this is the aerobic fermentation process, so all the things are and you know I told you that typical property of the living system they are very sensitive to the environment, so temperature and pH and because aerobic organism take take the oxygen that is dissolved in the media.

So this is to be maintained properly so that the growth of the organism takes place. When the fermentation is finished, fermentation broth is hydrolysed with hydrochloric acid and glutamic acid is obtained in a process analogous to that for the recovery of the protein hydrolysate. So it is the that we after the of the after the fermentation where we get the different cell mass and other products and that is hydrolysed by using the hydrochloric acid and we have seen in protein hydrolysate we use hydrochloric acid and during this hydrolysis we produce the glutamic acid, similar to that process is used.

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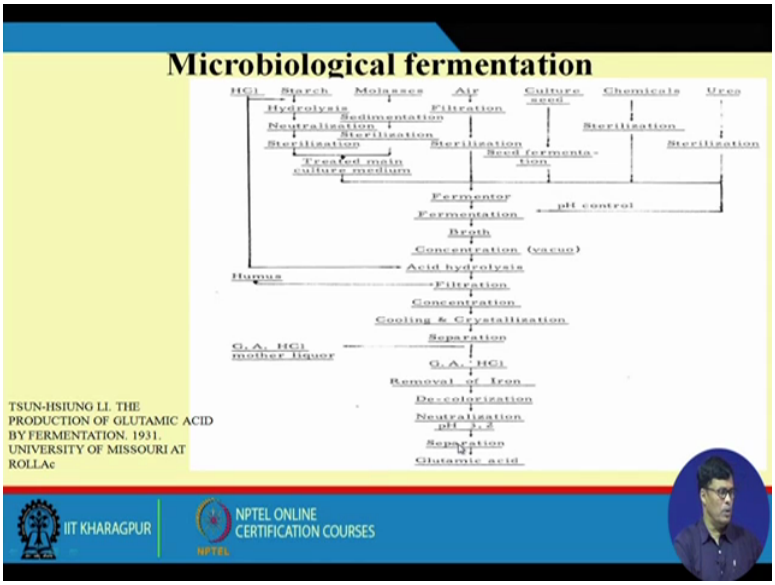

Now if you look at the steps involve for this process is like this, we use the different, we use the HCL, we use starch, hydrolysis we use the HCL and so that we get the soluble starch usually the insoluble molecules. Some soluble starch is also there but you know that that is to be degraded to the monomer or dimer so that your organism can utilise it. So then molasses it then it contains good amount of sugar we require air, because for in case the dissolved oxygen concentration the culture seed we require the chemicals, we require as per the required for the organism and urea is considered as the nitrogen source.

Now when hydrolyse that acid the first steps that we have the neutralisation, because when we hydrolyse with acid the pH of the solution will decrease to a great extent. So you have to adjust the pH and to the extent into the pH that is whatever is required for the fermentation process. Then we do the sterilisation and treated with main cultural media, because then we mix this together maybe all these things together and we sterilise that we that you know chemicals, urea we mixed together, take it to the fermenter then we sterilise then we pass air to it and then we add culture to it. So this is the first we add cultures, then we do the aeration then we do the pH control as per the requirement of the process.

Now after fermentation is over after 36 to 48 hours we get the fermentation broth then we concentrate by vacuum that and then we again put the acid for acid hydrolysis. And then here also like protein we have seen that gluten and other molecule that when you do the acid hydrolysis humus carbonaceous material like humus formation is taking place. So that humus you separate it out through the filtration process then filtrate you concentrate cooled and

crystallise and separation takes place where you can get 2 type of material after the separation of glutamic acid. Crystals of hydrochloride you will get in the crystal form, also you get good amount of glutamic acid hydrochloride in the soluble form in the mother liquor this you can put it back here and concentrated further so that more glutamic acid can be recovered.

And here also like protein hydrolysis we have to remove the iron by using sodium sulphide, you have to use the decolourisation by using activated charcoal, then you have to do the neutralisation of 3.2 as well similar to activated in case of hydrolysis of protein, then separation we get the glutamic acid, so these are the process through which we can get the glutamic acid.

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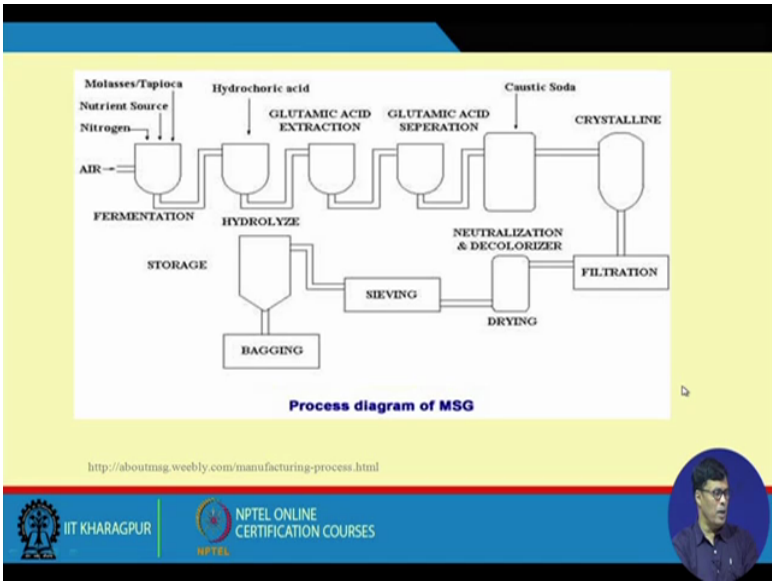

Now if you look at the process flow diagram, how we produce in actually in practice we have molasses, we have topioca or then we have nutrient source, we have nitrogen, we have air then we take it to the glutamic acid this is carried out in a fermenter; this is the aerobic fermentation process. Then we had hydrochloric acid and this is the glutamic acid extractor, then glutamic acid separator, we adjust the pH with caustic soda, then crystallisation of the glutamic acid it takes place filtration you dry the crystals, sieving it and hydrolyse and then we put it in the plastic bags for storage. And this is how the glutamic acid is produced in the industry because this is the process flow diagram how it is produced.

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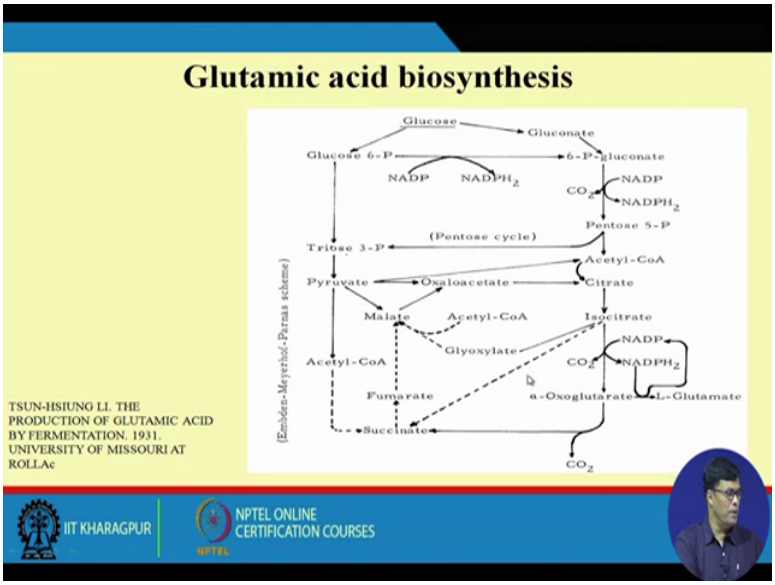

And if you look at the biochemical pathways, how the glutamic acid production takes place, that also very interesting that we know the TCA cycle, because in the TCA cycle first glucose when you take the glucose first for the aerobic and anaerobic organism they go first through the Indole Miro Pathway, after Indole Miro Pathway they produce the pyruvate acid and after pyruvate acid they form the acetyl CoA, then we have malate glyoxylate, the iso-citrate, citric acid, oxaloacetate, acetyl CoA, in combination they produce citric acid and this iso-citrate acid, and then this is form oxoglutarate and this oxoglutarate it produce the L-glutamate acid.

This is how through the biochemical pathways how we can how it is produces the glutamic acid and this is what is in practice in the industry. So this is all about the glutamic acid fermentation process, because I told you that glutamic acid is a amino acids. Amino acid is largely used as a food supplement, not only it is used as a flavour enhancer. Several product we use this glutamic acid and both glutamic acid can be produced by both biological means, by chemical means.

Biologically we produced by using protein hydrolysis as well as microbial fermentation process. When we use the protein hydrolysis, we use the gluten, we use different type of proteinaceous material, we hydrolyse with H2SO4 and that I told you that wheat starch is a good source of gluten, because whatever protein is present that in the wheat starch is the wheat is the gluten. That gluten when you hydrolyse with HCL then we glutamic acid we produced, then we separate out this humus from that and then we concentrate it and then we crystallise this and when we do the crystallisation then we can separate out the glutamic acid

and the mother liquor. Mother liquid also contains good amount of concentrated glutamic acid.

Now similarly microbial fermentation the glutamic acid the fermentation process also we can use for this is the bacterial fermentation process we use for the glutamic acid fermentation process. Main purpose is to produce the cell mass and then we dry it and hydrolyse with HCL, after hydrolysis of HCL then we usually follow the same process as we use for the protein hydrolysis and only the thing is that we use the sodium sulphide to remove the iron content in the fermentation broth and also we use the activated charcoal to remove the colour from that.

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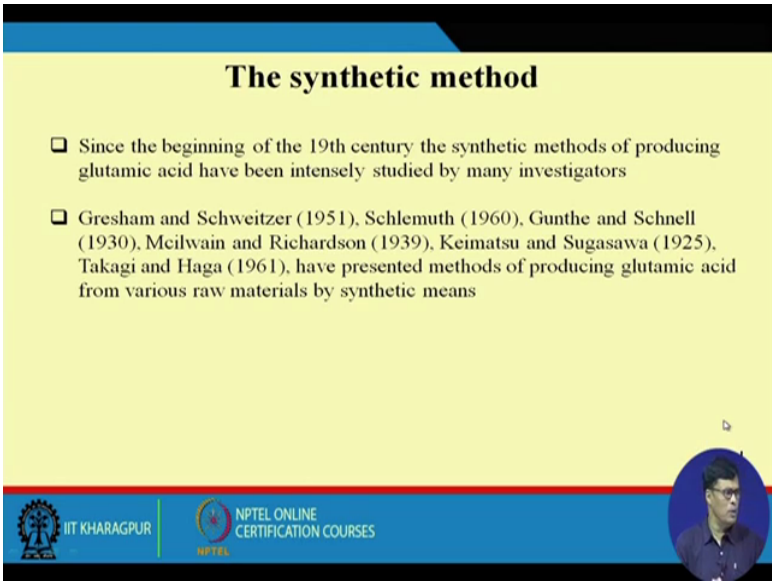

So only I can give you the small thing about the chemical process that you know the synthetic process that is used by the industry. Since the beginning in the 19th centuries the synthetic method of producing glutamic acid have been intensely studied by many investigators like Greshm and the Schlemuth and Gunthe they walk together and they found out have presented the method of producing glutamic acid from various raw material and by synthetic means. But I am not going into details about the chemical process because main purpose of the course is described the biotechnology process.

So we in short I can tell you that, like glutamic acid we can produce lysine also and we know that lysine is we stay in a country where rice is a staple food and rice the protein content protein has a lack of lysine and lysine if we supplement it with the rice, the protein neutralisation efficiency of rice increases to a great extent. So protein that particularly amino

acid, pre-amino acids we use in a different forms, we use not only by addition with the food products but we also doctors sometime recommends in the form of tonic that we use when we are we have some health problems they give this because there is some lack of amino acid might be deficiency is there then they add some in the tonic contains some kind of amino acid is required for a particular person. Thank you very much.