ENZYME SCIENCE AND ENGINEERING

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LECTURE – 26

APPLICATION OF IMMOBILIZED ENZYMES IN PROCESS INDUSTRIES

So far we have discussed various facets of enzymes starting from the chemical and functional nature of the enzymes, reaction kinetics, methods of immobilization of enzymes followed by the design and analysis of immobilized enzyme reactors merely from the point of view of their application in process industries and today we will take some examples of these applications particularly in process industries.

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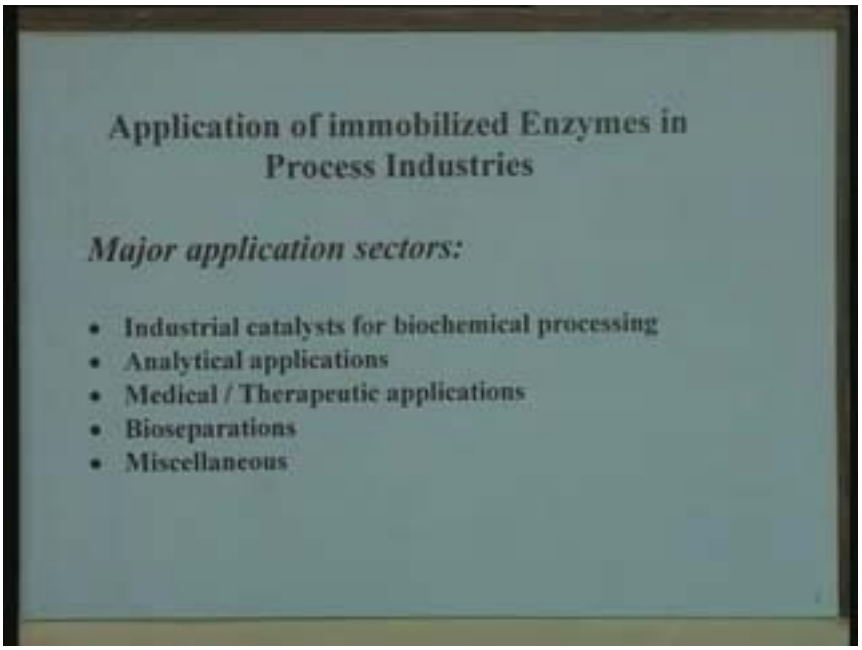

That means the use of immobilized enzymes as industrial biocatalyst in the industries. Well I will try to illustrate with a couple of examples which will give you an idea as to why we have been looking at the use of immobilized enzyme compared to either soluble enzyme or chemical catalyst. You will also appreciate that the use of enzymes particularly for an industrial application are often limited to the sections that are listed here. A major application sector is industrial catalysis whereby we replace a chemical catalyst for carrying out a chemical transformation to produce a desired product. Typical examples are hydrolysis of starch, hydrolysis of penicillin to produce six amino

penicillanic acid. Incidentally bulk of the applications are in the field of hydrolysis that means hydrolytic reactions. Although there are examples where other groups of enzymes have been used in an immobilized form for carrying out industrial processing their large scale applications have been so far limited. Bulk of the commercial application have been in the area of hydrolytic enzymes with the exception of glucose isomerase which is in the isomerase category.

The other group of application sector is analytical applications. Enzymes here have a very important role to play because of their specificity and many of the analytical situations which are enormously difficult in the case of chemical analysis can be handled very accurately and dependably by use of enzymes and to economize the operation of the analysis, the role of immobilized enzymes becomes more important. The immobilized enzymes will also find use in sensors that means the electrodes if you want to have an analytical tool in the form of an online sensor in that case one looks for the immobilized enzyme preparations which can detect a particular product of interest.

Then another area where probably cost or the process of economics is not important is the medical or therapeutic applications where the effectiveness is a very important aspect and in many cases even immobilized enzymes have found way in medical or therapeutic applications. The typical examples are the urease waste kidney that means dialysis unit, where dialysis units have been modified to include immobilized urease to facilitate the dialysis for patients who are suffering from kidney failure. The other one is on the use of aspergillus (4:58) for leukemia patients. Another application sector is bioseparations; the typical example is affinity separation which are based on the covalent coupling of one of the ligand for effecting the separation process. A very specific method is applicable either for very high value products or where the number of stages required are very large, they can be minimized to a small number. You have a whole range of small applications in different sectors probably the most prominent among the miscellaneous groups I will like to place is biological …… (5:42) treatment. Many of the specific systems require intervention of enzymatic catalysis for safe disposal of the waste emerging from a particular industry. There might be very small applications here and there where immobilized enzymes might find useful applications.

Today we will be looking at the first application sector where they are used mainly as industrial catalyst.

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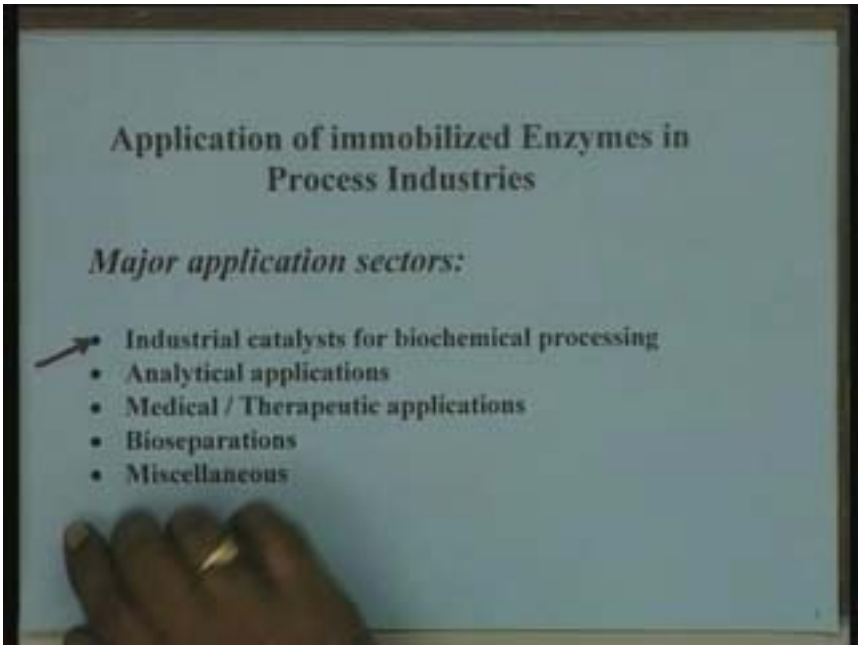

For their use as a industrial catalyst the important factors which have to be kept in mind are the yield of enzyme from native source; the original enzyme itself has to be chosen which is desirable for any industrial process both interms of operational characteristics as well as the effect of other impurity or other conditions which prevail in the feed stream. They must be able to tolerate it and the enzyme must be available when you say yield I am referring to a term which incorporates the cost part; because higher the yield, the cost can be lower. Then the second factor is cost of carrier and reagents for immobilization. The immobilization method, the carrier and the reagents required for immobilization is another factor which contributes significantly to the suitability of the use of an immobilized enzyme preparations for industrial processing. Then activity of immobilized biocatalyst, the final activity which has to be used for catalysis and operational stability of immobilized biocatalyst, the factor which determines the whole process economics. Finally regenerability of the carrier is a factor you will notice in one of the examples which I have given today that although the carrier is very expensive the cost factor here as we have mentioned may be high but if it is regenerable the cost can be taken care and does not contribute very significantly to the overall cost of the process.

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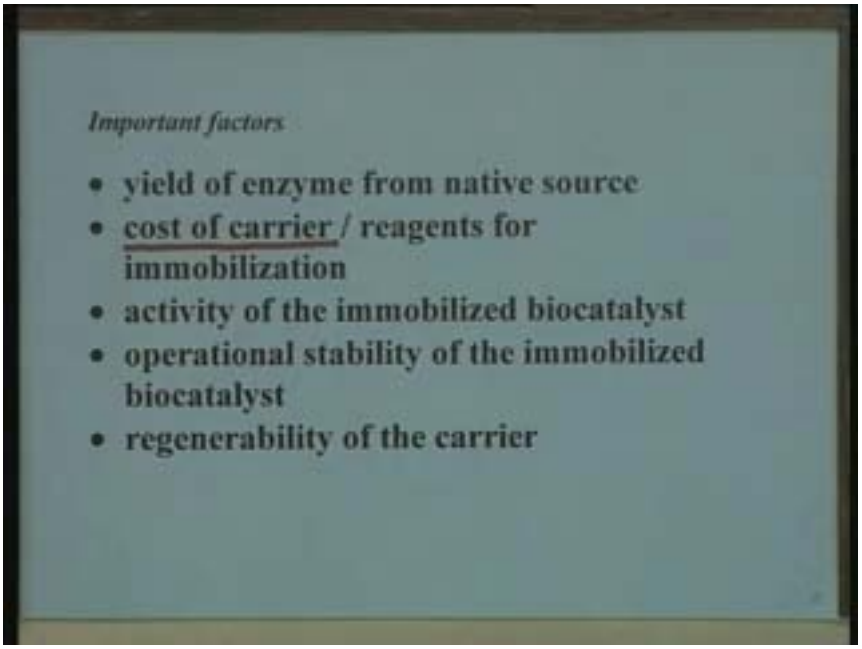

So regenerability can be a very, very positive feature and in most cases that has been the key factor.

If you look at variety of industrial processing that go in processing of foods, animal feeds those industrial factors which use primarily natural products as the feed stocks our enzymes are going to play a major role in those industries where the feed stock or raw materials are from natural source. That means the contents, carbohydrates, lipids and proteins or some other biomolecules which are often attackable by the enzyme molecules.

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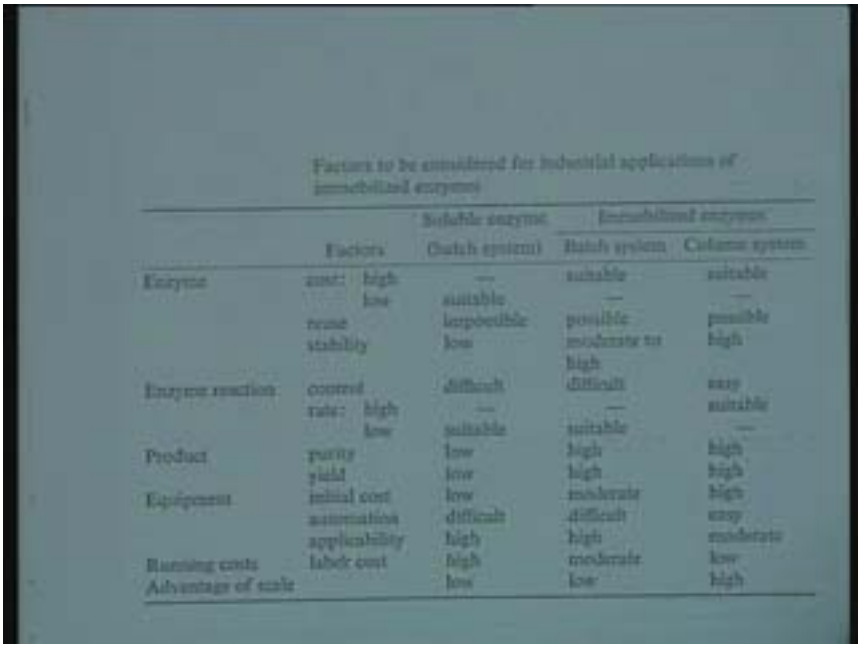

Some of the factors which in addition to the earlier components of an immobilized enzyme process which have emerged by virtue of experience in various processes are also like this. For example if you consider the enzyme and the three factors the cost, reuse and stability then the immobilized enzyme systems are very, very suitable for high cost enzymes; the cost of the enzyme is very high. In the case of enzymes with low cost even the batch system soluble enzyme may be suitable. An enzyme like alpha amylase is one of the example for low cost enzymes and is used in soluble form in batch reactor because you can afford to discard it; you need not recover it whereas high value enzymes are most suitable to be used in immobilized form and here when I say column system I am covering both either by packed bed or even it could be depending on the process a CSTR also; but here the column gives only column.

The reaction control in the case of soluble enzyme and the batch immobilized enzyme is usually difficult which makes it very easy in the case of a continuous flow immobilized enzyme reactor. The control of the enzyme reactors is rather difficult in the case of soluble enzyme batch processes or even immobilized enzyme batch processes and the control is much more effective in the case of a continuous flow reactor using immobilized enzyme. The product purity also has a role to play in decision making of the use of immobilized enzyme otherwise product purity can be very high in the case of a immobilized enzyme used in continuous reactors because there is no contamination from the enzyme protein. Some of the side reactions are likely to take place in the case of the continuous reactors and the residence times are usually lesser than the soluble enzymes or the batch reactors because the advantage of the smaller residence time in the continuous reactor as well as the absence of any contamination from the enzyme protein provides you a product which is much more pure compared to soluble enzymes or an immobilized enzyme in the batch systems.

The equipment part is slightly unfavorable in the sense that in the case of continuous flow immobilized enzyme reactor the initial capital investment or equipment may be comparatively higher compared to a batch soluble process or a batch immobilized process. The same thing applies to automation. In the case of batch processes there is no automation whereas continuous processes can be automated and which can make a lot of saving in the labor cost and running cost that means the labor cost are related by per unit weight of volume of the product are relatively low in the case of continuous reactor systems using immobilized enzyme and the processes with immobilized system can be easily scaled up compared to the batch processes which might find variations from batch to batch.

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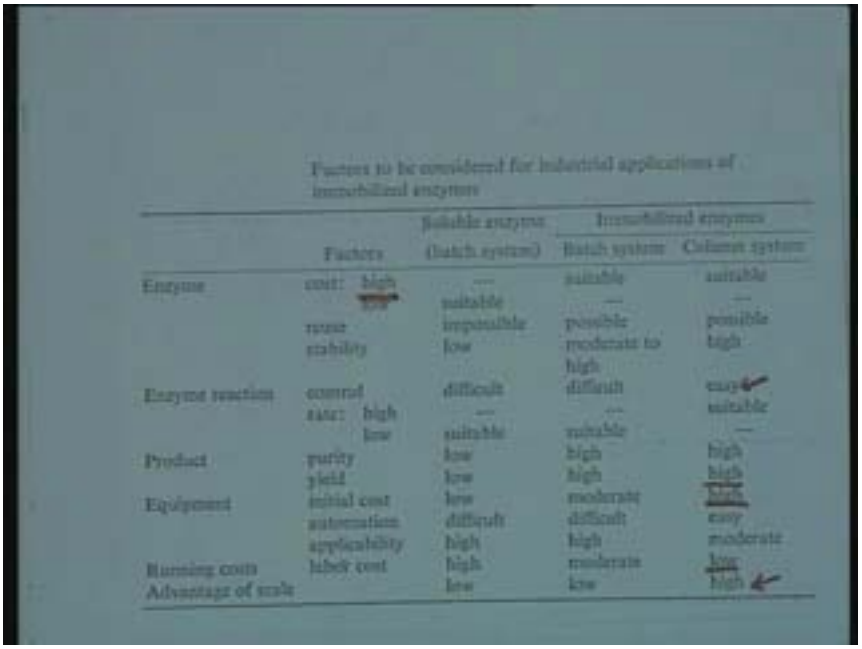

These are the various parameters like the enzyme itself, the reaction, product, equipment, running cost and advantage of scale and one has to analyze a given process on the basis of these parameters and then take a decision whether it is desirable to go for a soluble enzyme batch system, immobilized enzyme batch system or immobilized enzyme continuous system.

The first example that I want to illustrate with the use of immobilized enzyme is the production of L amino acids by immobilized amino acylase. This example probably was the first commercial process used anywhere in the world using immobilized enzymes.

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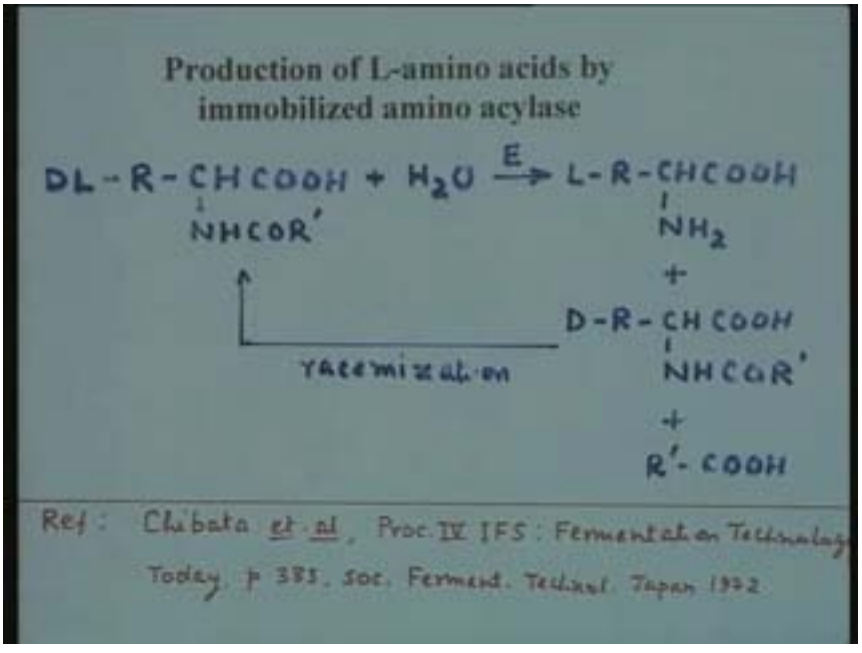

The same process was used in Japan using a soluble batch enzyme over a period of about ten years and they were producing L amino acids by using the amino acylase soluble enzyme produced from the microbial resources but in those soluble enzyme batch processes, the problem of purification was very, very severe. That means usually the product stream after the batch processes will be contaminated with an enzyme. It has to be removed because these L amino acids which were produced were useful either for food applications or animal feed or sometime pharmaceuticals and therefore you cannot leave unconverted or unused proteins in the feed stream and the number of stages involved in purifications will lead to loss of yield for the amino acid as high as fifty to sixty percent. Therefore when you are going to loose almost fifty percent amino acid, in recovery process the cost is going to be double plus the cost involved in the purification itself. Therefore there the shift from the soluble enzyme to immobilized enzyme became a very great incentive to bring down various facets of cost and having a much cleaner process this can give a better quality product, much higher yield, ease of purification and all those advantages.

Normally when these amino acids are synthesized by chemical route, they are residual mixture of D&L and we get in final stage as DL-acyl amino acid. When I am saying L-acyl amino acid this R could be anything the whole range of amino acids specifically in most cases depending on the amino acids there is a corresponding amino acylase. Some of the enzymes are little less specific. That means they can act up on two or three different amino acids but most of them are very specific to a particular amino acid and they are produced and the DL-acyl amino acid can be hydrolyzed by the suitable enzyme which hydrolyzes or …. the particular amino acids to give the L-amino acid; it gets deacylated and you get L-amino acid and D form remains untouched. You get a mixture of L-amino acid and D-acyl amino acid. These two molecules have a very distinct difference in their solubility at different pH; very distinct. So adjustment of pH and taking advantage of solubility the L-amino acids can be easily crystallized and produced in a very pure state without much of head ache of removing proteins or deactivation of the enzymes or separation of unconverted D form.

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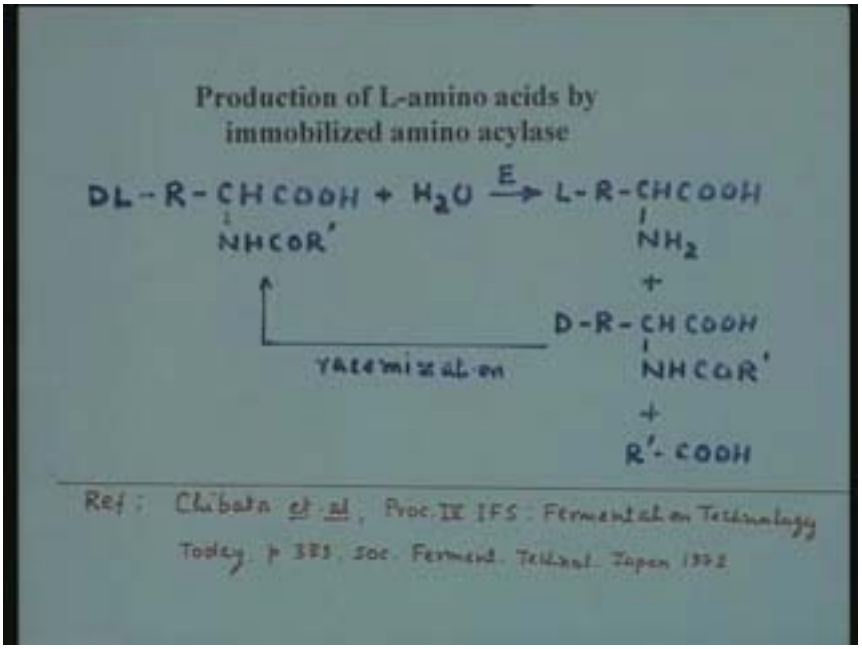

The same process also gave a side effect or rather a very important tool to produce D-amino acids because if the D-acyl amino acid separates out after crystallization, it can even be chemically hydrolyzed to produce D-amino acid. There is no problem and D-amino acids are used as a raw material for many of the keto acids which are used as a raw material in pharmaceutical industries. Until then from the residual mixture we were isolating L-amino acid and the balance was almost wasted. Here the process of economics becomes very attractive because while you are able to isolate L-amino acid with a much better yield you are also able to use the residual material D-amino acid either if it is required for keto acids or alternatively this can again be racemised chemically or just by change of pH it gets racemised again to a DL mixture and the whole process can be repeated till almost hundred percent of the amino acid E is recovered in the L form and you get the corresponding fatty acid also produced in the system which is separated from the mixture.

Stable refers to that the enzyme, the amino acylase from aspergillus species was isolated and one of the first enzyme which was isolated was based on methionine acylase. Methionine acylase from aspergillus orgy was isolated and immobilized by using a variety of methods and if you look into list they had used physical adsorption, ionic binding, covalent binding, cross linking in the presence of carrier, lattice entrapment and micro casual entrapment. Almost all the methods are employed to immobilize the methionine acylase.

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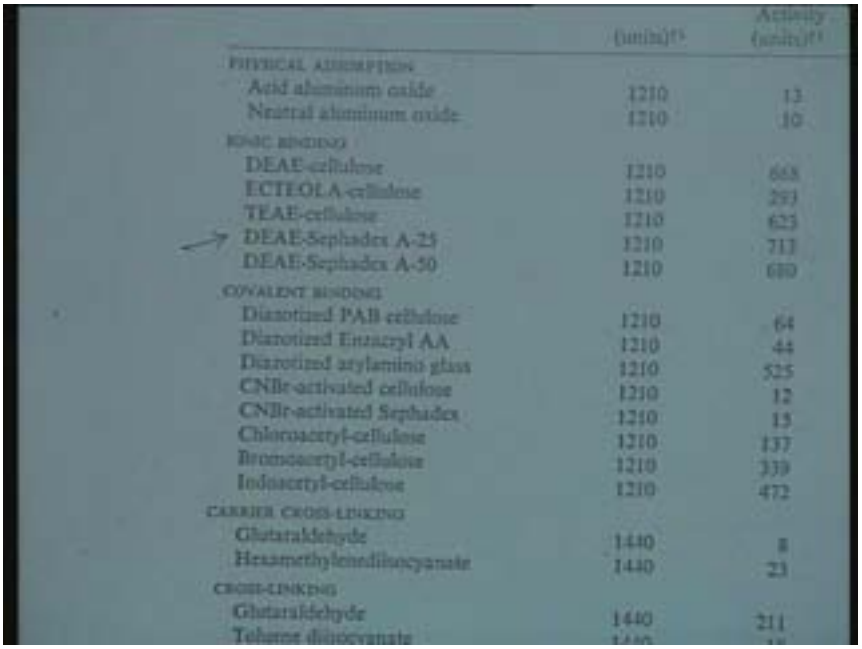

	(units)[a]	Activity (units)[a]
PHYSICAL ADSORPTION		
Acid aluminum oxide	1210	13
Neutral aluminum oxide	1210	10
IONIC BINDING		
DEAE-cellulose	1210	668
ECTEOLA-cellulose	1210	293
TEAE-cellulose	1210	623
DEAE-Sephadex A-25	1210	713
DEAE-Sephadex A-50	1210	680
COVALENT BINDING		
Diazotized PAB cellulose	1210	64
Diazotized Enzacryl AA	1210	44
Diazotized arylamino glass	1210	525
CNBr-activated cellulose	1210	12
CNBr-activated Sephadex	1210	15
Chloroacetyl-cellulose	1210	137
Bromoacetyl-cellulose	1210	339
Iodoacetyl-cellulose	1210	472
CARRIER CROSS-LINKING		
Glutaraldehyde	1440	8
Hexamethylenediisocyanate	1440	23
CROSS-LINKING		
Glutaraldehyde	1440	211
Toluene diisocyanate		

If you compare all the methods based on the initial quantity of amino acylase used, the activity recovered in the immobilized form and the yield level activity, the method based on immobilization of amino acylase by ionic binding to DEAE sephadex proved to be the best in terms of its enzyme activity; seven thirteen units per gram or what ever unit they have used and with “fifty eight point nine percent” yield of the recovery of the activity from the soluble enzyme as compared to various methods where the activity recovery as well as the final activity of immobilized preparation was much lower. The next comparable ones were entrapment in polyacrylamide gel, covalent binding to some aryl amino glass, diazotization, but if you look into the cost of immobilization in terms of cost of reagents, covalent binding was expensive. Gel entrapment was cheaper than Sephadex but when you look into the operational advantage you will notice that even expensive carrier was desirable and acceptable.

For designing the whole commercial process they looked at two major parameters effectively although we have much better strategy and understanding of the various design parameters for the immobilized enzyme reactor but in the process two more parameters were considered; the effect of space velocity on the concentration of amino acid in effluent. A space velocity is analogous to dilution rate in the CSTR and so you will notice here that the same enzyme was able to act on methionine as well as phenylalanine. So in the two cases on the right hand side is the hydrolysis of L form. In fact at a concentration of point one molar the reaction is stopped when almost hundred percent hydrolysis of the L isomer was completed and the kind of profile you get is almost a sigmoidal profile and at a space velocity of approximately about eight hour inverse the reaction was complete and that means hundred percent of the L isomer was hydrolyzed and recovered.

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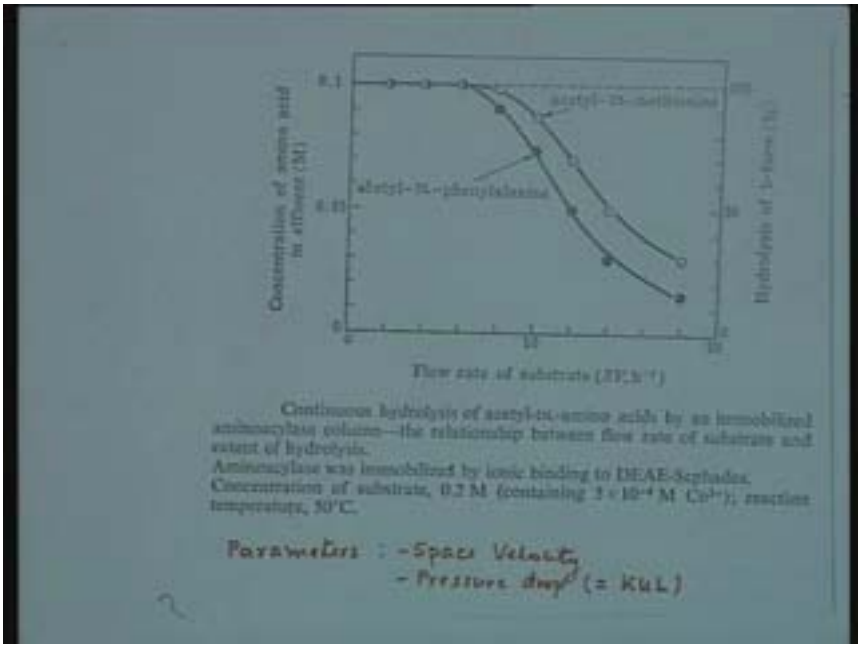

If you notice here some of the experimental conditions, amino acylase was immobilized by ionic binding to DEAE sephadex “0.2M” concentration of substrate was used containing 5x 10^{-4} M cobalt salt which acts as stabilizing agents, stabilizer for enzymes and reaction temperature was 50 deg C. Another parameter which they considered was the pressure drop; probably that they considered to find out the height of the column and they noted also that the pressure drop across the column bed followed very typical fluid dynamics expressions which are available and it was proportional to the space velocity as well as height of the column. They selected the height of the column based on the pressure drop limitations so that don’t have to face very severe pressure drops. Particle size was chosen according to that. They did not make any further analysis based on mass transfer or anything else. Space velocity and pressure drop was the key parameters that were studied based on which the whole reactor system was designed and operated continuously.

This is a process flow diagram that is still in operation in Japan and has been in operation since 1963, the first system which came into being and is still being operated. As you notice here this is the storage tank for the racemic mixture which comes from chemical synthesis. Here we pass it through a heat exchanger just to maintain the temperature fifty degrees and pass through the immobilized enzyme column. There are arrangements for temperature, pH and flow rate control. Then the final product from the packed bed column goes to a concentrator where it is concentrated just to take advantage of solubility and crystallization and the L-amino acid gets crystallized and the crystalline L-amino acid is separated.

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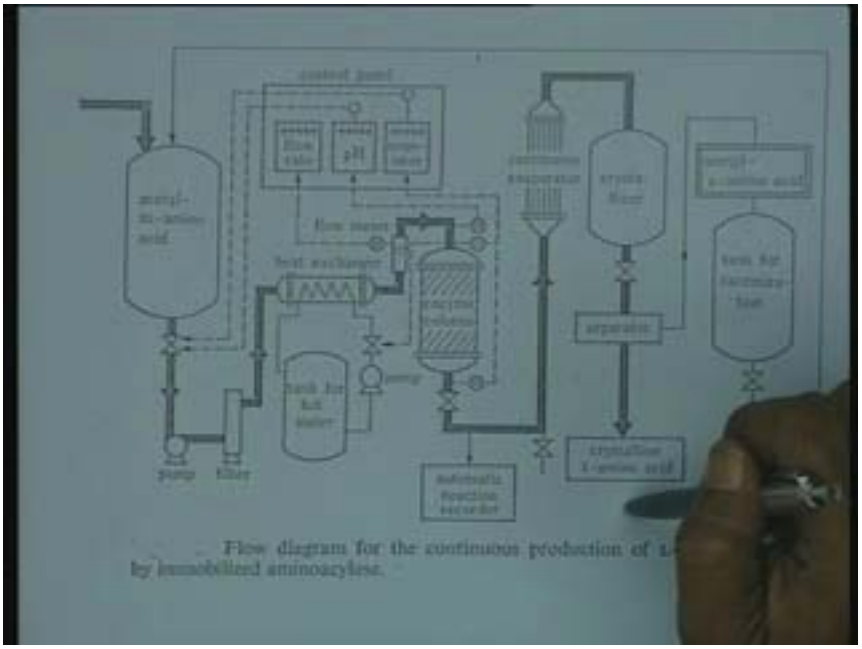

Flow diagram for the continuous production of L- by immobilized aminoacylase.

Crystallization is being used as the method which is a very favorable method for purity of the product. The D-acyl amino acid is then separated and is taken to a separate tank for racemization where pH is changed and racemization occurs and this mixture is again fed back to this tank as a racemic mixture and the whole process produces almost hundred percent conversion of DL to L-amino acids. Alternatively there have been attempts where the D-acyl amino acid can be chemical hydrolyzed to produce D-amino acids which are also raw material for keto acids in the pharmaceutical industry

The operational stability and the regeneration of the carrier are the points which I was addressing. The actual data is that they have quite a stable performance in the sense that in about thirty two days of time of continuous operation more than 70% of the activity is retained. As per their plan they regenerated their material. After one cycle when 72% of the activity was lost they stopped the reactor; added more enzymes whatever loss has taken place 28% enzyme was additionally added and it accumulated so that the enzyme reaches to the original loading capacity. Again the reactor was started and this can also be done by using multiple columns and instead of using a single column of large capacity one can breakdown the total volume in smaller capacities and stagger (25:52) operation. We will try to look at the operational part also subsequently. Although while they operated, they operated at a single column with the break in between of the operation but today most of the immobilization enzyme columns are operated in a multiple column and staggered start up mode so that the productivity you don't have to make a brake in between and the productivity and conversion can be maintained almost constant within a narrow range over a period of time. The regenerated enzyme again went down and it can be again regenerated and this cycle is continued over almost about thirty two days period.

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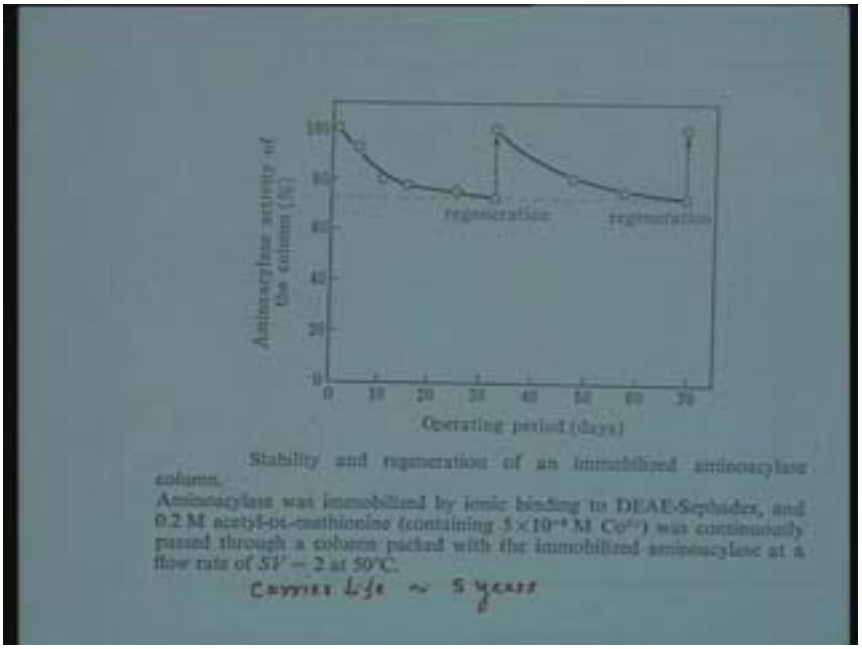

Stability and regeneration of an immobilized aminoacylase column.
Aminoacylase was immobilized by ionic binding to DEAE-Sephadex, and 0.2 M acetyl-DL-methionine (containing 5×10^{-4} M Co^{2+}) was continuously passed through a column packed with the immobilized aminoacylase at a flow rate of $SV = 2$ at 50°C.
Carrier Life ~ 5 years

Look at the cost part because it is process economics and this was the data presented by the authors themselves who carried out the commercial operation and if you compare the production cost of L-amino acids by batch which was being operated originally that means prior to 1963 it was a batch soluble enzyme process and a continuous immobilized enzyme process. This is the comparison and this is the fraction of the relative cost. First point to be noted is that the cost of the production of the product has come down to 59%, whatever was the original cost 100% of the L-amino acid. This is in the case of methionine and similar analysis applies to almost all amino acids. The cost of production per unit quantity of the product comes down by over sixty nine point of total cost.

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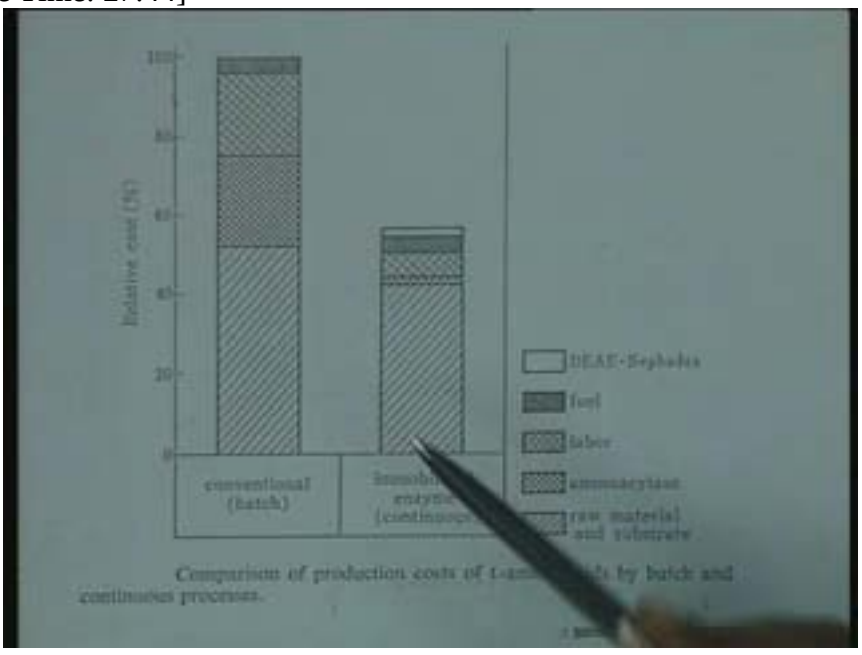

Comparison of production costs of L-amino acids by batch and continuous processes.

If you see the break up you will notice that the there is a significant change in the cost as a result of the raw material. That means racemic mixture of amino acids, D-acylamino acid. The cost is reduced significantly and that is bottom shaded portion and that is because of the increase in yield. The recovery has improved so per unit cost as a result of raw material consumption has decreased. The second part is the enzyme cost. As expected in the case of soluble enzyme there is no recovery of enzymes and the cost of the enzyme in the case of immobilized process is very, very small almost negligible compared to this. The third component there is significant reduction in the labor cost. Two major factors which contribute to reduction of the cost in immobilized processes are enzyme and labor. So that also gives us a clue about the enzymes which are more expensive. Immobilized processes are more favorable if let us say the enzyme cost is not very high this is not going to make a dent on your process.

Similarly the recovery process involving labour if it is very tedious again immobilized enzyme processes will be more favorable. So the labor and the enzyme cost are the major factors. Then the fuel cost; it makes no difference. Another factor is the blank portion; the cost of the carrier which is an additional input in the case of immobilized enzyme which does not exist in the case of soluble enzyme. Although DEAE sephadex is very expensive, considered to be probably one of the most expensive carrier that is tested on all materials, the total life of the carrier which they were able to use is almost five years.
If you distribute on the total product formation that cost contribution is very, very small. They can regenerate the carrier as and when the enzyme activity comes down; even they can dissolve; they can re-adsorb it and the carrier is fairly sturdy and it doesn't get degenerated. It can attain its own adsorption and they were able to use easily for periods as high as five years. During process there might be some handling losses and which might reduce some quantity and this factor is primarily because of the handling losses during the regeneration process. So this gives you a picture of the process economics and the contribution of different factors in the operation of an immobilized enzyme processes.

Another example that I like to illustrate is also a normal molecule, production of L-aspartic acid. L-aspartic acid is a molecule which is used in the food industry and pharmaceutical industry and this can be produced by an aspartase, enzyme lyase which acts on fumaric acid and ammonia to give you the L-aspartic acid and the work was reported by Tosa and others in Biotech Bioengineering in 1973.

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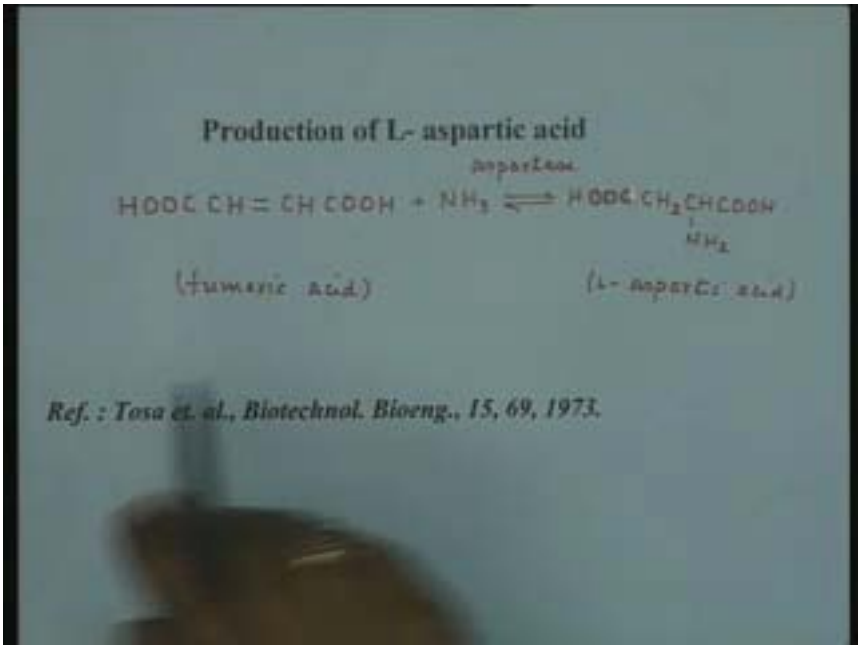

I think you may like to go through and I suggest some of the references; you just go through them; you will find the experimental details as to how they arrived at the final decisions.

If you look at the same data, as we looked in the case of amino acylase, in the case of aspartase they also carried out variety of immobilization methods including the physical absorption, ionic binding, covalent binding and entrapments.

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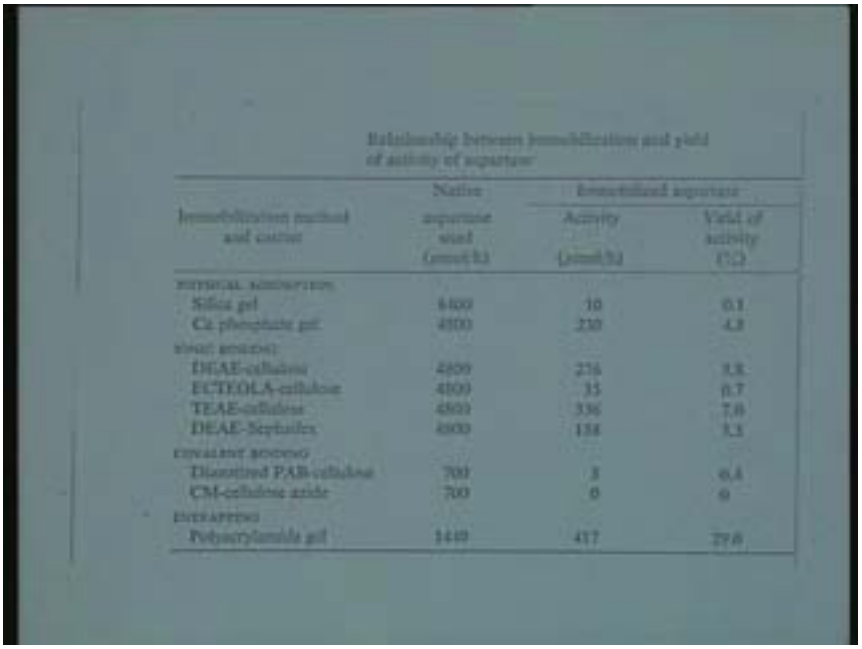

The activities of the final enzyme preparations and the percentage yield of the activity from the …. immobilization are relatively very low here compared to the original enzyme used. If you notice that the percentage recovery are very, very low excepting in the case of entrapment in polyacrylamide gel which resulted in the yield of over 29% compared to amino acylase where the activity recovery was of the order of 50% or more. Here we have 29% but still 29% is the highest and probably one has to accept it. Although they started using immobilized aspartase for producing aspartic acid they discontinued very shortly and shifted to an immobilized cell process where these losses were not there; they immobilized the whole cell itself and the whole activity was retained and the process was more much more economical than an immobilized enzyme process.

This is just one example where I want to illustrate in what conditions you have to shift over. If there is enzyme which is intercellular and the recovery or purification cost is very high probably immobilized cells will provide an answer. On the other hand if the immobilization method is not able to produce or you are not able to get a good method which can recover a good yield of enzyme or the final activity in high amounts in that case also immobilized cells may be an easy or a suitable answer. The same enzyme aspartase continuous operation in the packed bed reactor gives you the percentage of the L-aspartic acid recovered in the product stream and they carried out at two different substrate concentration 0.2M and 1M ammonium fumarate and the performance over the operating time is totally different as a function of new substrate concentration.

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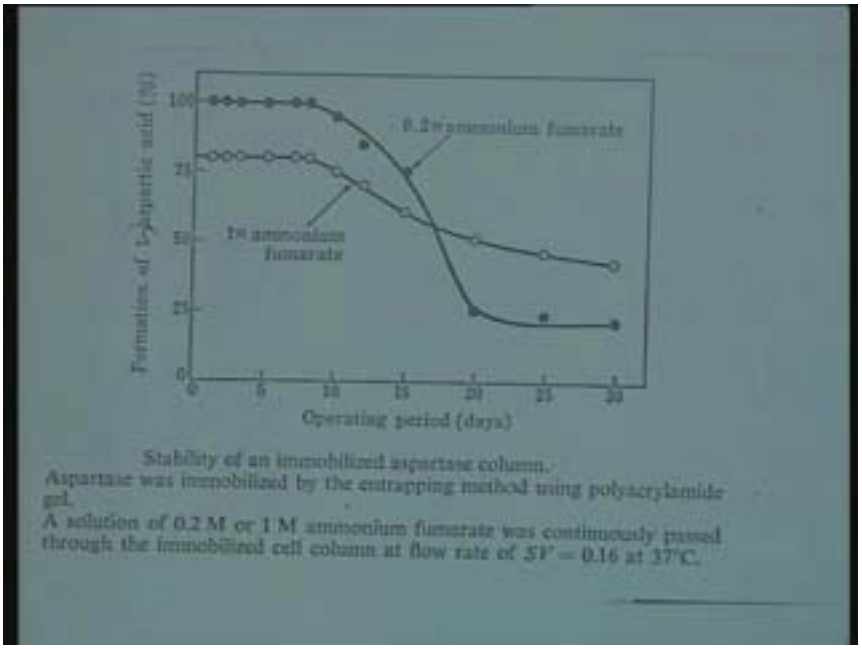

Stability of an immobilized aspartase column.
Aspartase was immobilized by the entrapping method using polyacrylamide gel.
A solution of 0.2 M or 1 M ammonium fumarate was continuously passed through the immobilized cell column at flow rate of $5V = 0.16$ at 37°C.

In the case of 1M ammonium fumarate it goes down by 50% activity in twenty days time. When you take lower substrate concentration, 0.2M the activity loss is much, much higher and over the same period of time instead of 50% it reaches to about 25% and this is the case where if you recall we discussed the substrate dependent enzyme decay. We

discussed two cases of enzyme deactivations during continuous operation of the reactor one was substrate independent where in the earlier case of aminoacylase substrate concentration plays no role and the deactivation constant is predominant but in the case of the aspartase, it is substrate dependent deactivation and higher the concentration of the substrate the operational life is comparatively better. This is the operational profile. For 0.2M, it deactivates much faster and you see that in twenty days time only 25% of the enzyme is left, 75% has got deactivated and here in the same period almost 50% of the enzyme is present at a higher substrate concentration. So therefore one has to also look about the deactivation kinetics because that will also play an important role.

The third and perhaps a very simplistic example is of the hydrolysis of starch for the production of glucose. The hydrolysis of starch goes through three different stages; gelatinization usually a heat treatment process, starch gel which is acted upon by alpha amylase which is very, very economical or very cheap enzyme cost wise. When I say cheap I am referring to comparable cost of other enzymes and therefore it is used usually in soluble batch process at a very high temperature because the alpha amylase is a very thermo stable enzyme and can be operated at temperatures even hundred plus and therefore the gel gets immediately liquefied.

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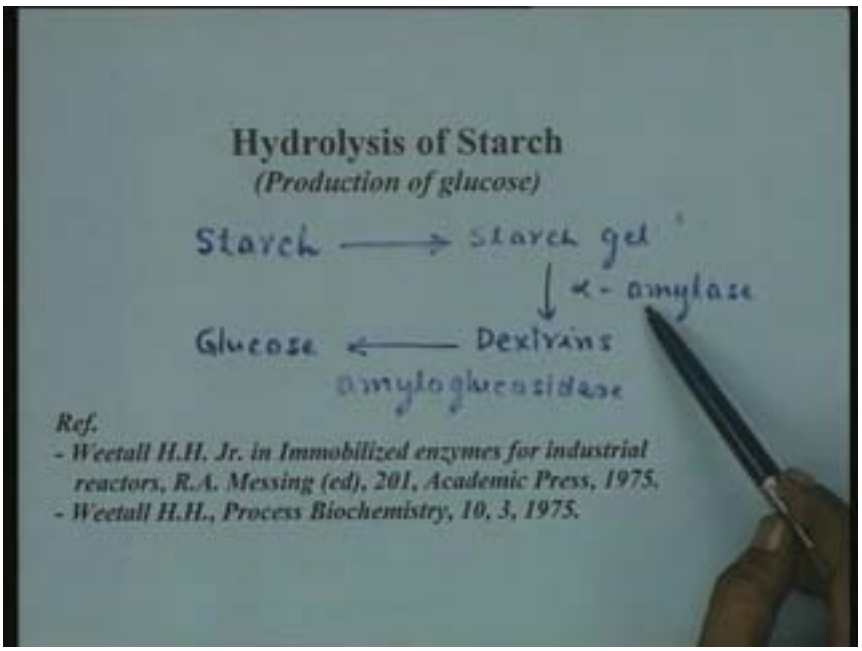

It's a very fast enzyme the specific activity is very high and therefore the enzyme very speedily converts the gel into a liquefied starch. This is a mixture of dextrins, a small chain length starch molecules; chain length not more than ten, usually six to eight but the dextrins should not have more than ten glucose units linked together and this is the step which is the rate limiting step in the whole process, the dextrins to glucose by amyloglucosidase or glucoamylase; it is called as glucoamylase also. This step has been

carried out using immobilized enzyme in continuous reactors so that one can take advantage and the whole process can be controlled by this step.

A very large number of studies have been carried out on immobilization of glucoamylase. The process was designed in US by Weetall and group which was based on adsorption of glucoamylase on to porous silica.

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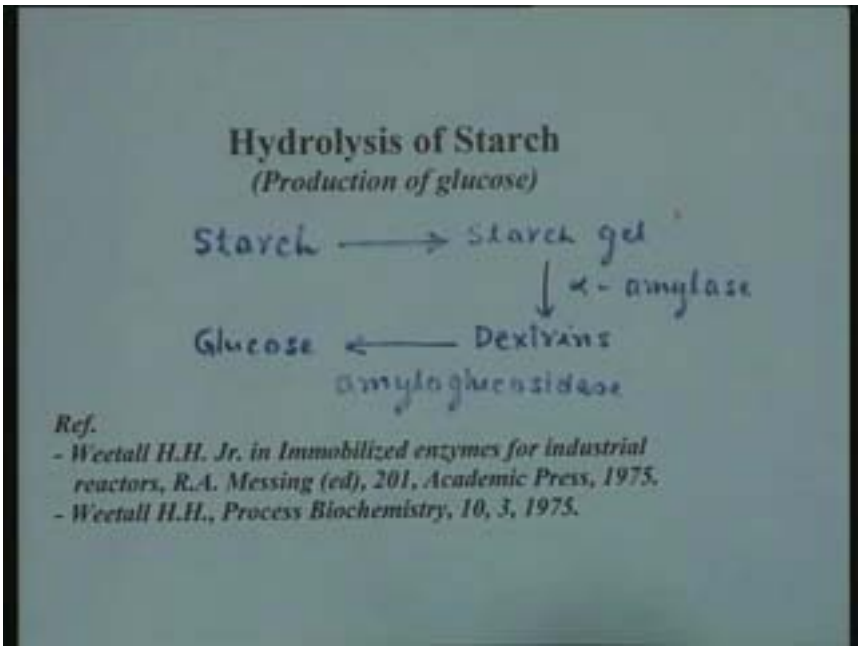

Porous silica beads were taken and the enzyme was adsorbed on to that and the cost of the product is not very high so that one can afford this immobilization method; even if the operational stability is not very high it doesn't matter. One of the major issues with hydrolysis of starch is in the form of the viscosity of the feed because starch hydrolysis is usually used at very high substrate concentration usually 30-40% dissolved solids which makes very viscous syrup during operation and this viscosity causes a significant problem as for the pressure drop is concerned in packed bed reactors and therefore not many packed bed reactors for these steps are operational today. Because use of packed bed reactors with an almost 40% total solids syrup is highly energy intensive and is usually avoided.

As an alternative some people have even proposed to use a CSTR where by one can use a stirred vessel, a classical system where one can maintain the substrate feed at a constant reservoir and an agitator pedal, a stone filter just to retain the immobilized enzyme particles and the material is filtered through this stone filter and passes through a peristaltic pump to a charcoal column which removes carbon matter or other impurities. Depending upon the further application it may be deionized and other operations may be done, concentrated and you can collect the product and this was the system which was reported for glucoamylase immobilized on DEAE cellulose by ionic binding. The DEAE

cellulose also is a very stable carrier which can be regenerated although no such commercial process is operational in the case of glucoamylase mainly because of the process economics.

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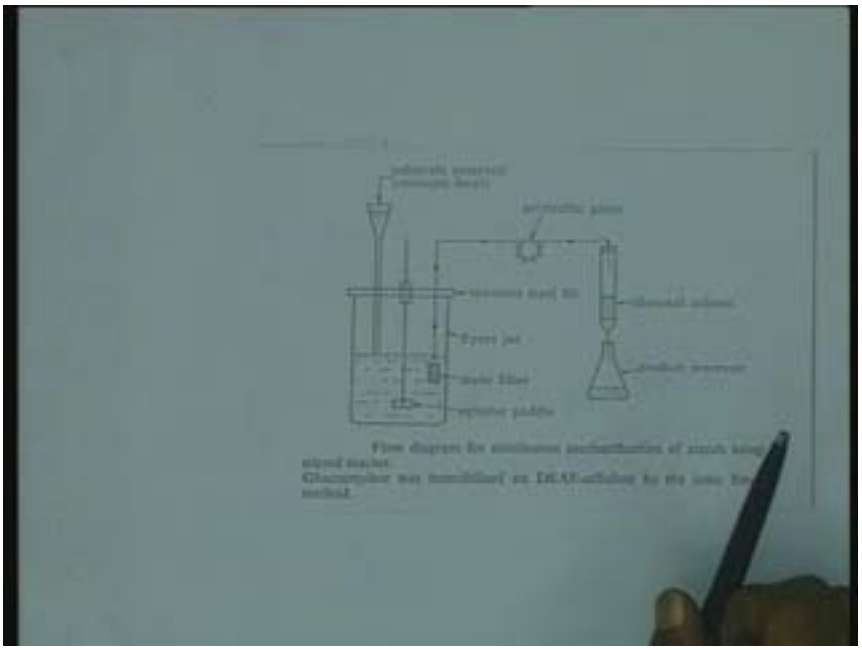

Most of the process which are in use they are using adsorption onto clays, bentonite or some other clay; not any expensive or well defined matrices. So these are some of the illustrated examples not that these are the only processes which are operational; a large number of other processes which are operational commercially all over the world are one is penicillin acylase. Hydrolysis of Pen G or V goes to six APA and the six APA is an intermediate for variety of antibiotics, the semi synthetic antibiotic and they are commonly used. Many of the immobilized penicillin acylase system have been produced wide vied and marketed. A large number of products are available commercially. India also is a major producer of six APA and produces significant quantity of six APA but bulk of the immobilized enzyme preparations are imported. The products are available from many countries …… in Sweden, or …. in UK, …. in Japan. Some industries which are making more immobilized enzymes here in house they are also importing the carrier. It makes no difference if you go to the carrier bulk of the cost is already paid and and this is another example where immobilized enzymes are used commercially and in very large quantities.

Then you have inversion of sucrose a very typical illustrative example from the point of view of India. World over the production of a glucose fructose syrup is mediated through corn. Corn as the starting material; Corn is hydrolyzed to produce glucose, corn ….. starch or starch to glucose and glucose isomerisation to fructose and an equimolar mixture of glucose and fructose but in India a good quality corn meant for this purpose what we call as sweet corn is usually not available since the relative cost of the corn is

also high in India, being the staple food. So it doesn't meet the requisite commercial requirements. But still to produce glucose fructose syrup for many of the food industry applications and the major consumer of glucose fructose syrup in India is the beverage industry. In fact most of the products for example the beverages particularly Pepsi and Coke they are based on glucose fructose syrup; they don't use sucrose as a sweetener because of the use of glucose and fructose syrup in their parent countries their processes need glucose and fructose syrup. Otherwise the product specification will change and so in India they are using the inversion of sucrose using invertase because sucrose is available at fairly low cost and inversion of sucrose by invertase is the process which is used in India and again in the immobilized form. There are at least three or four major units which supply glucose fructose syrup produced from sugar.

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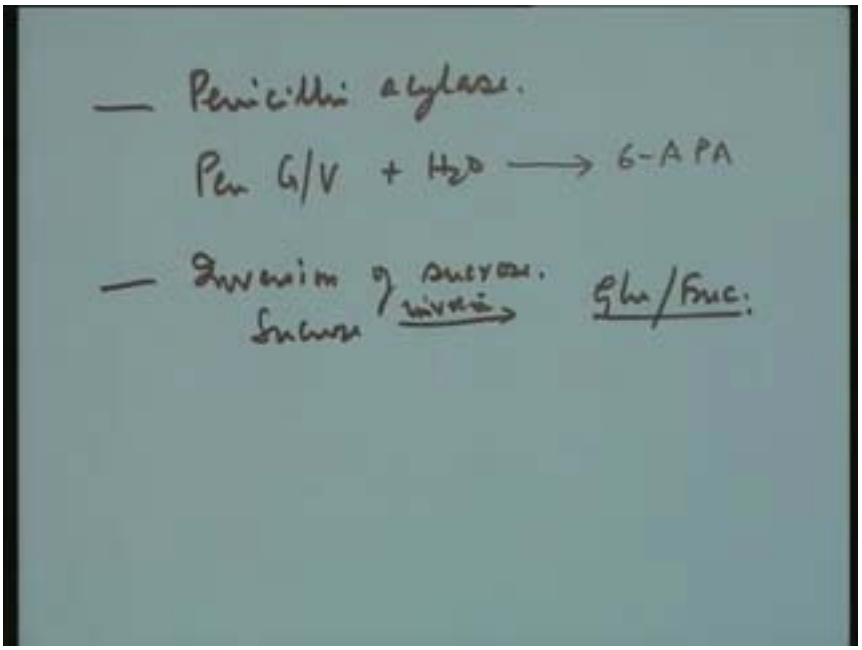

Then another process is for malic acid from fumaric acid. The fumaric acid on hydrolysis by fumerase gives L-maleic acid which finds applications in food industry as a food additive. As a matter of fact the conventional method other than this for maleic acid is only isolation from fruits which has to be very, very expensive compared to this method but fumaric acid is available as synthetic organic acid and fumaric acid can be converted to maleic acids for variety of applications. Then the brewing industry uses for chill proofing of beer. During the stages of beer after fermentation is over it is clarified and is stored for sometime. During storage it develops a haze and this haze is as a result of reaction between polyphenolics and polypeptides because both these molecules comes from the basic raw materials. You use barley or any other cereal …. which have initially started to make the mess. They contain certain polyphenolics and polypeptides and they react during storage to give you a haze and hazy beer nobody likes. One wants a sparkling clear beer and this clarification or what they call as the term chill proofing is done by immobilized papain, a proteinase enzyme which is immobilized and used in a

column because it degrades polypeptides or even polyphenol oxidase also have been used or a mixture of the two so as to degrade those molecules and remove the haze which can be filtered and clarified beer is available.

Then another application in food sector has been on lactose free milk. Milk contains primarily lactose as a principle sugar and this lactose is an undesirable sugar for certain adults. In India we are used to take milk and sugar is assimilated. But in many countries the population is not able to assimilate lactose and it causes diarrhea and other kind of clinical symptoms and therefore people cannot take milk. In those cases they produce lactose free milk by the action of beta galactosidase on milk which hydrolyses the lactose into galactose and glucose.

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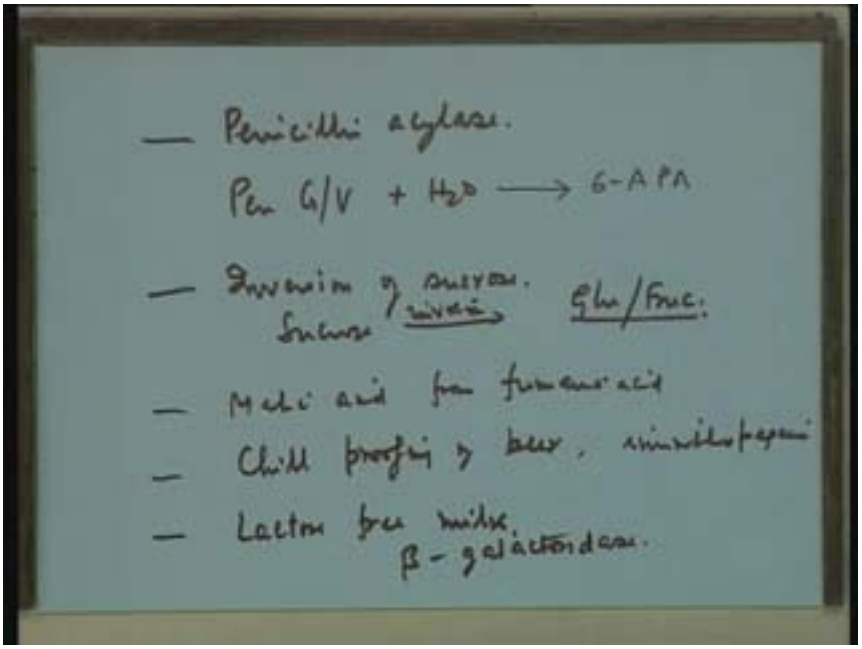

Galactose and glucose are assimilable or the monosaccharides which can be assimilated and without loosing any of the nutritive value the milk can be made free from lactose and are available for consumption in some of the countries. There are many other smaller applications but these applications require the idea of methods of immobilization and their application for reactor design keeping in view the variety of issue that we have discussed in the past.