Biomass Conversion and Biorefinery
Prof. Kaustubha Mohanty
Department of Chemical Engineering
Indian Institute of Technology - Guwahati

Lecture – 26
Cellulase Production, SSF and CBP

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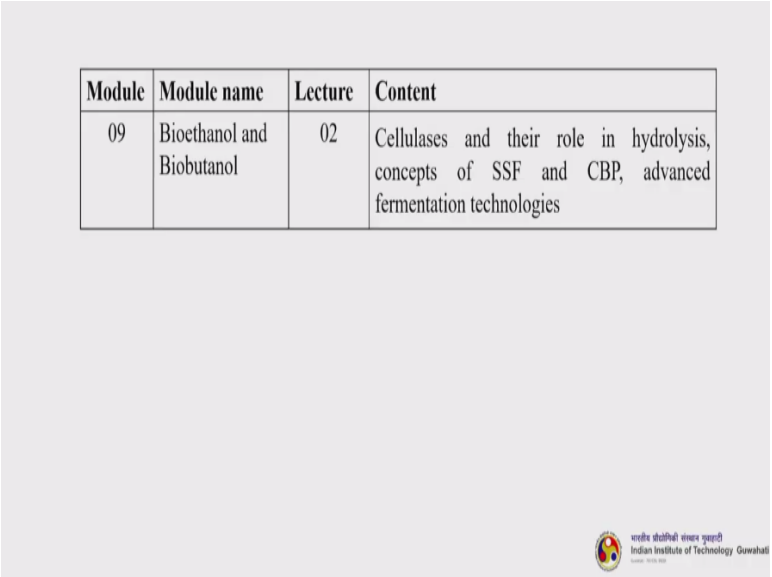

Good morning students. This is lecture 2 under module 9. As you know that we have been discussing bioethanol and biobutanol under this particular section. So, we will learn today about cellulases - the enzymes - their role in hydrolysis, concepts of SSF and CBP and advanced fermentation technologies. So, let us begin.

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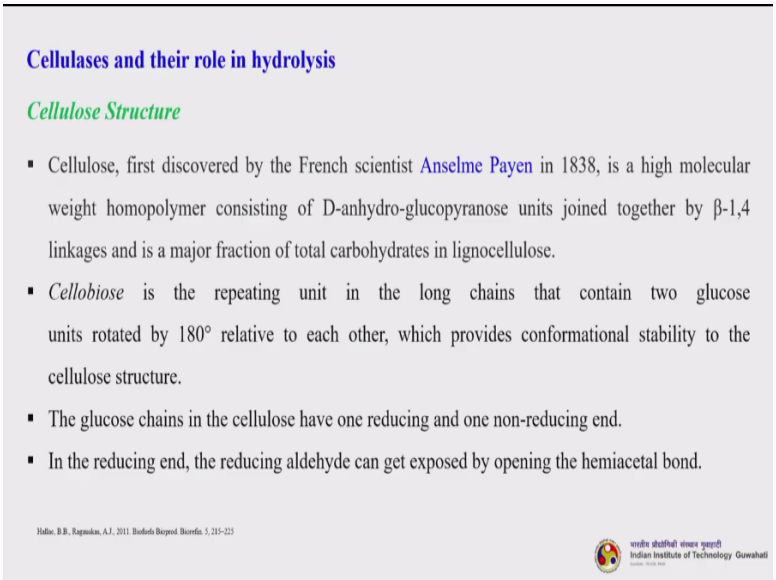

Cellulases and their role in hydrolysis. Though we have discussed, but we will again quickly go through the cellulose structure. Cellulose first discovered by the French scientist Anselme Payen in 1838 is a high molecular weight homopolymer consisting of D-anhydro-glucopyranose units joined together by beta 1, 4 linkages and is a major fraction of the total carbohydrate in lignocellulose.

Cellobiose is the repeating unit in the long chains that contain 2 glucose units rotated by 180 degree relative to each other which provides conformational stability to the cellulose structure. The glucose chains in the cellulose have one reducing and one non-reducing end. The reducing aldehyde can get exposed by opening the hemiacetal bond.

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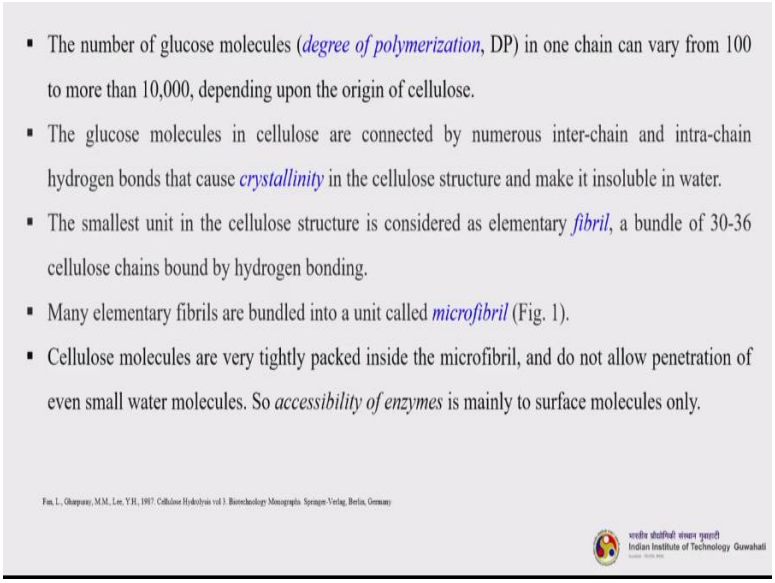

So, the number of glucose molecules which we call as the degree of polymerization in one chain can vary from 100 to more than 10,000 depending upon the origin of the cellulose. The glucose molecules in the cellulose are connected by numerous inter-chain and intra-chain hydrogen bonds that cause the crystallinity in the cellulose structure and make it insoluble in water. The smallest unit in the cellulose structure is considered as elementary fibril, a bundle of around 30 to 36 cellulose chains bound by hydrogen bonding. Now, many elementary fibrils are bundled into a unit called as microfibril. I will show you the image in next slide. Cellulose molecules are very tightly packed inside the microfibril and do not allow penetration of even small water molecules. Thus, accessibility of enzymes is mainly restricted to the surface molecules only.

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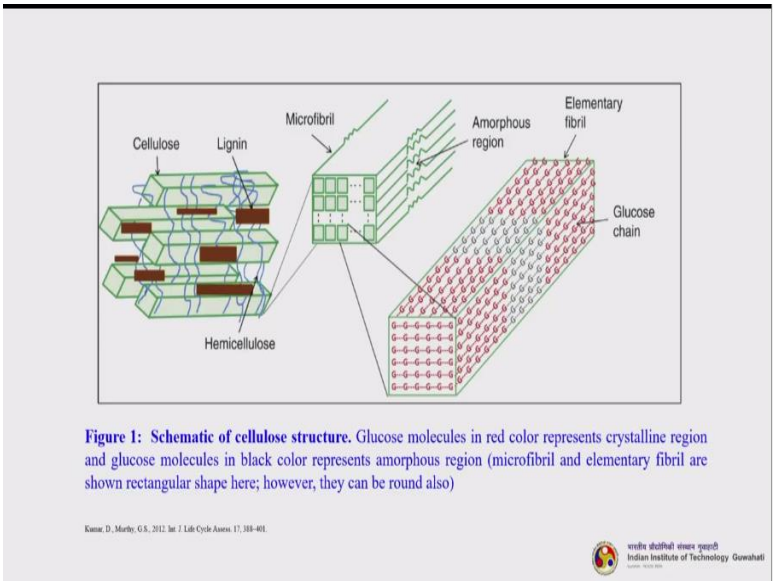

Figure 1: Schematic of cellulose structure. Glucose molecules in red color represents crystalline region and glucose molecules in black color represents amorphous region (microfibril and elementary fibril are shown rectangular shape here; however, they can be round also)

Kumar, D., Murthy, G.S., 2012. Int. J. Life Cycle Assess. 17, 388–401.

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You can see this is the schematic representation of the cellulose structure. Now, this we have discussed earlier also, but we will just as I told you that just to understand the role of cellulases it is also very important to again understand the cellulose structure. So you can see this. The green color things these are actually the cellulose fibrils and lignin is this brown color one and the blue color small lines which have been indicated here everywhere are hemicelluloses.

So cellulose, hemicellulose and lignin are very intricately interlinked or bound together. So, if you see here how it looks like, let us consider a small microfibril and this is expanded here, you will see that whatever is written in G, these are actually glucose chain and this is a single elementary fibril. So the red color, the glucose molecules in red color represents the crystalline region.

Whatever it is mentioned in the black color are amorphous region. So, in crystalline region, it is difficult for the enzymes to act on. So, usually they first act on the amorphous region, then start the cleavaging. So this we will discuss.

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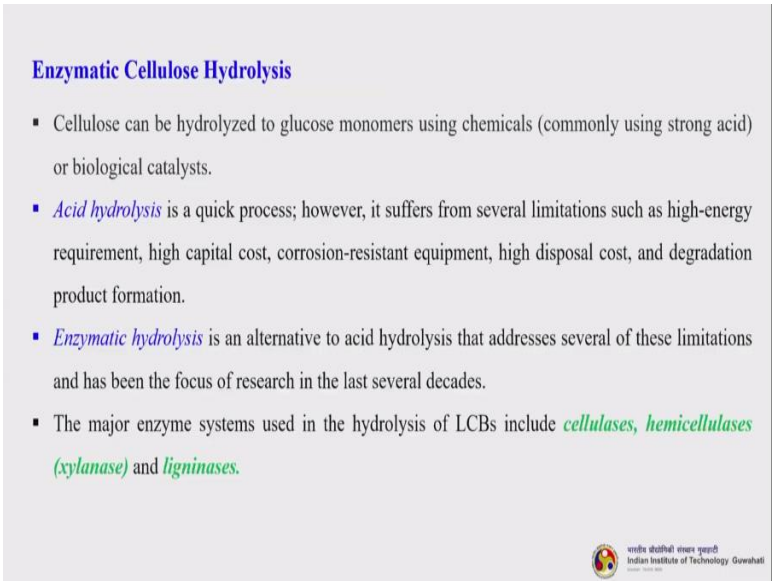

Let us discuss about the enzymatic cellulose hydrolysis. Cellulose can be hydrolyzed to glucose monomers using chemicals, commonly using strong acid or biological catalysts. Acid hydrolysis is a quick process. However, it suffers from several limitations such as high energy requirement, high capital cost, corrosion resistant equipment, high disposal cost and degradation product formation.

Now, enzymatic hydrolysis is an alternative to acid hydrolysis that addresses several of these limitations and has been the focus of research in the last several decades. The major enzyme systems used in the hydrolysis of the lignocellulosic biomass include cellulases, hemicellulases for example xylanase and ligninases; ligninases and the enzymes that will degrade lignin.

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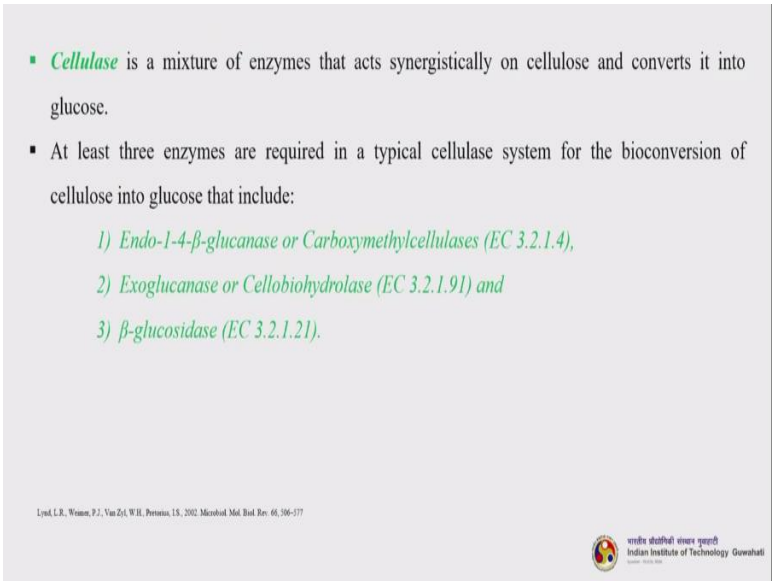

Cellulase is a mixture of enzymes, it is a cocktail basically, that acts synergistically on cellulose and converts it into glucose. At least 3 enzymes are required in a typical cellulase system for the bioconversion of cellulose into glucose that include and that must include these 3: Endo-1-4-beta-glucanase or Carboxymethylcellulases; Exoglucanase or Cellobiohydrolase – CBH; then Beta-glucosidase. Every single enzyme has their different role and we will discuss what is their role.

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- *Endoglucanases* mainly act in the *amorphous region* on the surface glucose chains of cellulase and hydrolyze the β-1,4 linkages in a random manner.
- This random hydrolysis of bonds in glucose chains results in a rapid decrease in the DP of cellulose.
- *Exoglucanase* enzymes act from the *chain ends* (CBH I from the reducing end; CBH II from the nonreducing end) in a processive manner (move along the same chain after hydrolyzing the bond) and produce mainly *cellobiose* as product.
- ***β-Glucosidases*** *hydrolyze the soluble oligomers and cellobiose to glucose* and complete the hydrolysis process.
- Structure and action pattern of major three classes of cellulases enzymes on surface layer of cellulose is shown in Figure 2.

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Now for example, endoglucanases mainly act in the amorphous region on the surface glucose chains of the **cellulose** and hydrolyze the beta 1-4 linkages in a random manner. So we will see how it works.

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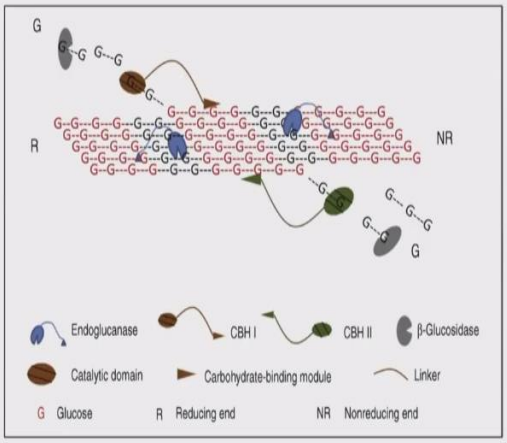

Figure 2: Structure and action pattern of major three classes of cellulases enzymes on surface layer of cellulose

Kumar, D., Murthy, G.S., 2013. Biotechnol. Biofuels 6, 63.

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So, this is a very typical representation of how the different enzymes act on glucose microfibril. So, this is you can see endoglucanase, this is CBH 1, this is CBH 2, then this is beta glucosidase. Now, endoglucanase as I told that it is working only on the amorphous region. So, you can see that it is working on the amorphous region which is represented by the black lines. Let us go back. So, this random hydrolysis of bonds in glucose chain results in a rapid decrease in the degree of polymerization of the cellulose.

Now, we will understand what is the role of exoglucanase. Now, these enzymes act from the chain ends like CBH 1 from the reducing end and CBH 2 from the non-reducing end in a processive manner. So, that means they move along the same chain after hydrolyzing the bond and produce mainly cellobiose as the product.

So, let us see how it happens. This end is the reducing end, this is the non-reducing end and you can see that here CBH 1 is acting on the reducing end and CBH 2 is acting on the non-reducing end. From this side and from this side they keep on doing the clevage or acting on that particular chain. What is the product? So, product is cellobiose, cellobiose is a disaccharide.

So, beta glucosidases hydrolyze the soluble oligomers or cellobiose to glucose and complete the hydrolysis process. Now, the last one to act is actually this beta glucosidase. So, this works on the cellobiose that is being produced by this CBH 1, CBH 2 and the oligomers. You can see here how it is working and then they form the glucose.

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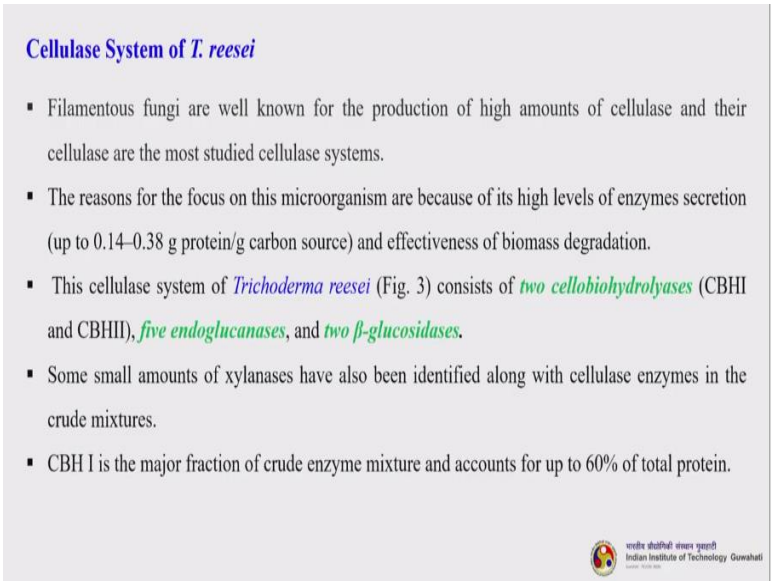

So, we will try to understand the cellulase system of the *Trichoderma reesei* which is one of the most widely adapted filamentous fungi for the production of the high amounts of cellulase and their cellulase are the most studied cellulase systems. The reasons for the focus on this particular microorganism are because of its high levels of enzymes secretion, it is almost 0.14 to 0.38 grams protein per gram of carbon source which is very good actually and the effectiveness of the biomass degradation.

So, this cellulase system of the *Trichoderma reesei* consists of two cellobiohydrolyases (so CBH 1 and CBH 2), five endoglucanases and two beta glucosidases. Now, some amounts of xylanases have also been identified along with cellulase enzymes in the crude mixtures. CBH 1 is the major fraction of the crude enzyme mixture and accounts for up to 60% of the total protein.

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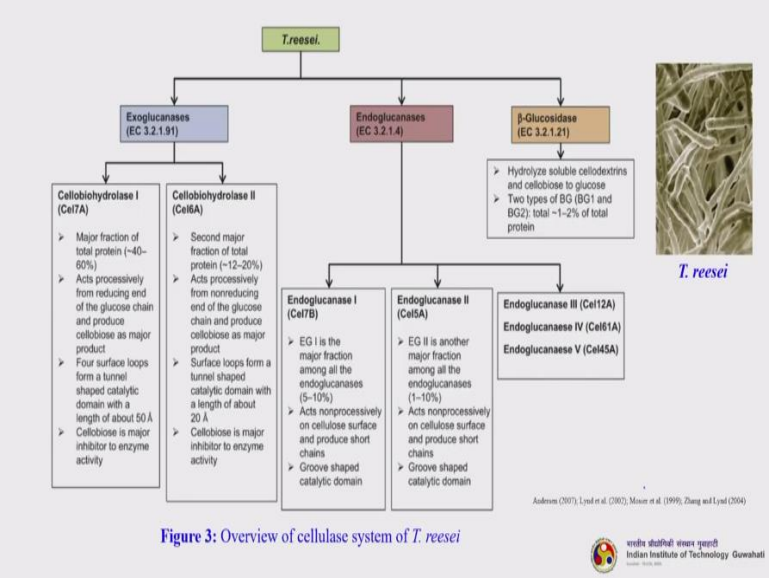

Figure 3: Overview of cellulase system of *T. reesei*

So, this particular slide will make you understand about the different types of enzymes that is being produced from the *Trichoderma reesei* and what is their role. So, you can see that this is the CBH 1, this is CBH 2. As you know that CBH 1 progressively acts from the reducing end, CBH 2 progressively acts from the non-reducing end. Then endoglucanase, endoglucanase 1 and endoglucanase 2 - so that acts nonprocessively on cellulose surface and produce short chains, very short chains. Whereas endoglucanase 2 acts nonprocessively on cellulose structure and produce again the short chains, but different actions. Then we have beta glucosidases, they hydrolyze soluble cellodextrins and cellobiose to glucose - that is the final cleavage.

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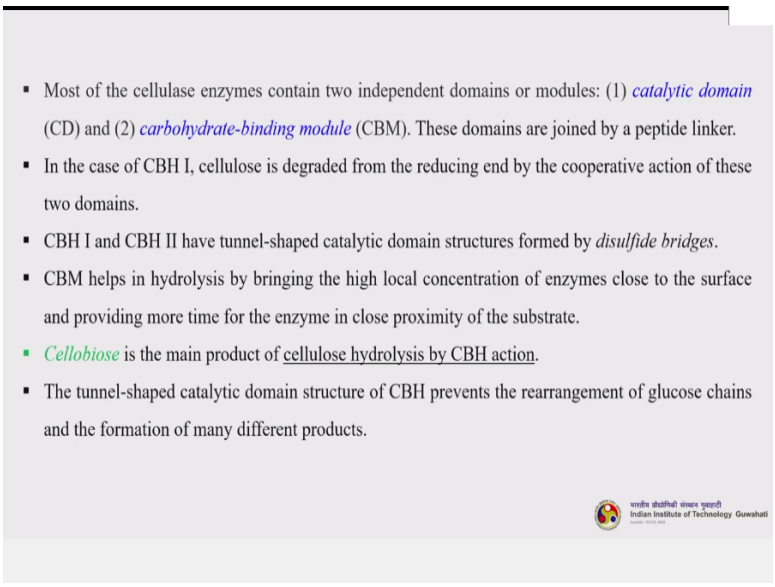

So, most of the cellulase enzymes contain two independent domains or modules. The first one is the catalytic domain which is sortly known as CD and the second one is the CBM which is carbohydrate-binding module. Now, these domains are joined by a peptide linker. In the case of CBH 1 cellulose is degraded from the reducing end by the cooperative action of these two domains. CBH 1 and CBH 2 have tunnel-shaped catalytic domain structures formed by disulfide bridges.

CBM helps in hydrolysis by bringing the high local concentrations of enzyme close to the surface and providing more time for the enzyme in close proximity of the substrate. Cellobiose is the main product of the cellulose hydrolysis by CBH action. The tunnel-shaped catalytic domain structure of CBH prevents the rearrangement of glucose chains and the formation of many different products.

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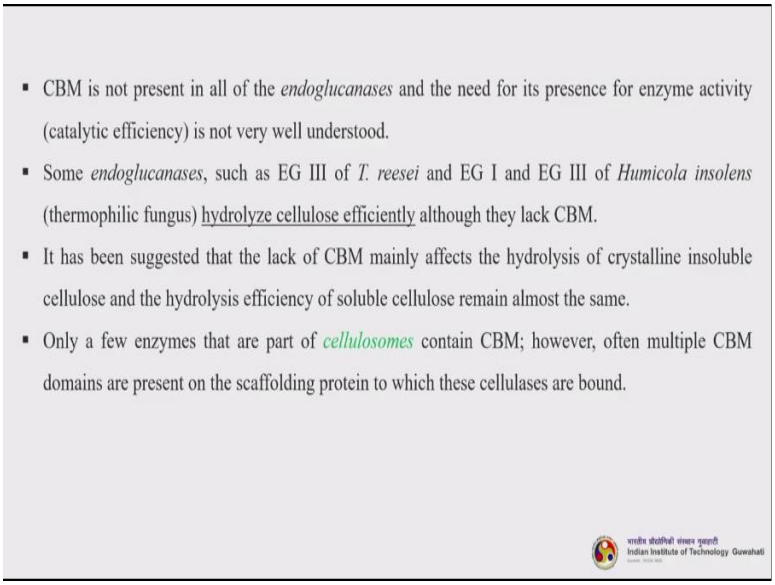

Now CBM is not present in all of the endoglucanases and the need for its presence for enzymatic activities, that is the catalytic efficiency, is not very well understood. Some endoglucanases such as EG 3 of the *Trichoderma reesei* and EG 1 and EG 3 of *Humicola insolens*, that is another thermophilic fungus, they hydrolyze cellulose efficiently although they are lacking CBM.

Now, it has been suggested that the lack of CBM mainly affects the hydrolysis of the crystalline insoluble cellulose and the hydrolysis efficiency of soluble cellulose remains almost the same. Only a few enzymes that are part of cellulosomes contain CBM. However, often multiple CBM domains are present on the scaffolding protein to which these cellulases are bound.

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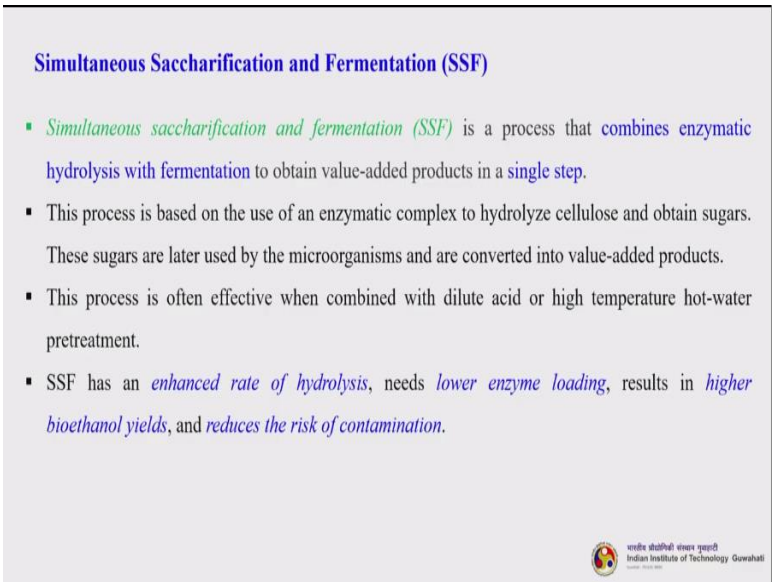

So, now let us move ahead and discuss what is SSF. So, we have discussed in a nutshell SSF long back, so let us try to understand how it works actually. Simultaneous saccharification and fermentation is a process that combines enzymatic hydrolysis with fermentation to obtain value-added products in a single step; underline single step, very important. Now, this process is based on the use of an enzymatic complex to hydrolyze cellulose and obtained sugars.

These sugars are later used by the microorganisms and are converted into value-added products. This process is often effective when combined with dilute acid or high temperature hot water pretreatment. SSF has an enhanced rate of hydrolysis, needs lower enzyme loading, results in higher bioethanol yields and reduces the risk of contamination.

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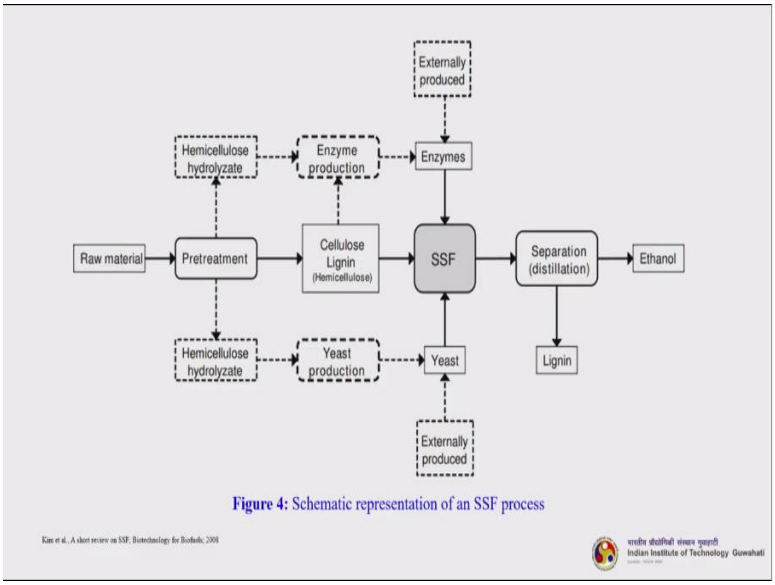

Now, this is the classical schematic representation of an SSF process and mostly SSF follows this route for the bioethanol production or ethanol production. Now, let us understand how it happens. So, the raw material biomass - it undergoes pretreatment. It can be different types of pretreatment depending upon what the biomass is. Then the hemicellulose part and you take it out.

The cellulose and lignin goes to another unit where cellulose is actually again basically recovered and fed back to the simultaneous saccharification and fermentation reactor, it is a single reactor. Now, you will have to add enzymes. Now, enzymes can be externally produced or it can be in-situ produced also. And whatever the yeast that is being used to

degrade this hemicellulose can also be used here, either externally produced or also can be supplied.

So, once the saccharification and fermentation happens, both the reactions, then the product can be taken out and it goes to a separation unit - usually distillation in case of ethanol. So, then you get a lignin product here, basically lignin rich product and you get a pure ethanol there. You may need some intermediate steps which are not shown here as I told you many times. This is just to try to understand what are the basic units or unit operations that is required for SSF process.

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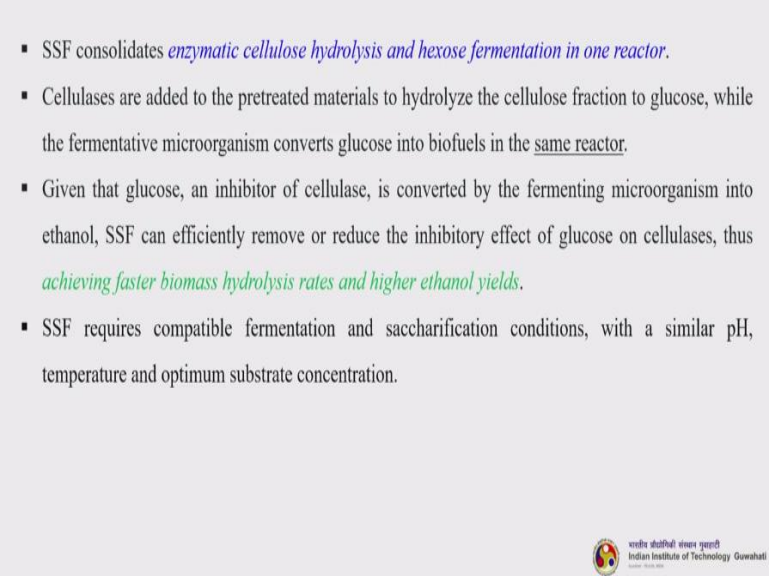

SSF consolidates enzymatic cellulose hydrolysis and hexose fermentation in one reactor. Cellulases are added to the pretreated materials to hydrolyze the cellulose fraction to glucose, while the fermentative microorganism converts glucose into biofuels in the same reactor. Given that glucose - an inhibitor of cellulase, is converted by the fermenting microorganisms into ethanol, SSF can efficiently remove or reduce the inhibitory effect of glucose on cellulases, thus achieving faster biomass hydrolysis rates and higher ethanol yields. So this is one of the most important factor of SSF or you can say the advantage of SSF. SSF requires compatible fermentation and saccharification conditions with a similar pH, temperature and optimum substrate concentration which is again a little drawback or disadvantage of the SSF system because you need to optimize it properly.

Two things are happening, saccharification and fermentation, and their optimized process parameters may differ, but when you are carrying out both in a single reactor you have to optimize in such a way that both the processes are efficiently happening to a certain extent.

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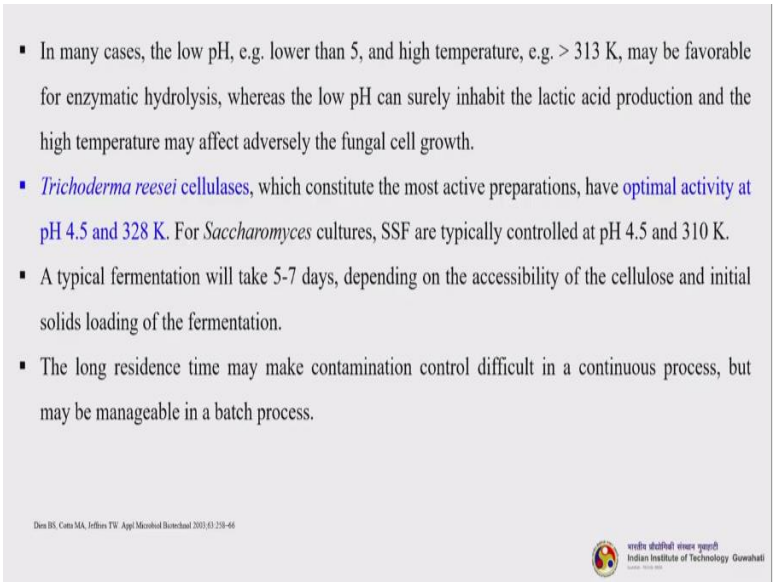

Now, in many cases the low pH as for example lower than 5 and high temperature for example greater than 313 Kelvin may be favorable for enzymatic hydrolysis, whereas the low pH can surely inhibit the lactic acid production and the high temperature may affect adversely the fungal cell growth. *Trichoderma reesei* cellulases which constitute the most active preparations have optimal activity at pH 4.5 and 328 Kelvin temperature.

Similarly, for *Saccharomyces* cultures, SSF are typically controlled at pH 4.5, but the temperature is a little low at 310 Kelvin. A typical fermentation will usually take 5 to 7 days depending on the accessibility of the cellulose and initial solids loading of the fermentation. The long residence time make contamination control difficult in a continuous process, but may be manageable when you go for a batch process.

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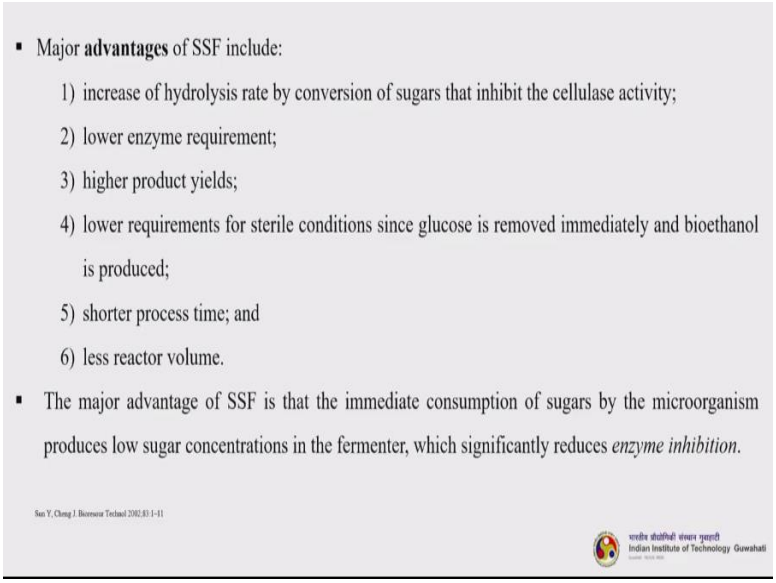

So, let us look for the advantages of this SSF. Now, there are many advantages. So, the first one is increase of hydrolysis rate by conversion of sugars that inhibit the cellulase activity. This is one of the most important thing when you talk about fermentation perspective. Now, lower enzyme requirement, higher product yields, lower requirements for sterile conditions - since glucose is removed immediately and being consumed and bioethanol is produced, shorter process time and less reactor volume. The major advantage of SSF is that the immediate consumption of sugars by the microorganisms produces low sugar concentrations in the fermenter which significantly reduces the enzyme inhibition.

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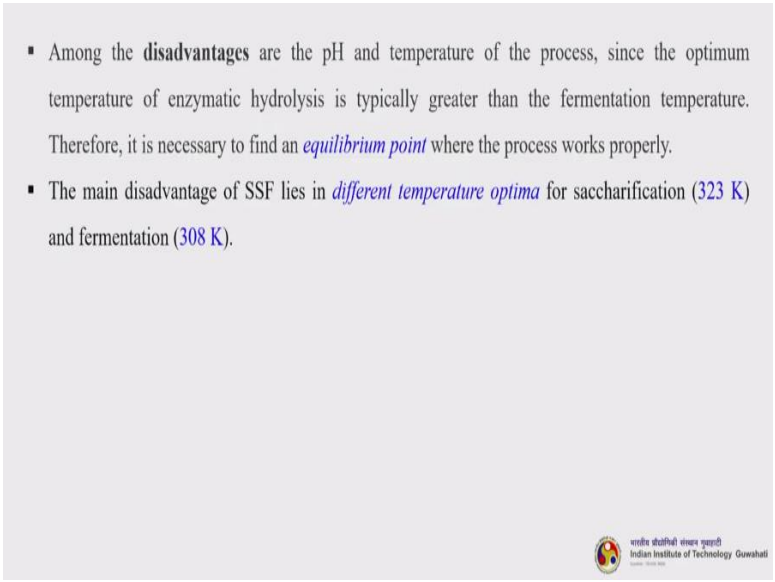

However, the major disadvantages are the pH and temperature of the process - this is what I was mentioning - since the optimum temperature of enzymatic hydrolysis is typically greater

than the fermentation temperature. Therefore, it is necessary to find an equilibrium point where the process works properly that means both fermentation and saccharification.

The main disadvantages of SSF lies in the different temperature optima for saccharification, it is 323 Kelvin and fermentation 308 Kelvin. So, some intermediate range you have to ensure so that both are happening in a particular efficient manner.

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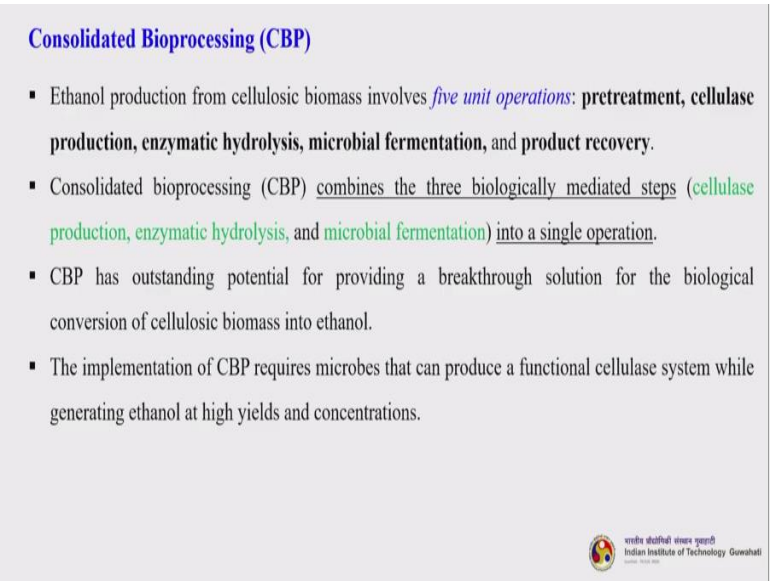

So, then another system which is called CBP, the consolidated bioprocessing. Now, ethanol production from cellulosic biomass involves 5 unit operations. What are those? First is pretreatment, second is cellulase production, third is enzymatic hydrolysis, fourth is the microbial fermentation and fifth is product recovery. Now, consolidated bioprocessing combines the 3 biologically mediated steps. What are those?

Cellulase production, enzymatic hydrolysis and microbial fermentation into a single operation - please underline single operation. So, that means 3 microbial processes are clubbed together, pretreatment is separate and your product recovery is separate. Now, CBP has outstanding potential for providing a breakthrough solution for the biological conversion of cellulosic biomass into ethanol.

The implementation of CBP requires microbes that can produce a functional cellulase system while generating ethanol at high yields and concentrations.

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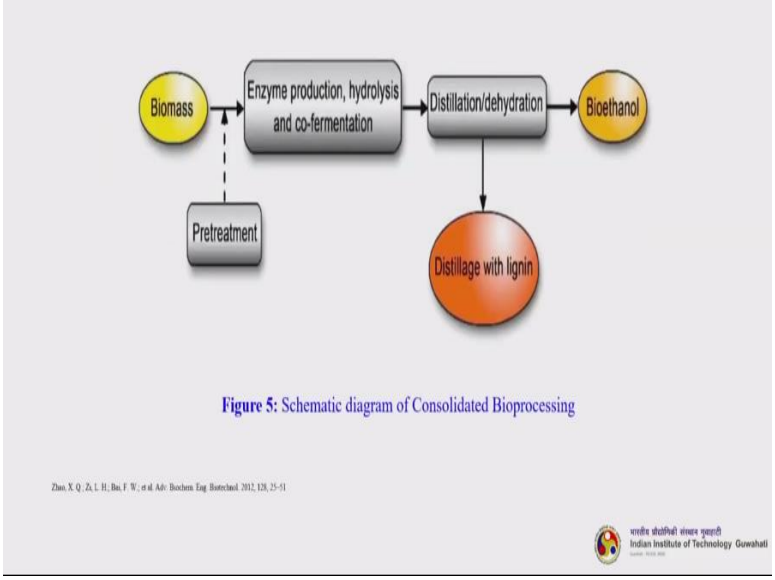

This is a typical schematic representation of the CBP system. Biomass, so of course it undergoes here, we have not seen here. So, biomass of course will undergo some different pretreatment processes. Then the next step is enzymatic production, hydrolysis and co-fermentation. Next is distillation and dehydration where you purify bioethanol and the distillage which is enriched with lignin can be converted and further processed to recover the lignin as you know that lignin is a high value product.

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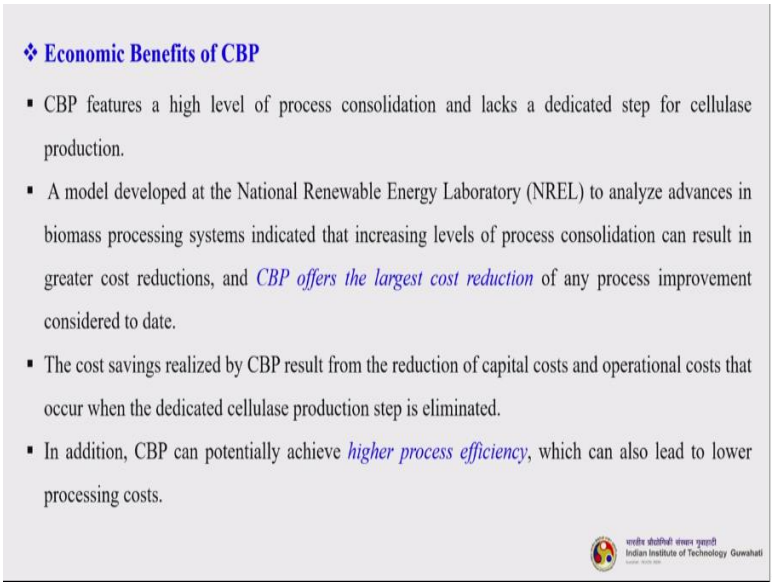

So, let us understand the economic benefits of the CBP process. Now, CBP features a high level of process consolidation and lacks a dedicated step for cellulase production. A model developed at the National Renewable Energy Laboratory to analyze the advances in biomass processing systems indicated that increasing levels of process consolidation can result in greater cost reductions.

And CBP offers the largest cost reduction of any process improvement considered to date. The costs savings realized by CBP result from the reduction of capital costs and operational costs that occur when that dedicated cellulase production step is eliminated. In addition, CBP can potentially achieve higher process efficiency, which can also lead to lower processing cost.

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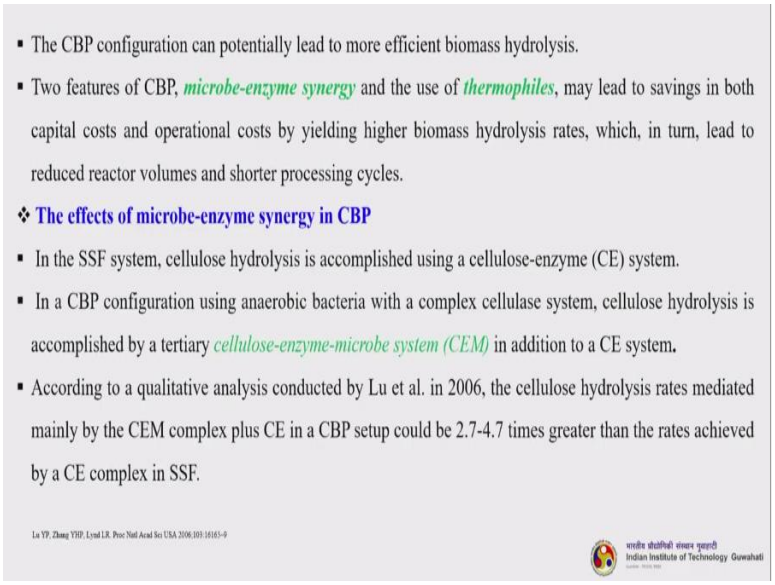

The CBP configuration can potentially lead to more efficient biomass hydrolysis. Two features of the CBP, one is the microbe-enzyme synergy and the other one is the use of thermophiles, may lead to savings in both capital cost and operational cost by yielding higher biomass hydrolysis rate which in turn lead to reduced reactor volumes and shorter processing cycles. Now we will try to understand the effects of the microbe-enzyme synergy in the CBP.

In the SSF system, cellulose hydrolysis is accomplished using a cellulose enzyme system. In a CBP configuration using anaerobic bacteria with a complex cellulase system, cellulose hydrolysis is accomplished by a tertiary cellulose-enzyme-microbe system which is called as CEM in addition to the CE system. So CE is just a cellulose-enzyme system, CEM is cellulose-enzyme-microbe system.

Now according to a qualitative analysis conducted by Lu et al in 2006, the cellulose hydrolysis rates mediated mainly by the CEM complex plus CE in a CBP setup could be 2.7 to 4.7 times greater than the rates achieved by CE complex in SSF. So that is the beauty of CBP.

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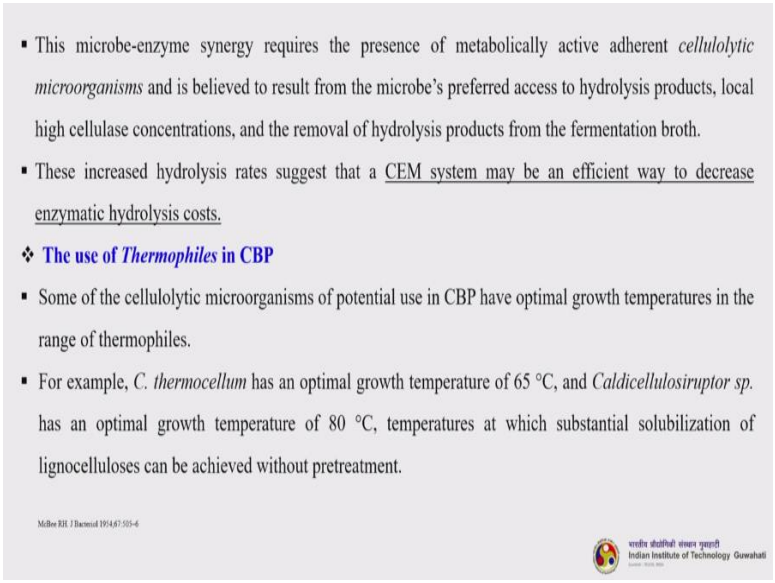

Now this microbe-enzyme synergy requires the presence of metabolically active adherent cellulolytic microorganisms and is believed to result from the microbe's preferred access to hydrolysis products, local high celulase concentrations and the removal of hydrolysis product from the fermentation broth. Now, these increased hydrolysis rates suggest that a CEM system may be an efficient way to decrease enzymatic hydrolysis cost.

Now we will try to understand the another important feature of the CBP which is the use of the thermophiles. Some of the cellulolytic microorganisms of potential use in CBP have optimal growth temperatures in the range of thermophiles. For example, *C. thermocellum* has an optimal growth temperature of 65 degrees centigrade whereas *Caldicellulosiruptor* species has an optimal growth temperature of 80 degrees centigrade, temperatures at which substantial solubilization of lignocelluloses can be achieved without pretreatment.

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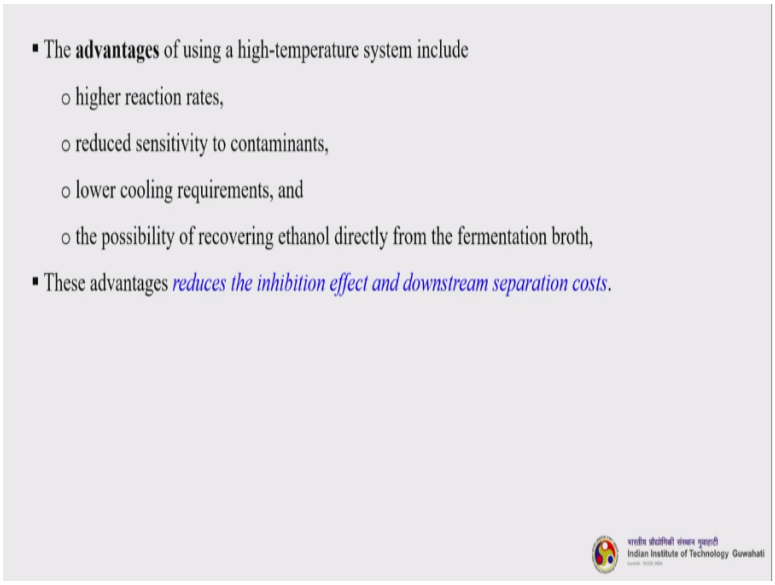

Now, the advantages of using a high-temperature system include - higher reaction rates, reduced sensitivity to contaminants, then lower cooling requirements and the possibility of recovering ethanol directly from the fermentation broth because it is at an elevated temperature and mostly it is in the vapor phase. Easy to collect it. Now, the advantages reduces the inhibition effect and the downstream separation cost.

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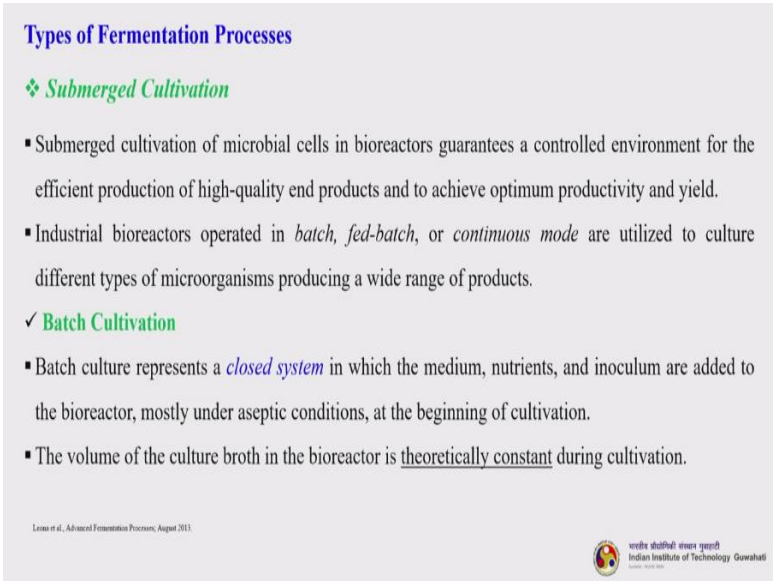

So, let us then understand the different types of fermentation processes. So, Submerged cultivation; now submerged cultivation of microbial cells in bioreactors guarantees a controlled environment for the efficient production of high quality end products and to achieve optimal productivity and yield. Industrial bioreactors operated in batch, fed-batch and continuous mode are utilized to culture different types of microorganisms producing a wide range of products.

So, we will discuss these 3 briefly. Batch cultivation: Now batch culture represents a closed system in which the medium, nutrients and inoculum are added to the bioreactor mostly under aseptic conditions at the beginning of cultivation. The volume of the culture broth in the bioreactor is theoretically constant during the cultivation.

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✓ Fed-Batch Cultivation

- Fed-batch culture represents a *semi-open system* in which one or more nutrients are aseptically and gradually added to the bioreactor while the product is retained inside.
- The volume of the culture broth in the bioreactor increases within this time.
- The main **advantages** of fed-batch over batch cultures are:
  a) the possibility to prolong product synthesis,
  b) the ability to achieve higher cell densities and thus increase the amount of the product, which is usually proportional to the concentration of the biomass,
  c) the capacity to enhance yield or productivity by controlled sequential addition of nutrients, and
  d) the feature of prolonged productive cultivation over the "unprofitable periods" when the bioreactor would normally be prepared for a new batch.

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So, fed-batch cultivation: Now fed-batch culture represents a semi-open system.

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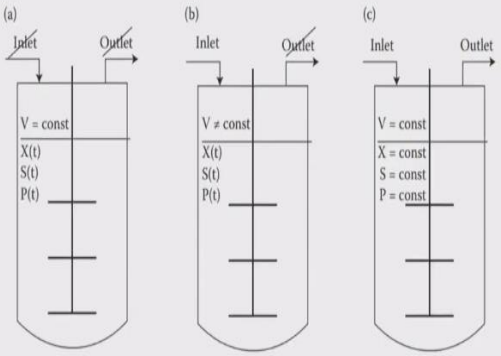

Figure 6: Simplified scheme of (a) batch, (b) fed-batch, and (c) continuous cultivation.

Leona et al., Advanced Fermentation Processes, August 2013.

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So, let us see the schematic representation. So this is batch, this is fed-batch and this is continuous. So, in the batch no inlet, no outlet. It is fed and closed, right. It is a completely closed system, volume is constant. And here in the fed-batch there is no outlet, but you supply intermittently the feed. Volume is not constant, right. In a continuous system

cultivation you have a continuous supply of feed and continuous withdrawal of the product, volume is maintained constant.

So, the fed-batch culture represents a semi-open system in which one or more nutrients are aseptically and gradually added to the bioreactor while the product is retained inside. The volume of the culture broth increases with time. Now, the main advantages of fed-batch over batch culture are that the possibility to prolong product synthesis; the ability to achieve higher cell densities and thus increase the amount of the product which is usually proportional to the concentration of the biomass; the capacity to enhance yield or productivity by controlled sequential addition of nutrients and; the feature of prolonged productive cultivation over the unprofitable periods when the bioreactor would normally be prepared for a new batch.

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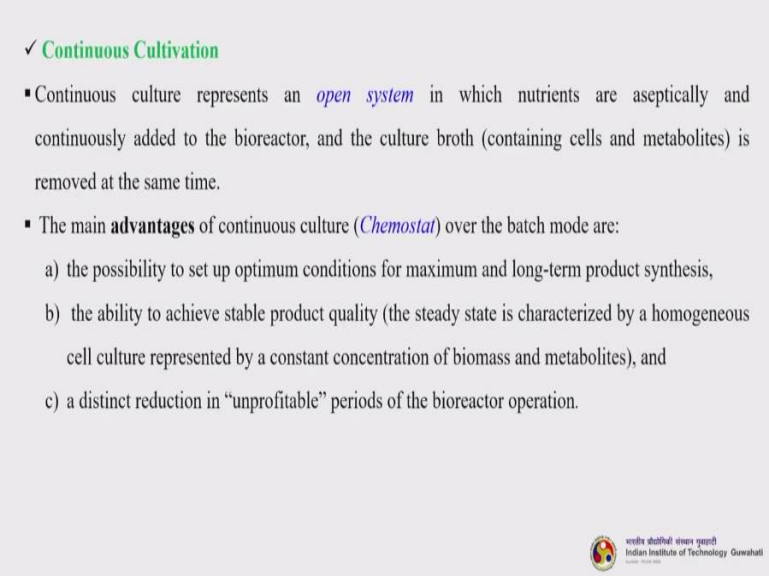

Continuous cultivation: Now continuous culture represents an open system in which nutrients are aseptically and continuously added to the bioreactor, and the culture broth that contains cells and metabolites that is moved at that same time. Now, the main advantage of continuous culture which is known as the chemostat over the batch mode are: the possibility to set up optimum conditions for maximum and long term product synthesis; The ability to achieve stable product quality - that is the steady state is characterized by a homogeneous cell culture represented by a constant concentration of biomass and metabolites and; a distinct reduction in unprofitable periods of the bioreactor operation.

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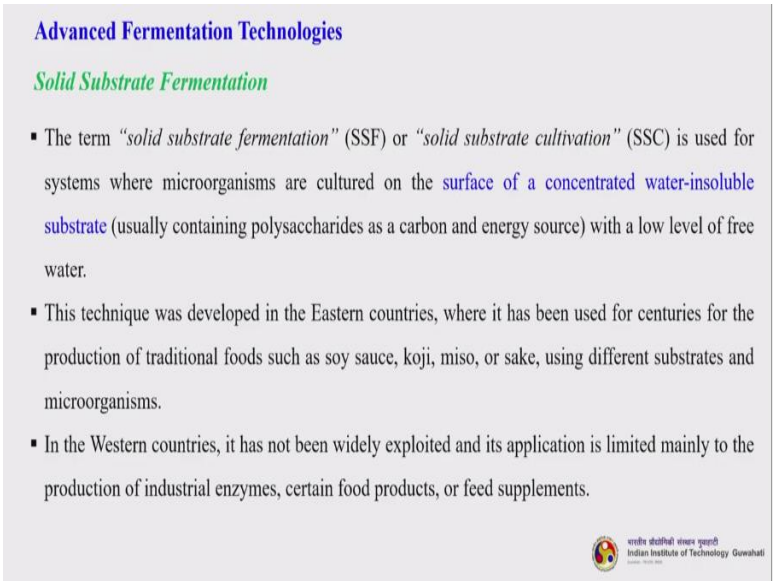

Let us move and discuss about the different other advanced fermentation technology. The first one is the solid state fermentation. The term solid state fermentation or solid substrate cultivation is used for systems where microorganisms are cultured on the surface of a concentrated water insoluble substrate (usually containing polysaccharides as a carbon and energy source) with a low level of free water.

Now, this technique was developed in the Eastern countries where it has been used for centuries for the production of traditional foods such as soy sauce, koji, miso or sake using different substrates and microorganisms. However, in the Western countries it has not been widely exploited and its application is limited mainly to the production of industrial enzymes, certain food products and feed supplements.

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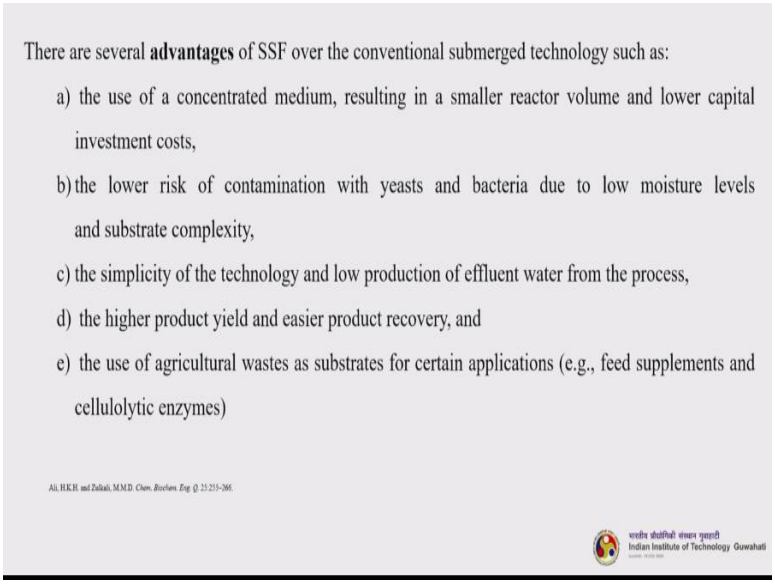

There are several advantages of this SSF over the conventional submerged technology such as: the use of the concentrated medium resulting in a small reactor volume and lower capital investment costs; The lower risk of contamination with yeasts - that is one of the most important thing - and bacteria of course due to low moisture levels and substrate complexity; The simplicity of the technology and low production of effluent water from the process (underline, that is very important, low production of effluent water); The higher product yield and easier product recovery and; the use of agricultural wastes as substitutes for certain applications. As for example feed supplements and cellulolytic enzymes.

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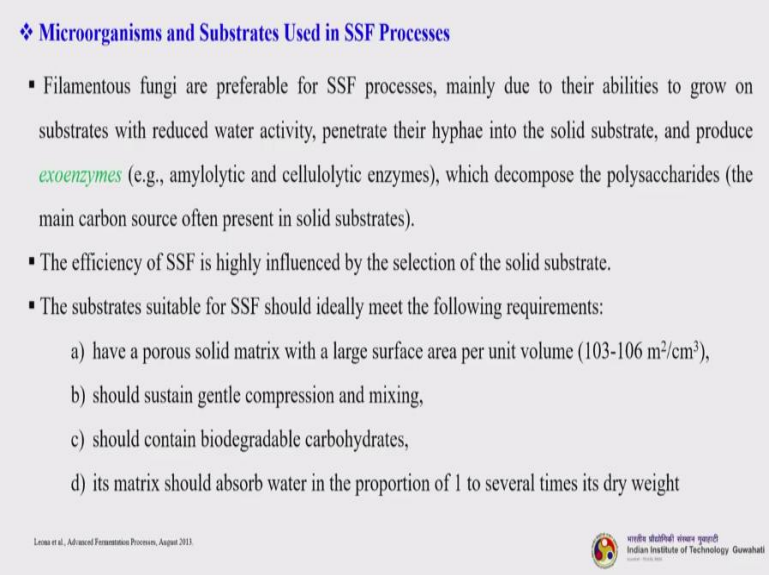

We will then discuss about the microorganisms and the substrates that are used in this SSF process. Filamentous fungi are preferred for this SSF processes mainly due to their abilities to grow on the substrates with the reduced water activity, penetrate their hyphae into the solid substrate and produce exoenzymes - for example amylolytic or cellulolytic enzymes, which decompose the polysaccharides - that is the main carbon source often present in the solid substrates.

The efficiency of SSF is highly influenced by the selection of solid substrate. The substrate suitable for SSF should ideally meet the following requirements. First it should have a porous solid matrix with a large surface area per unit volume almost in the range of 103 to 106 meter square per centimeter cube. It should sustain gentle compression and mixing. It should contain biodegradable carbohydrates. Its matrix should absorb water in the proportion of one to several times its dry weight.

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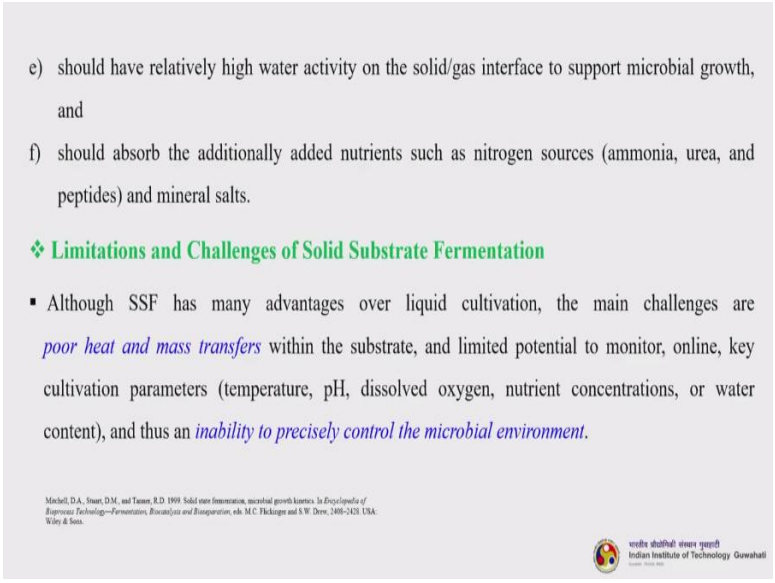

And also should have a relatively high water activity on the solid/gas interface to support microbial growth and should absorb the additionally added nutrients such as nitrogen sources ammonia, urea and peptides and mineral salts. Let us understand the limitations and challenges of the solid substrate fermentation. Although SSF has many advantages over liquid cultivation, the main challenges are poor heat and mass transfers within the substrate.

And limited potential to monitor, online and key cultivation parameters as for example temperature, pH, dissolved oxygen, nutrient concentration, water content, etc. Thus, an inability to precisely control the microbial environment.

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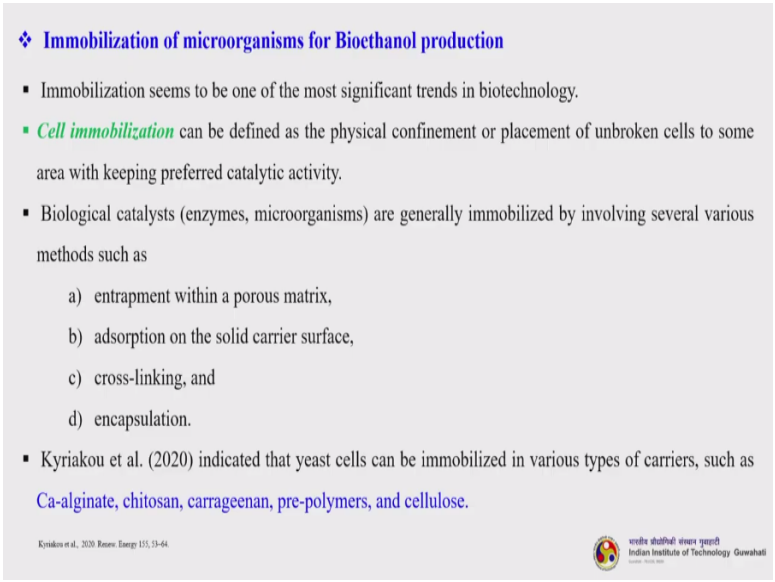

To overcome this, immobilization has been studied widely for bioethanol production. Now immobilization seems to be one of the most significant trends in the biotechnology industries.

Cell immobilization can be defined as the physical confinement or placement of unbroken cells to some area with keeping preferred catalytic activity.

Biological catalysts as for example enzymes, microorganisms are generally immobilized by involving several various methods such as entrapment within a porous matrix, adsorption on the solid carrier surface, crosslinking and encapsulation. Kyriakou et al indicated that yeast cells can be immobilized in various types of carriers such as calcium alginate, chitosan, carrageenan, pre-polymers and cellulose.

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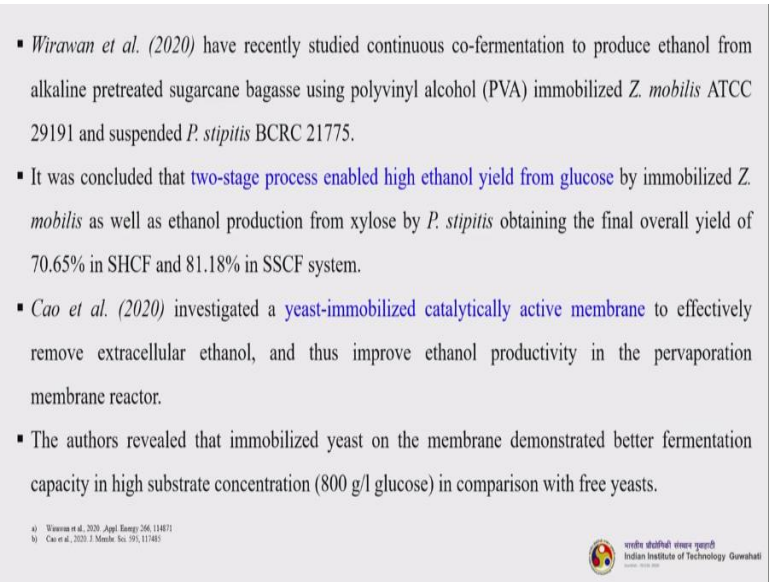

Wirawan et al have recently studied continuous co-fermentation to produce ethanol from alkaline pretreated sugarcane bagasse using polyvinyl alcohol immobilized *Zymomonas mobilis* ATCC 29191 and suspended *P. stipitis* BCRC 21775. These are the strains. Now, it was concluded that two-stage process enabled high ethanol yield from glucose by the immobilized *Zymomonas mobilis* as well as ethanol production from xylose by *P. stipitis* obtaining the final overall yield of almost 70.65% in SHCF and 81.18% in SSCF systems, quite a significant improvement. Now Cao et al investigated yeast-immobilized catalytically active membrane to effectively remove extracellular ethanol and thus improve ethanol productivity in the pervaporation membrane reactor. It is simultaneously getting removed so that there will be less inhibition.

Now, the authors revealed that immobilized yeast on the membrane demonstrated better fermentation capacity in high substrate concentrations almost 800 grams per liter of glucose in comparison with free yeasts.

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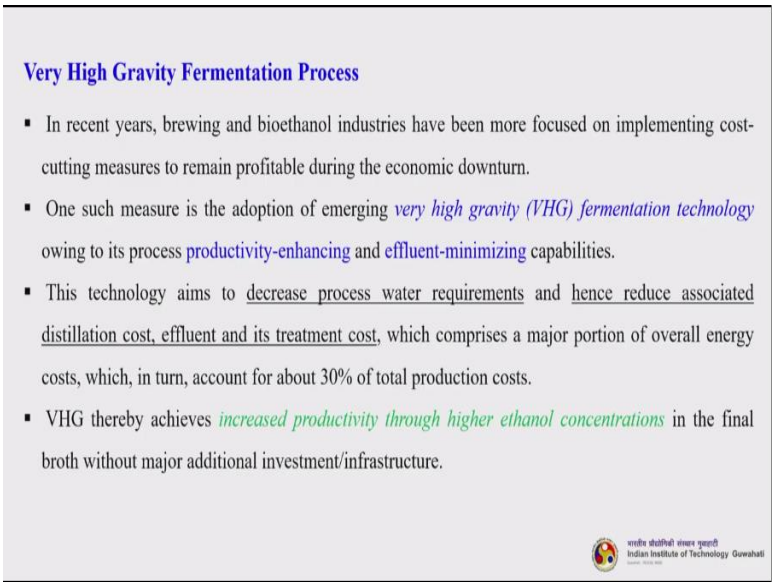

Now, another process which is known as Very high gravity fermentation process, VHG process. Now, in the recent years brewing and bioethanol industry have been more focused on implementing cost cutting measures to remain profitable during the economic downturn. One such measure is the adaptation of emerging very high gravity fermentation technology owing to its process productivity-enhancing and effluent-minimizing capabilities.

Very important is that effluent minimizing - because if you reduce your effluent so much downstream processing part and wastewater treatment steps can be eliminated. Now, this technology aims to decrease process water requirements and hence reduce associated distillation cost, effluent and its treatment cost which comprises a major portion of the overall energy cost which in turn accounts for about almost 30% of the total production cost. VHG thereby achieves increased productivity through higher ethanol concentrations in the final broth without major additional investment or infrastructure requirement.

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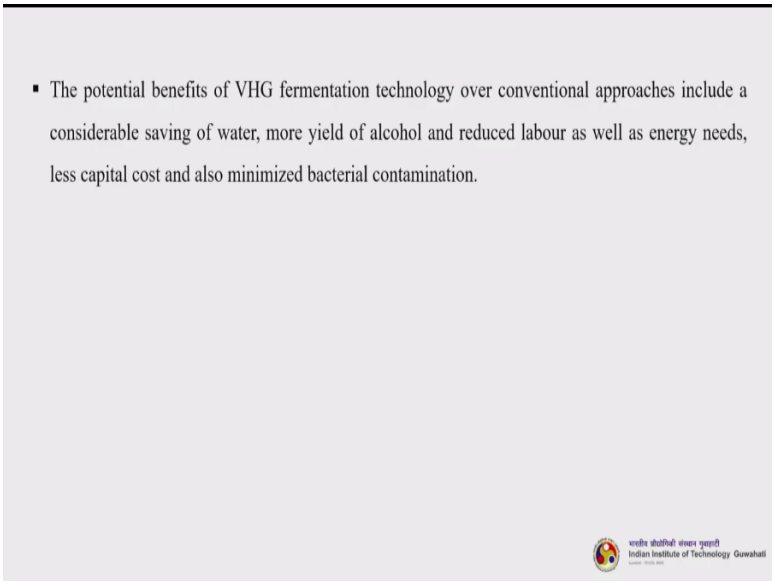

The potential benefits of VHG fermentation technology over conventional approaches include a considerable savings of water, more yield of alcohol and reduced labour as well as energy needs, less capital cost and also minimize bacterial contamination.

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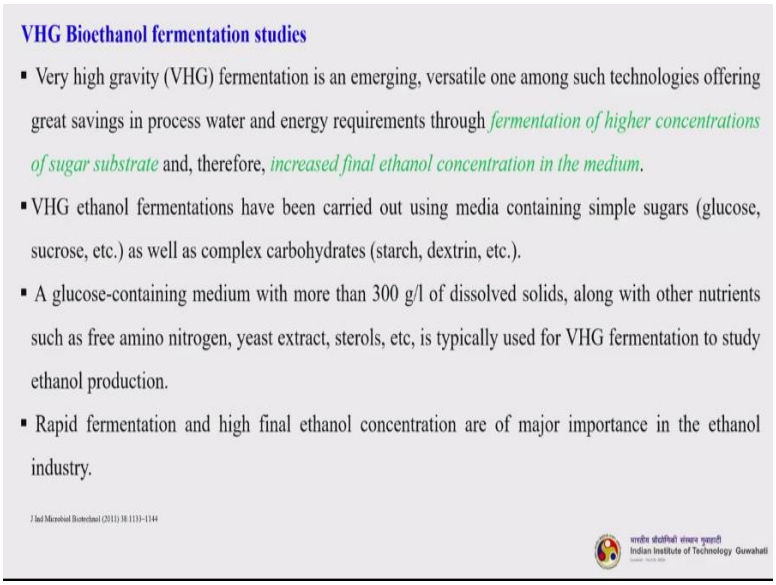

So, let us understand the VHG bioethanol fermentation. So VHG fermentation is an emerging versatile one among such technologies offering great savings in the process water and energy requirements through fermentation of higher concentrations of sugar substrate and therefore increased final ethanol concentration in the medium. VHG ethanol fermentations have been carried out using media containing simple sugars, like glucose, sucrose, etc., as well as complex carbohydrates also such as starch, dextrin, etc. A glucose-containing medium with more than 300 grams per liter of dissolved solids along with other nutrients such as free amino nitrogen, yeast extracts, sterols, etc., is typically used for the VHG fermentation to

study ethanol production. Rapid fermentation and high final ethanol concentration are of the major importance in the ethanol industry.

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Table 1: VHG ethanol fermentation studies using different starch substrates

Substrate	Conc. of dissolved solids	Maximum ethanol produced	Fermentation time (h)
Wheat mash	35% (w/v)	17.1% (v/v)	72
Wheat mash	37.9% (w/v)	23.8% (v/v)	130
Hull-less barley	32% (w/v)	17.1% (v/v)	96
Oats (hull-less)	>30% (w/v)	353.2 ± 3.7 l/t (dry wt.)	72
Rye	32–34% (w/v)	434.5 ± 5.1 l/t (dry wt.)	48
Rye and triticale	>30% (w/v)	15.7–16.1% (v/v)	96–120
Sweet & grain sorghum	34% (w/v)	16.8% (v/v)	96
Corn mash	35% (w/w)	126–130 (g/kg)	72
Malto-dextrin	>30% (w/v)	129 (g/l)	72
Pearl millet	35% (w/v)	16.8% (v/v)	72
Corn mash	>30% (w/v)	17% (v/v)	48
Barley (dehulled bold)	30% (w/w)	14.3% (v/v)	72
Potato mash	>30% (w/v)	16.61% (v/v)	72
Finger millet mash	>30% (w/v)	15.6% (v/v)	72
Cassava starch	40% (w/v)	15.03% (v/v)	72
Sweet potato mash	>30% (w/v)	~17.0% (v/v)	36

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So, this table will make you understand the ethanol production via VHG fermentation using different starch substrates. You can look at closely for different substrates such as wheat, maize, hull-less barley, oats, rye, then sweet and grain sorghum, then corn mash, potato mash, sweet potato mash. You see that just go to the third one, third column you can see the maximum ethanol produced.

You can see in percentage it almost varies from 14 to 17%. One or two cases it is 23.8% in case of wheat mash, where the concentration of dissolved solids is 37.9% and fermentation time is 130 hours. So enhanced fermentation time or more or less the maximum ethanol that is produced is in that same range volume by volume almost 15 to 17%.

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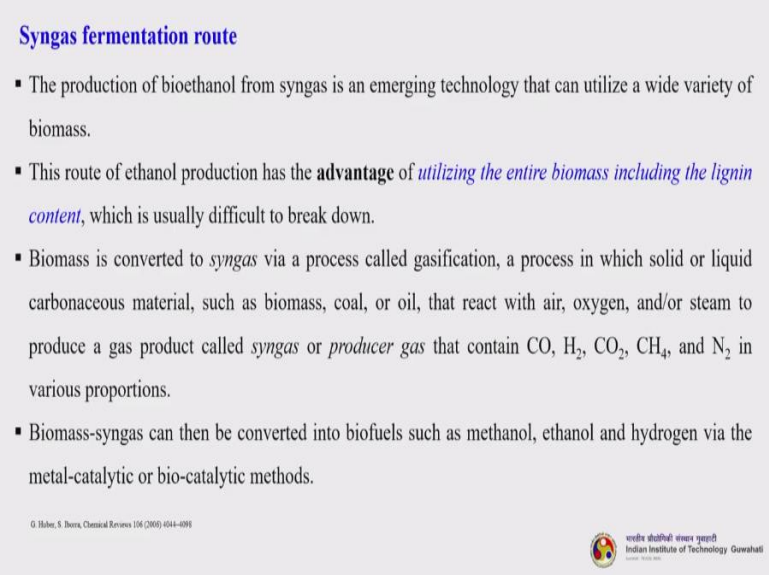

So, then the next is syngas fermentation route. Now, the production of bioethanol from syngas is an emerging technology that can utilize a wide variety of biomass and it fits exactly very nicely in a biorefinery concept. The route of ethanol production has the advantage of utilizing the entire biomass including the lignin content, which is usually difficult to break down and it is not so in case of your SSFs or CBP.

Now, biomass is converted into syngas via a process called gasification (which we have discussed in detail earlier), a process in which solid or liquid carbonaceous material such as biomass, coal or oil that react with air, oxygen and/or steam to produce a gas product called syngas or producer gas that contains carbon monoxide, hydrogen, carbon dioxide, methane and nitrogen in various proportions. Biomass syngas can then be converted into biofuels such as methanol, ethanol and hydrogen via the metal-catalytic or biocatalytic methods.

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❖ *Process*

- The initial stage is ethanol production via syngas is biomass gasification.
- Cleaned, gas is cooled to the normal ambient temperature and stored at a high pressure.
- Cooled and cleaned gas is fed into an ethanol conversion chamber where microbes ferment the gas into ethanol and acetic acid.
- After fermentation is complete, the liquid is distilled to separate ethanol from other products.
- The ethanol produced is then dehydrated to produce fuel quality ethanol (Fig. 7).
- The cell mass can be recycled to the gasifier, while it is not approved as animal feed.
- A large number of bacterial strains have been isolated that have the ability to ferment producer gas (the CO, CO_2, and H_2 components) to ethanol, acetic acid, and other useful liquid.

P. Dwivedi, J.R.R. Alavalapati, P. Lal, Energy for Sustainable Development 13 (2009) 174–182

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Let us understand the process. The initial stage is ethanol production via syngas is biomass gasification. Cleaned gas is cooled to the normal ambient temperature and stored at a high pressure. Cooled and cleaned gas is fed into an ethanol conversion chamber where microbes ferment the gas into ethanol and acetic acid. After fermentation is complete, the liquid is distilled to separate ethanol from other products.

The ethanol produced is then dehydrated to produce fuel quality ethanol. I will show you the figure 7, then we can understand it better. The cell mass can be recycled to the gasifier while it is not approved as animal feed in case. Now, a large number of bacterial strains have been isolated that have the ability to ferment producer gas to ethanol, acetic acid and other useful liquid.

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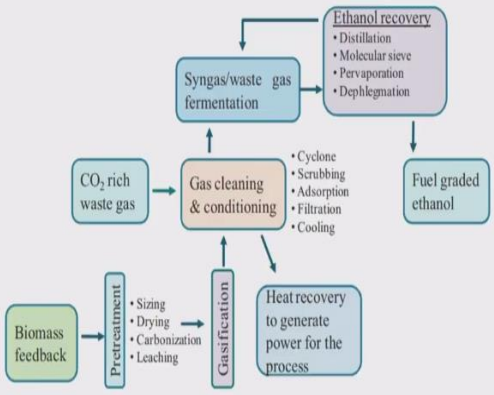

Figure 7: Production of ethanol by microbial fermentation of biomass-derived syngas

H.N. Abubackar, M.C. Veiga, C. Kennes, Bioproducts and Bioenfining 5 (2011) 93–114

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Now, this is the schematic of the production of ethanol by microbial fermentation of the biomass-derived syngas. So, you can see the biomass. Then we have the pretreatment, any sorts of physicochemical pretreatment processes. Then it goes to the gasification. Now, after the gasification is over, the gas goes to the gas cleaning and conditioning step where the heat can be recovered to generate power for the process and again being reused in this process.

Carbon dioxide rich waste gas may be supplied for the cleaning purposes. Then whatever you get the syngas or waste gas it goes to the fermentation here. This is the fermenter. Then you do the ethanol recovery. Part of ethanol can be feedback. Then you go for what you can call the downstream processing part - distillation and other processing - to get the fuel graded ethanol.

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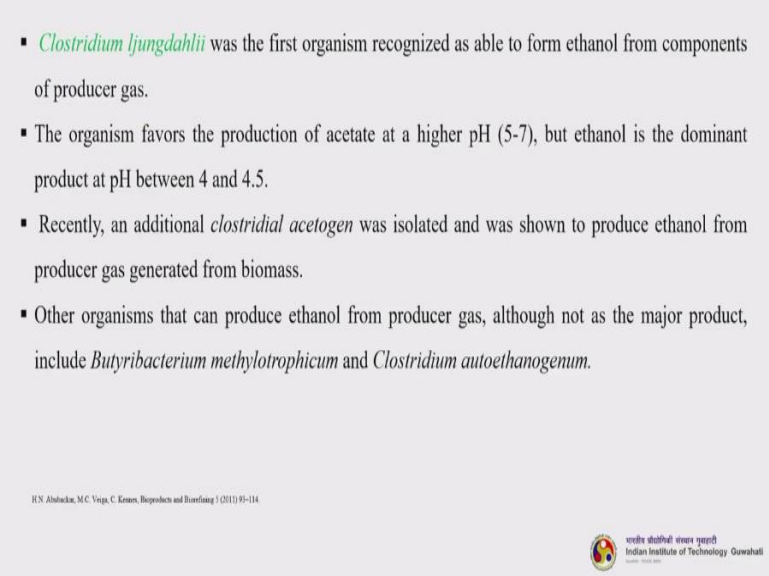

So, *Clostridium ljungdahlii* was the first organism recognized as able to form ethanol from components of producer gas. The organism favors the production of acetate at a higher pH, but ethanol is the dominant product at pH between 4 and 4.5. Some optimization needs to be done. Now recently, an additional *clostridial acetogen* was isolated and was shown to produce ethanol from producer gas generated from biomass.

Other organisms that can produce ethanol from producer gas although not as the major product include *Butyribacterium methylotrophicum* and *Clostridium autoethanogenum*.

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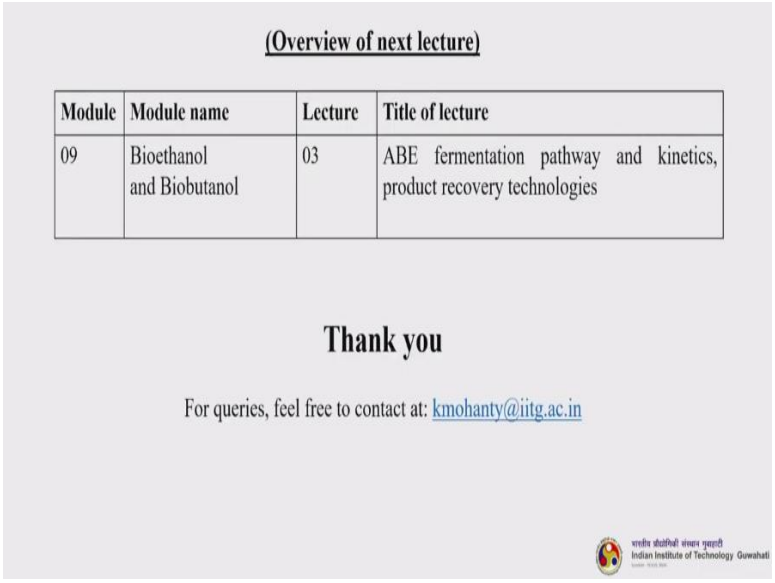

So with this, I conclude today's lecture. In our next lecture that is lecture 3 under module 9, we will be discussing about biobutanol that is the ABE fermentation pathway, its kinetics and how do you recover biobutanol. So in case you have any query, please feel free to register it in the Swayam portal or you can drop a mail to me at kmohanty@iitg.ac.in. Thank you.