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

Lecture – 27
ABE Fermentation Pathway and Kinetics, Product Recovery Technologies

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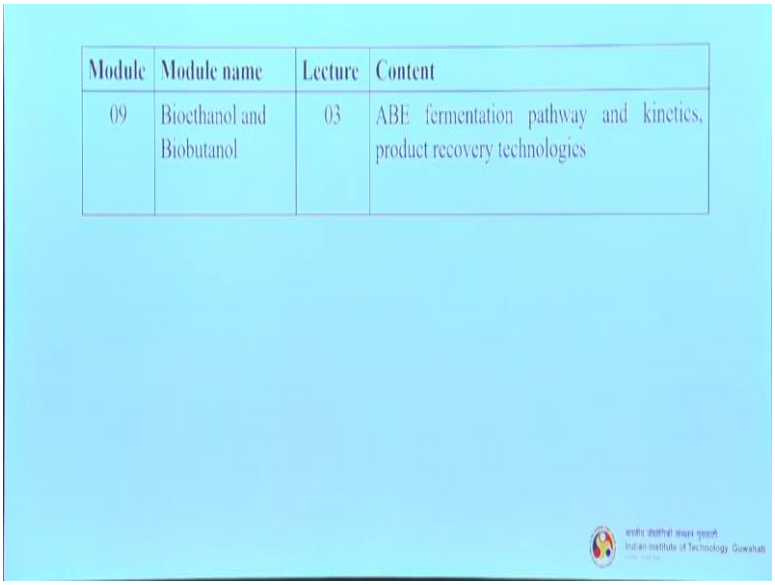

Good morning students. This is lecture 3 under module 9. And in today's lecture we will be discussing in detail about the ABE fermentation, one of the most important fermentation processes that actually produces acetone, butanol and ethanol, but the major aim can be to produce butanol as a fuel additive. So let us begin.

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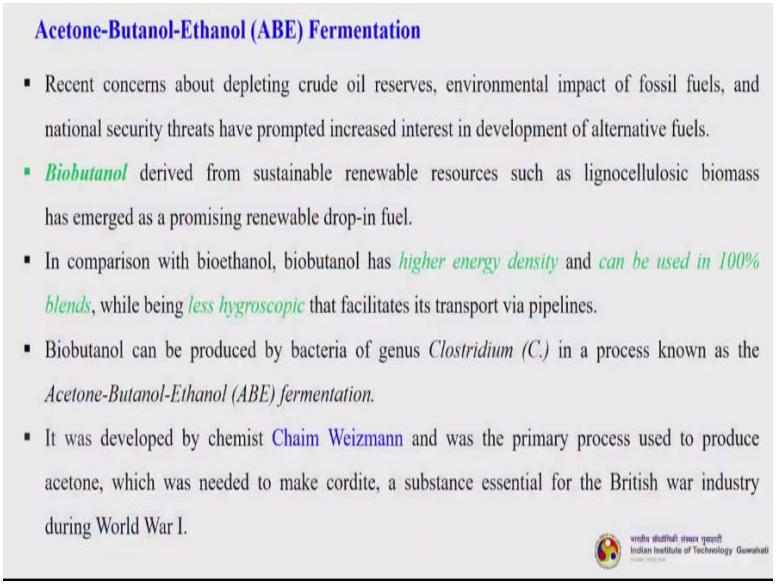

So, recent concerns about depleting crude oil reserves, environmental impact of fossil fuels and national security threats have prompted increased interest in the development of alternative fuels. Biobutanol derived from sustainable renewable sources such as lignocellulosic biomass has emerged as a promising renewable drop-in fuel. In comparison with bioethanol, biobutanol has a higher energy density and can be used in 100% blends, while being less hygroscopic that facilitates its transport via pipelines.

Biobutanol can be produced by a bacteria of genus *Clostridium* in a process known as the Acetone-Butanol-Ethanol fermentation - in short ABE fermentation. It was developed by chemist Chaim Weizmann and was the primary process used to produce acetone, which was needed to make cordite, a substance essential for the British war industry during the World War 1.

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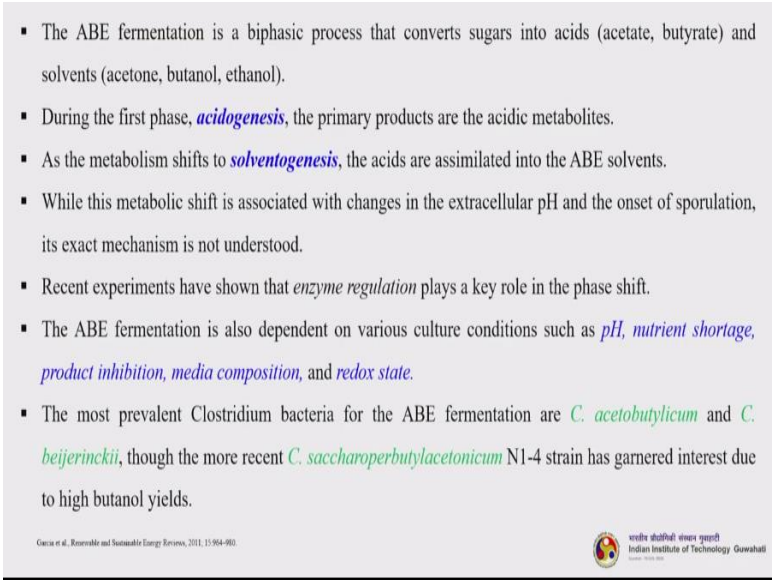

The ABE fermentation is a biphasic process that converts sugars into acids, basically your acetate and butyrate, and then solvents acetone, butanol and ethanol. During the first phase which is also known as the acidogenesis phase the primary products are the acidic metabolites. As the metabolism shifts to solventogenesis, the acids are assimilated into the ABE solvents.

While this metabolic shift is associated with changes in the extracellular pH and the onset of sporulation, its exact mechanism is not well understood. Recent experiments have shown that enzyme regulation plays a key role in the phase shift. The ABE fermentation is also

dependent on various culture conditions such as pH, nutrient shortage, product inhibition, media composition as well as the redox state.

The most prevalent *Clostridium* bacteria for the ABE fermentation are *Clostridium acetobutylicum* and *Clostridium beijerinckii*, though the more recent species mentioned here the *Clostridium saccharoperbutylacetonicum* N1-4 strain garnered a lot of interest due to its high butanol yield capacity.

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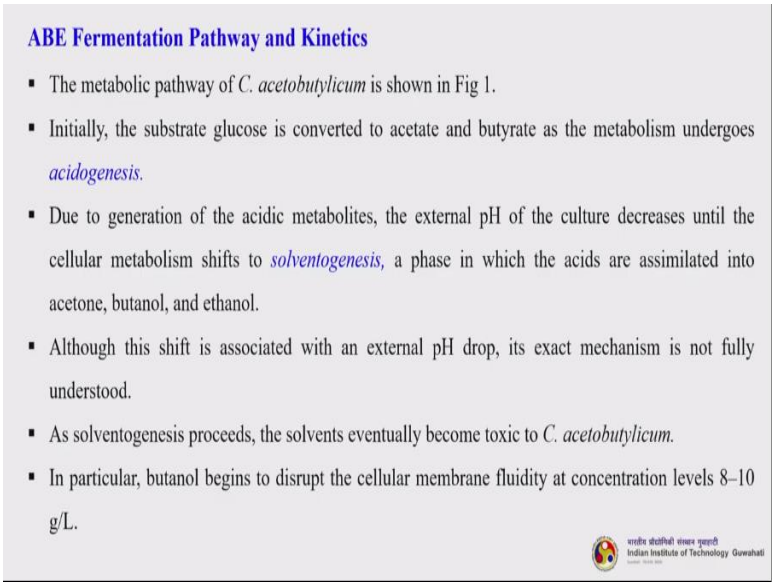

So, we will discuss about the fermentation pathway and the kinetics of the ABE fermentation route. The metabolic pathway I have shown here, we will just go to the next slide.

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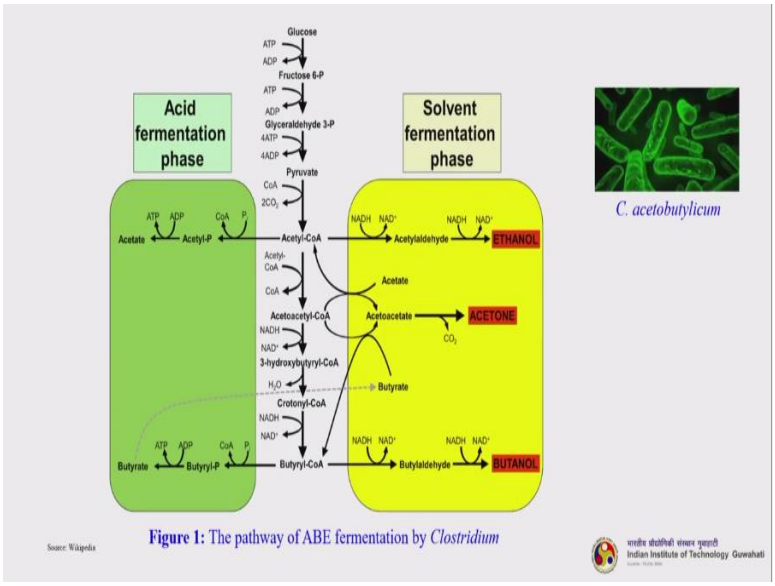

You can have a close look here. So this is the metabolic pathway of the ABE fermentation that is the *Clostridium acetobutylicum* strain image, microscopic image. So, this is the acid fermentation phase here where we are getting acetate and butyrate and here the yellow portion is the solventogenesis phase or the solvent fermentation phase in which we are getting the solvents of our interest ethanol, acetone and butanol.

So, let us discuss how it is happening basically. So, initially the substrate glucose is converted to acetate and butyrate as the metabolism undergoes the acidogenesis. Due to the generation of the acidic metabolites, the external pH of the culture decreases until the cellular metabolism shifts to solventogenesis, a phase in which the acids are assimilated into acetone, butanol and ethanol.

Although the shift is associated with an external pH drop, its exact mechanism is not fully understood. As solventogenesis proceeds, the solvents eventually become toxic to the *Clostridium* strain. Now in particular, butanol begins to disrupt the cellular membrane fluidity at concentration levels of just 8 to 10 grams per liter. So, let us see how it happens actually. This is the main metabolic pathway.

So the glucose get converted to fructose 6-P with the help of ATP, the adenosine triphosphate energy molecules and it again generates ADP. Now in the second phase, the fructose 6-P is getting converted to glyceraldehyde 3-P again with the help of ATP. And similarly that glyceraldehyde 3-P is getting converted to pyruvate the most important molecule of this fermentation pathway.

The pyruvate gets converted to acetyl coenzyme A with the help of enzyme coenzyme A and it produces two carbon dioxide molecules. Now this acetyl coenzyme A can get into different pathways. So, one pathway is this where we get actually the acetate and butyrate which is known as the acidogenesis path. Now, if you see this also there is a shift to here. Now, all these things depends upon the process conditions your pH and other parameters.

So, that goes to the solventogenesis phase where you get the ethanol, acetone and butanol. Now acetyl coenzyme A part also can be converted to acetoacetyl coenzyme A, then 3-hydroxybutyryl CoA, crotonyl coenzyme A and then finally butyryl coenzyme A. Now, this

butyryl coenzyme A again goes back to two different pathways. So, one is solventogenesis pathway where we directly get butanol and here you get butyrate under the acidogenesis path.

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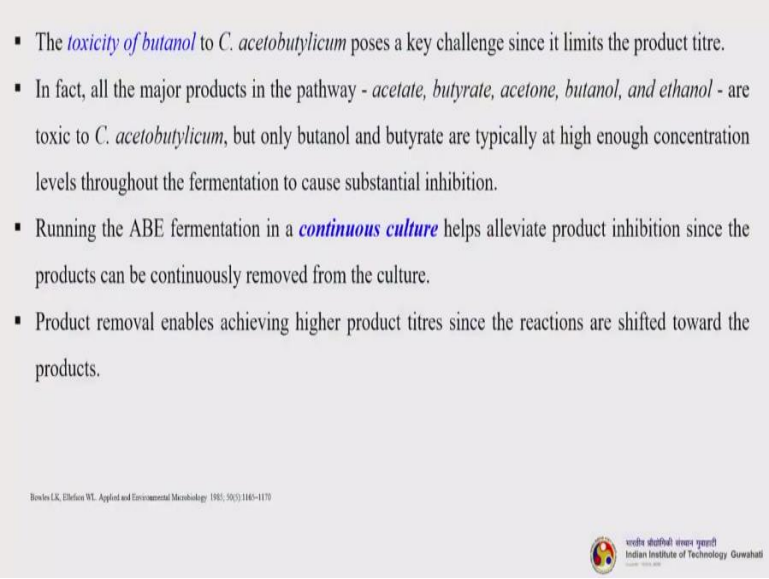

So, the toxicity of butanol to *Clostridium* poses a key challenge since it limits the product titre. In fact, all the major products in the pathway that is the acetate, butyrate, acetone, butanol and ethanol are toxic to the *Clostridium* species, but only butanol and butyrate are typically at high enough concentration levels throughout the fermentation to cause substantial inhibition. Because your acetone and ethanol, the rate of formation of those two are much less compared to that of the butanol.

Now, running the ABE fermentation in a continuous culture helps alleviate product inhibition since products can be continuously removed from the culture. Product removal enables achieving a higher product titre since the reactions are shifted towards the products and this is what actually we wish to happen. So, continuous culture of course solves that problem. However, it has also some inherent issues.

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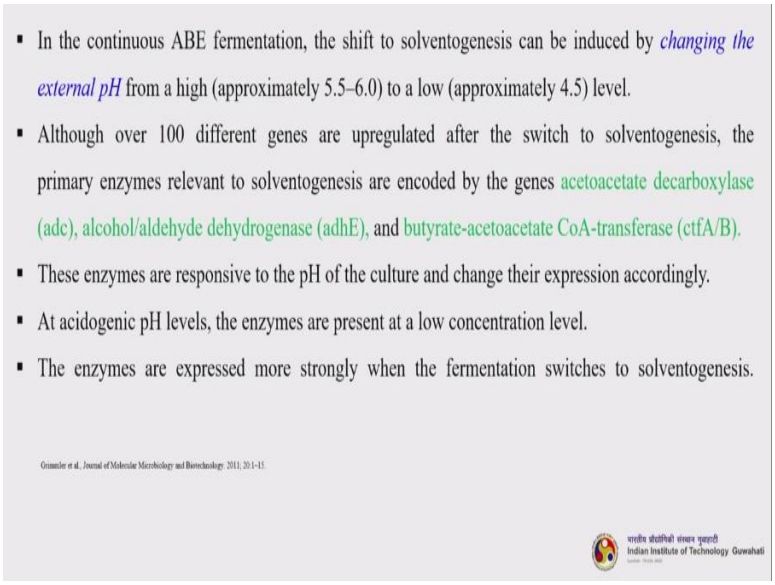

Now, in the continuous ABE fermentation, the shift to solventogenesis can be induced by changing the external pH from a very high approximately 5.5 to 6 to a low almost around 4.5 level. Now, although there are 100 different genes that are upregulated after the switch to solventogenesis, the primary enzymes relevant to solventogenesis are encoded by the genes acetoacetate decarboxylase which is known as the adc, alcohol aldehyde dehydrogenase (adhE) and then butyrate-acetoacetate CoA transferase (ctfA/B). Now, these enzymes are responsive to the pH of the culture and change their expression accordingly. So, please understand pH plays a very important role in the entire ABE fermentation process. Now, at acidogenic pH levels, the enzymes are present at low concentration level. The enzymes are expressed more strongly when the fermentation switches to solventogenesis.

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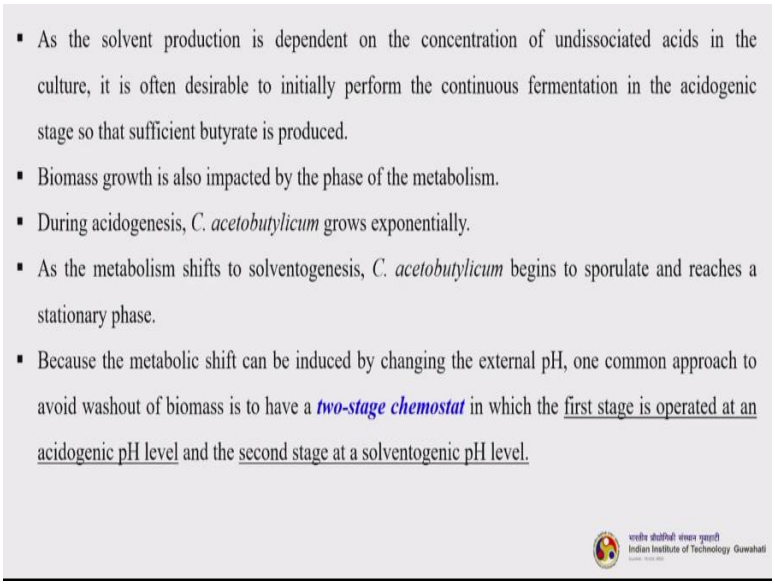

As the solvent production is dependent on the concentration of the undissociated acids in the culture, it is often desirable to initially perform the continuous fermentation in the acidogenic stage so that the sufficient butyrate is produced. Biomass growth is also impacted by the phase of metabolism. During acidogenesis, *Clostridium* grows exponentially. As the metabolism shifts to solventogenesis, *Clostridium* begins to sporulate and reaches a stationary phase.

Now, because the metabolic shift can be induced by changing the external pH, one common approach to avoid washout of biomass is to have a two-stage chemostat, two-stage continuous process basically in which the first stage is operated at an acidogenic pH level and the second stage at a solventogenesis pH level. Now if you do like that, then what will happen is that you can freely maintain the optimum culture conditions or environment for both acidogenesis as well as the solventogenesis.

So, in a single fermenter it is very difficult to maintain that. So, somewhere you have to optimize or while you are optimizing that in a single this one (fermenter) somewhere you have to make compromise.

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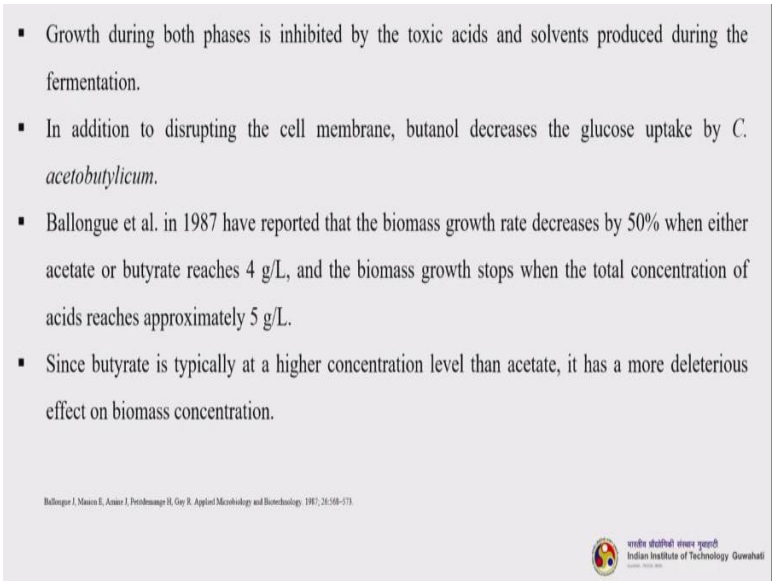

Now, growth during (both) phases is inhibited by the toxic acids and solvents produced during the fermentation. In addition to disrupting the cell membrane, butanol decreases the glucose uptake by *Clostridium acetobutylicum*. Now, Ballongue et al in 1987 have reported that biomass growth rate decreased by 50% when either acetate or butyrate reaches 4 grams per liter, that becomes toxic beyond that limit.

And that the biomass growth stops when that total concentration of acids reaches approximately 5 grams per liter. You can see such a narrow range. Now since butyrate is typically at a higher concentration level than acetate, it has more deleterious effect on the biomass concentration.

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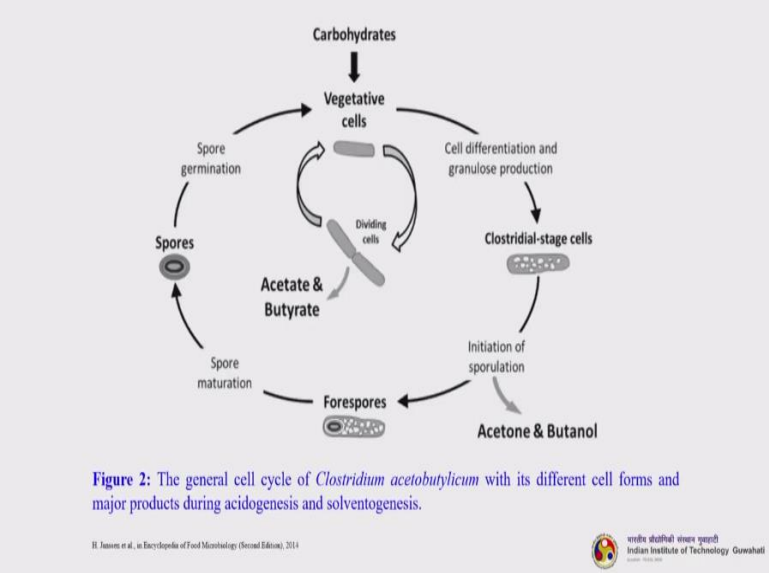

Figure 2: The general cell cycle of *Clostridium acetobutylicum* with its different cell forms and major products during acidogenesis and solventogenesis.

H. Jansen et al., in Encyclopedia of Food Microbiology (Second Edition), 2014

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So, let us understand the general cell cycle of the *Clostridium acetobutylicum* with its different cell forms and the major products during the acidogenesis and solventogenesis. How it actually happens that growth phase of the *Clostridium* species? So we can start that. Let us say the carbohydrate that is being fed to the vegetative cells which are of the *Clostridium* species. Then it goes in this cycle.

So, the cell differentiation and granulose production happens. We will get the Clostridial stage cells, then initiation of the sporulation. Now, at this stage acetone and butanol are getting formed. Now their growth is happening, so they started doing the sporulation. So, you can see the forespores here. Now, after that we have the spore maturation stage that will come. Now once that happens, then the spores will come out and then spore germination will happen, eventually they again get converted to vegetative cells.

Now in a secondary way, we can say that this vegetative cells are also dividing themselves during their growth. And when that is happening, you are getting acetate and butyrate. So, now I hope you can understand that how the cell division and the cell growth of the *Clostridium* species is happening and in the same time when they are producing basically our

product of interest acetone, butanol of course even acetate and butyrate to carry out their metabolic pathway.

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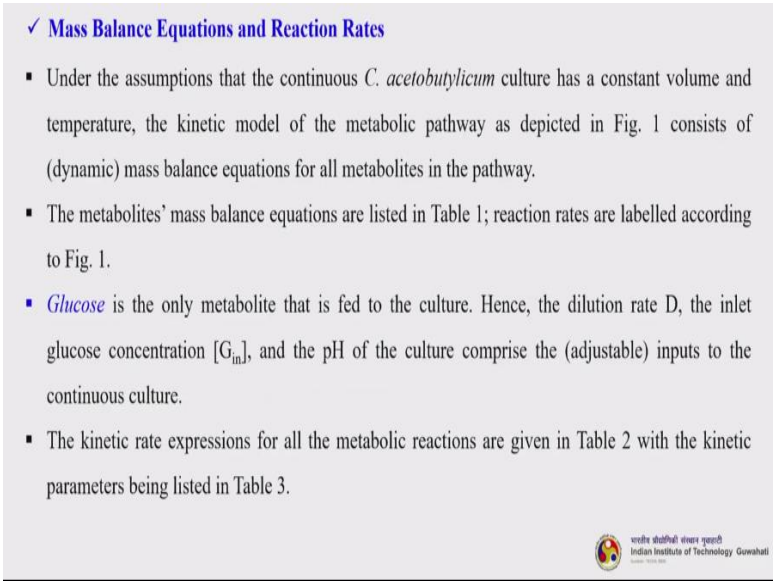

Let us talk about the mass balance equation and the reaction rates. Under the assumptions that the continuous *Clostridium acetobutylicum* culture has a constant volume and temperature, the kinetic model of the metabolic pathway as depicted in the figure 1 consists of a dynamic mass balance equations for all metabolites in the pathway. Whatever I have shown starting from your glucose, then it goes to pyruvate, from pyruvate to acetyl coenzyme A and then butyrate and all these things.

Now, glucose is the only metabolite that is fed to the culture. Hence, that dilution rate D, the inlet glucose concentration and the pH of the culture comprise the inputs to the continuous culture. These are the main important parameters. The kinetic rate expressions for all the metabolic reactions are given in table 2 in the next slide with the kinetic parameters are also given in that table 3.

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Table 1: The mass balance equations for all metabolites in the continuous *C. acetobutylicum* culture (see Fig 1)

Glucose : $\frac{d[G]}{dt} = -R_1 - R_4 - D([G] - [G_{in}])$	Acetate : $\frac{d[A]}{dt} = R_7 - R_8 - D[A]$
Fructose 6 – Phosphate : $\frac{d[F_{6P}]}{dt} = R_1 - R_2 - D[F_{6P}]$	Ethanol : $\frac{d[En]}{dt} = R_{10} - D[En]$
Glucose 3 – Phosphate : $\frac{d[G_{3P}]}{dt} = R_2 - R_3 - D[G_{3P}]$	Acetoacetyl – CoA : $\frac{d[AaC]}{dt} = R_3 - R_5 - R_{12} - R_{13} - D[AaC]$
Pyruvate : $\frac{d[Py]}{dt} = R_3 + R_1 - R_5 - R_6 - D[Py]$	Acetoacetate : $\frac{d[Aa]}{dt} = R_6 + R_{13} - R_{11} - D[Aa]$
Lactate : $\frac{d[Lac]}{dt} = R_5 - R_{14} - D[Lac]$	Butyryl – CoA : $\frac{d[BC]}{dt} = R_{12} + R_{13} - R_{14} - R_{15} - D[BC]$
Butyrate : $\frac{d[B]}{dt} = R_{14} - R_{13} - D[B]$	Acetyl – CoA : $\frac{d[AC]}{dt} = R_4 + R_5 - R_7 - R_9 - R_{10} - D[AC]$
Biomass : $\frac{d[X]}{dt} = R_x - R_d - D[X]$	Acetone : $\frac{d[An]}{dt} = R_{11} - D[An]$
Carbon Dioxide : $\frac{d[CO_2]}{dt} = R_6 + R_{11} - D[CO_2]$	Butanol : $\frac{d[Bn]}{dt} = R_{15} - D[Bn]$
Adc : $\frac{d[Ad]}{dt} = r_{Ad} + r^+_{Ad}H - D[Ad]$	CtfA/B : $\frac{d[Cf]}{dt} = r_{Cf} + r^+_{Cf}H - D[Cf]$
AdhE : $\frac{d[Ah]}{dt} = r_{Ah} + r^+_{Ah}H - D[Ah]$	

Note: In all equations, [ ] denotes metabolite concentration (mM), R refers to rate equations (mM h^{-1}), r is the base enzyme production rate during acidogenesis (mM h^{-1}), r+ is the upregulated enzyme production rate during solventogenesis (mM h^{-1}), H is defined in Eq (3), D is the dilution rate (h^{-1}), and $[G_{in}]$ is the inlet glucose concentration (mM). The rate equations are given in Table 2.

Buehler EA, Mesbah A (2016) Kinetic Study of Acetone-Butanol-Ethanol Fermentation in Continuous Culture

भारतीय प्रौद्योगिकी संस्थान गुवाहाटी
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So, you can refer to this. I have listed this. This is taken from a very interesting work by Buehler and Mesbah. It is already given, you can see the reference also. Now, the mass balance equations for all the metabolites in the continuous *Clostridium* culture according to the figure 1 which we have shown. So glucose mass balance equation, then fructose, pyruvate, lactate, butyrate, carbon dioxide, acetate, ethanol everything is given.

So, you can refer to it later on. So, we are not going to discuss this kinetics in detail, but I just thought that I will give this information so that you can understand that what actually is happening in a nutshell.

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- Most of the reactions in the metabolic pathway are represented by a typical enzyme-substrate reaction scheme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P. \qquad (1)$$

- The rate expressions for these reactions are described by the ***Michaelis-Menten kinetics***:

$$\frac{d[P]}{dt} = \frac{V_{max}[S]}{K_S + [S]}$$

- Where [S] and [P] denote the concentration of substrate S and product P, respectively (mM); V_{max} is the maximum reaction rate (h^{-1}); and K_S is the Michaelis constant (mM).

Michaelis L, Menten ML. Kinetik der Invertinwirkung. Biochemical Zeitung. 1913; 49:333-369.

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So, most of the reactions in the metabolic pathway are represented by the typical enzyme substrate reaction scheme. So, what is that? E + S, E is the enzyme, S is the substrate, so that

when they react, the kinetic constant is k1 and k –1. So it is a reversible reaction. So, they form ES, ES is the intermediate complex. Now when the ES undergoes further reaction, it gives us E + P, so P is your product.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P. \qquad (1)$$

So, this is the usual enzyme substrate reaction. Now, the rate expressions we can do by the famous Michaelis-Menten kinetics, MM kinetics. So, you can write d by dt of P = V max S by K s + S, now where S and P denote the concentration of the substrate S and product P respectively in millimoles and V max is the maximum reaction rate, h inverse or the hour inverse and K s is the Michaelis-Menten constant.

$$\frac{d[\mathrm{P}]}{dt} = \frac{V_{max}[\mathrm{S}]}{K_S + [\mathrm{S}]}$$

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Table 2: The reaction rates in the metabolites' mass balance equations listed in Table 1

$R_1 = \frac{2(V_1[G][X])}{K_1+[G]}F$	$R_7 = \frac{V_7[AC][X]}{K_7+[AC]}F$	$R_{13} = \alpha_{13}[B][AaC][Cf][X]$
$R_2 = \frac{V_2[F_{6P}][X]}{K_2+[F_{6P}]}F$	$R_8 = \alpha_8[A][AaC][Cf][X]$	$R_{14} = \frac{V_{14}[BC][X]}{K_{14}+[BC]}F$
$R_3 = \frac{V_3[G_{3P}][X]}{K_3+[G_{3P}]}F$	$R_9 = \frac{V_9[AC][X]}{2(K_9+[AC])}$	$R_{15} = \alpha_{15}[BC][Ah][X]F$
$R_4 = \frac{V_4[Lac][X]}{K_4+[Lac]}F$	$R_{10} = \alpha_{10}[AC][Ah][X]F$	$R_d = k_d[X]$
$R_5 = \frac{V_5[Py][X]}{K_5+[Py]}F$	$R_{11} = \alpha_{16}[Aa][Ad][X]$	$R_x = \frac{\mu[G][X]}{K_x+[G]}$
$R_6 = \frac{V_6[Py][X]}{K_6+[Py]}F$	$R_{12} = \frac{V_{12}[AaC][X]}{K_{12}+[AaC]}F$	

Note: F describes the effect of glucose inhibition (see Eq (2)). In all equations, V is the maximum reaction rate (mM h-1), K is the Michaelis constant (mM), α is the kinetic parameter described by Eq (4), k_d is the first order biomass death constant (h^{-1}), and μ is the specific biomass growth rate (h^{-1}).

Boehler EA, Meshab A (2016) Kinetic Study of Acetone-Butanol-Ethanol Fermentation in Continuous Culture

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Now table 2 gives the reaction rates in the metabolites' mass balance equations as listed in the table 1. So, R 1, R 2, R 3 all these and the corresponding equations. So, here the F, you can see there is F, so the F actually describes the effect of the glucose inhibition. In the equation 2 it is mentioned. In all equations V is the maximum reaction rate, K is the Michaelis-Menten constant, alpha is the kinetic parameter as described in the equation 4, k_d is the first order biomass death constant and μ is the specific biomass growth rate.

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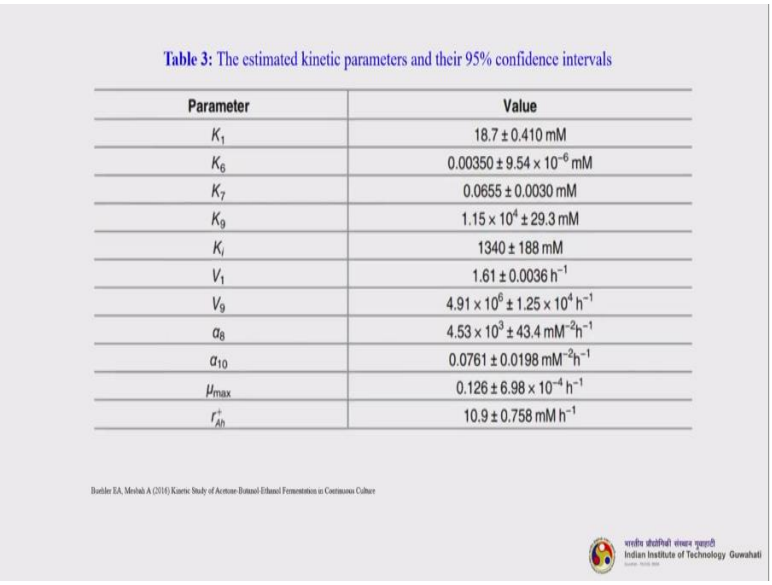

So, table 3 gives us the estimated kinetic parameters and their 95% confidence intervals. All the parameters K 1, K 6, K 7, K 9, K i, V 1, V 9, alpha 8, alpha 10, mu max and r ah plus. So, let us move ahead.

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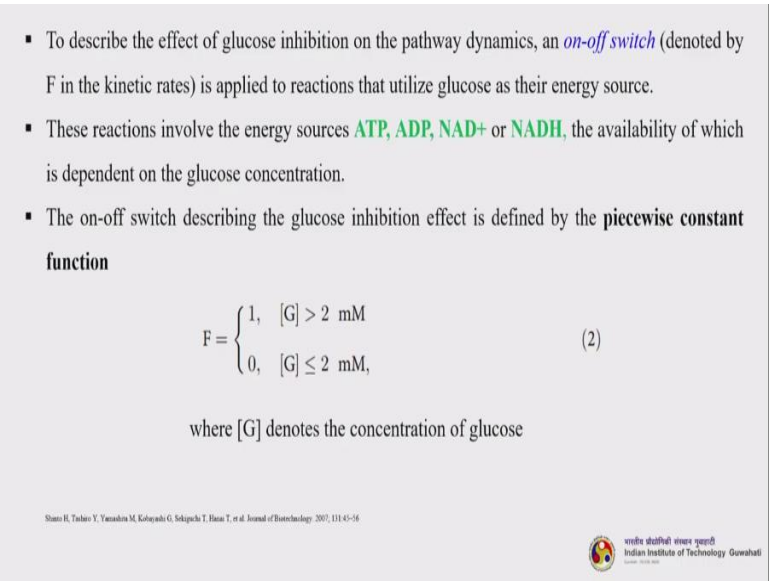

So, to describe the effect of glucose inhibition on the pathway dynamics, an on-off switch which is denoted by F in the kinetic rates is applied to reactions that utilizes glucose as their energy source. Now, these reactions involve the energy sources that is ATP, adenosine triphosphate, adenosine diphosphate, and nicotinamide adenine dinucleotide and NADH - the hydrogen, the availability of which is dependent on the glucose concentrations.

So, all these ATP, ADP, NAD, NADH these are the energy carriers. Now the on-off switch describing the glucose inhibition effect is defined by the piecewise constant function. So, F

you can see that F =1 when G is greater than 2 millimolar, F = 0 when G is much less than or equal to 2 millimolar where G denotes the concentration of the glucose.

$$F = \begin{cases} 1, & [G] > 2 \ \text{mM} \\ 0, & [G] \leq 2 \ \text{mM}, \end{cases} \qquad (2)$$

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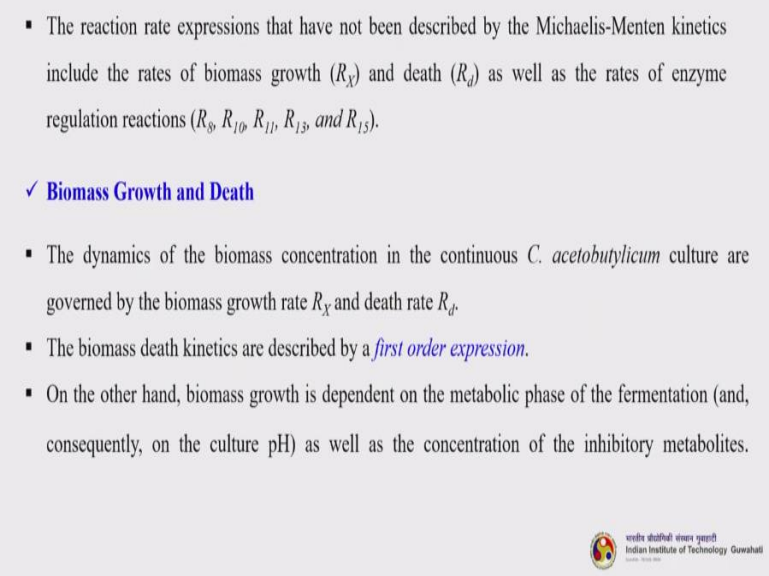

The reaction rate expressions that have not been described by the MM kinetics include the rate of the biomass growth, the death as well as the rate of the enzyme regulation reactions all these which are already there in the tables; R 8, R 10, R 11, R 13 and R 15. So, let us talk about the biomass growth and death. The dynamics of the biomass concentration in the continuous *Clostridium acetobutylicum* culture are governed by the biomass growth rate R x and the detath rate R d.

The biomass death kinetics are described by a first order expression. On the other hand, biomass growth is dependent on the metabolic phase of the fermentation and consequently on the culture pH as well as the concentration of the inhibitory metabolites.

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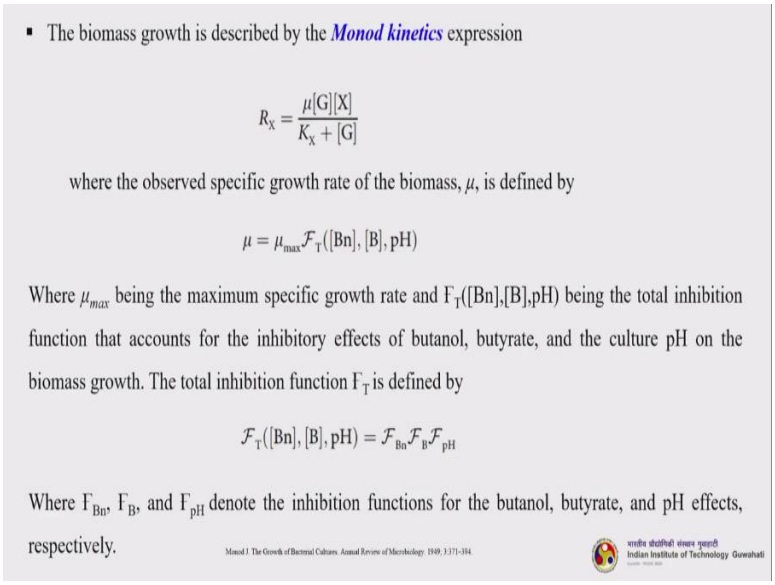

The biomass growth is described by the Monod kinetics expression R x = mu G X by K x + G,

$$R_X = \frac{\mu[G][X]}{K_X + [G]}$$

where the observed specific growth rate of the biomass mu is defined by mu = mu max F T, F is a function basically of the B n, B and pH.

$$\mu = \mu_{max}\mathcal{F}_T([Bn], [B], pH)$$

Now, mu max being the maximum specific growth rate and the function of that function of F T of that B n, B and pH being the total inhibition function that accounts for the inhibitory effects of the butanol, butyrate and the culture pH.

So, this function tells us the inhibitory effect of the three components. First is the butanol, second is the butyrate and third is the pH on biomass growth. So, the total inhibition function is defined as F T = F B n, F B and F pH

$$\mathcal{F}_T([Bn], [B], pH) = \mathcal{F}_{Bn}\mathcal{F}_B\mathcal{F}_{pH}$$

where F B n, F B and F pH denote the inhibition functions for the butanol, butyrate and pH effects respectively independently.

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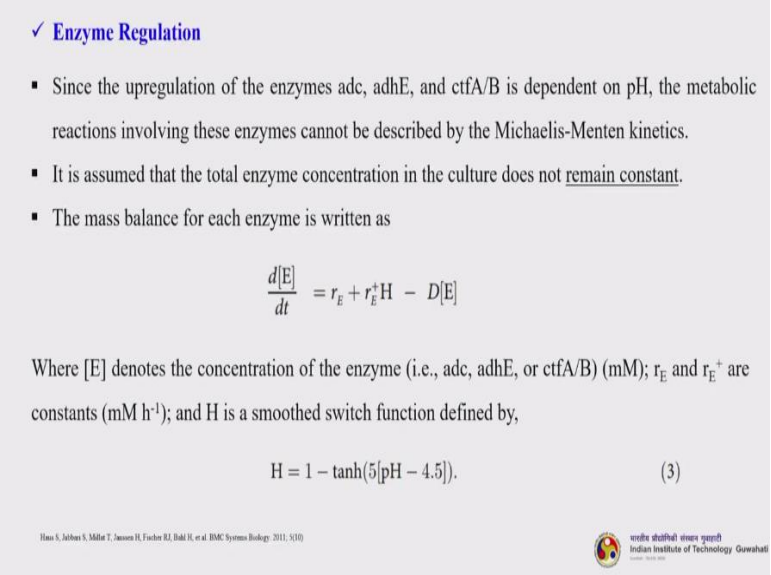

Let us now discuss about the enzyme regulation. Since the upregulation of the enzyme adc, adhE and ctfA/B is dependent on the pH, the metabolic reactions involving these enzymes cannot be described by the MM kinetics. Now, it is assumed that the total enzyme concentration in the culture does not remain constant. The mass balance for each enzyme is written as d by dt of E = r E + r E +H – D E

$$\frac{d[E]}{dt} = r_E + r_E^+ H - D[E]$$

now where E bracket denotes the concentration of the enzyme, r E and r E+ are the constants and H is the smoothed switch function denoted by H = 1 – tan hyperbolic 5 pH –4.5.

$$H = 1 - \tanh(5[pH - 4.5]). \quad (3)$$

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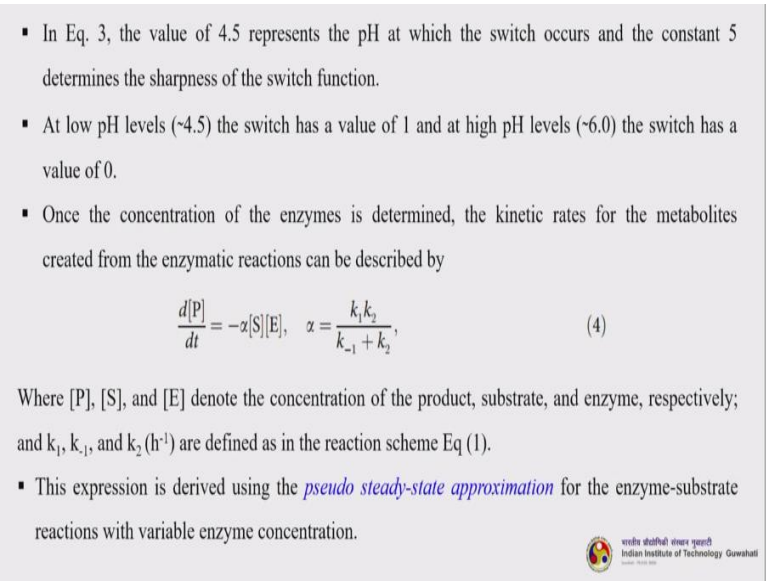

So, in equation 3 the value of 4.5 represents the pH at which the switch occurs and the constant 5 determines the sharpness of the switch function. At low pH levels that is approximately 4.5, the switch has a value of 1 and at high pH values greater than 6 the switch has a value of 0. Once the concentration of the enzyme is determined, the kinetic rates for the metabolites created from the enzymatic reactions can be described by d by dt of P = – alpha S E where alpha = k 1 k 2 divided by k –1 + k 2.

$$\frac{d[\mathrm{P}]}{dt} = -\alpha[\mathrm{S}][\mathrm{E}], \quad \alpha = \frac{k_1 k_2}{k_{-1} + k_2}, \qquad (4)$$

These are the rate constants. P, S, and E denote the concentration of the product, substrate and enzyme respectively; k 1, k –1 and k 2 are all reaction constants as we have defined already in the equation 1. Now, this expression is derived using the pseudo steady-state approximation for the enzyme-substrate reactions with variable enzyme concentration.

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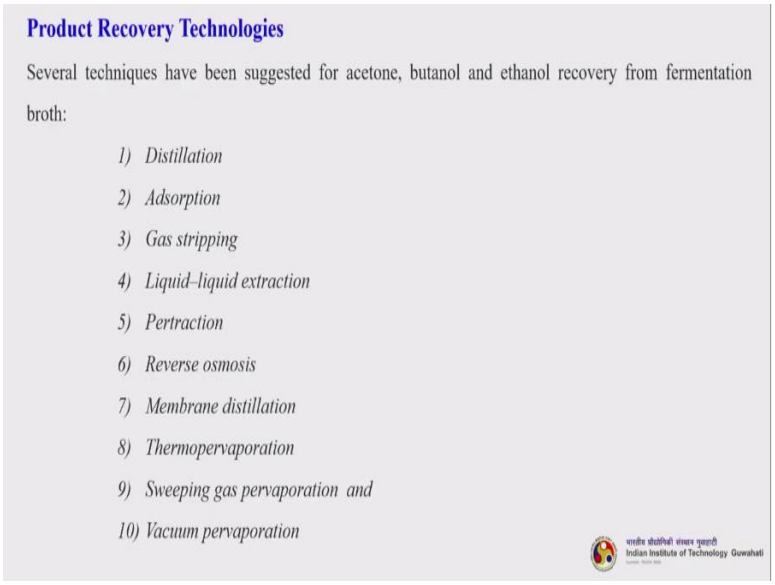

Now, we will talk about the product recovery technologies, the challenges also. See, let me tell you that when you talk about this ABE fermentation, please understand that ABE fermentation is not at all similar to that of when we talk about the SSF and SHF all these things to produce bioethanol basically. There are our target is to produce only bioethanol and whatever inhibitory compounds are produced we can control them easily.

Because their metabolism and their metabolic pathway is lesser complicated than this ABE fermentation. Now here in ABE, we are having 3 different types of products, the final products acetone, butanol, ethanol. Now, mostly butanol production is much higher than that of the acetone and your ethanol; this you can also regulate by doing the cleaving and changing the metabolic pathway in a particular direction so as to get more acetone than that of the ethanol or butanol.

See these things can be done, but the entire metabolic pathway of the ABE fermentation is quite complicated. And again you need to understand that a *Clostridium* is a strict anaerobic species, you have to maintain strict anaerobic conditions, otherwise your products will be something else. You do not get a proper yield of the product and the efficiency of the process will come down.

Now, let us come into the discussion of what we going to right now discuss is about the product recovery technology. So, as we have discussed it many times that once a certain product is produced in a fermentation pathway, there is much challenge because in a

fermentation broth there are so many things, the cells are also there, live cells. Apart from that there are metabolites and there are so many inhibitory compounds.

Now with that the product of interest is actually present in a aqueous phase. The recovery of that particular product of interest is extremely challenging when we are talking about a much more complicated process like that of the ABE fermentation. But there are excellent works have been reported and there are many processes which are being commercialized, but still a lot of scope exists to do more research on this particular product recovery technologies of even ethanol production also during your SSF, SHF and here also in the ABE fermentation also. So, let us discuss.

So, many processes are there. I have listed few which are mostly commercialized or going to be commercialized soon. Distillation, adsorption, gas stripping, liquid-liquid extraction, pertraction, reverse osmosis, membrane distillation, thermopervaporation, sweep gas pervaporation and vacuum pervaporation.

Let me tell you that these 4 are the classic unit operations. These are practiced in most of the refineries and biorefineries. So much of work has been done and these are commercial processes. When we start about from 5 onwards, all these, starting from pertraction, RO and all these things, these are all latest developments and these are all membrane based systems. We will quickly discuss about all these things. We will try to understand the basics.

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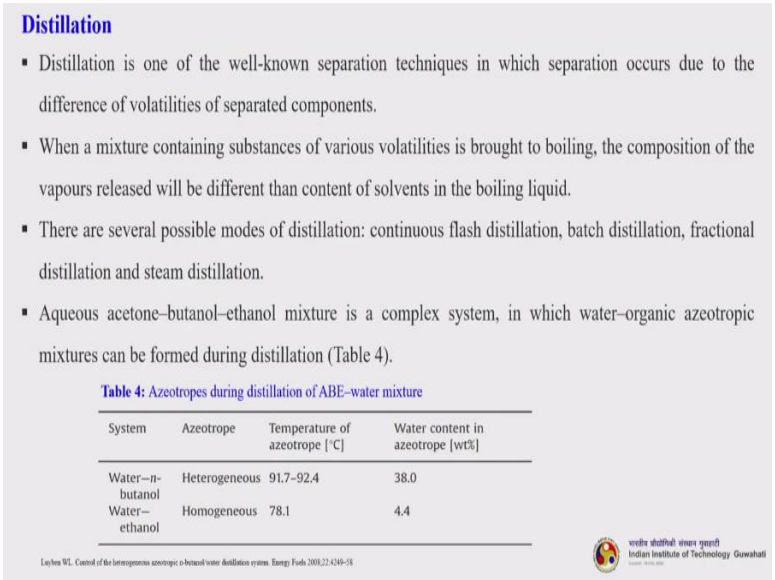

So, first is distillation. Now distillation is one of the well-known separation techniques in which separation occurs due to the difference of volatilities of the separated component. So, the vapour liquid equilibrium plays the important role. When a mixture containing substances of various volatilities is brought to boiling, the composition of the vapours released will be different than that of the content of solvents in the boiling liquid.

Now, there are several possible modes of distillation. We can have continuous flash distillation. We can have batch distillation. We have fractional distillation. We can have steam distillation. There are many other distillations also. Now, aqueous acetone-butanol-ethanol mixture is a complex system in which the water-organic azeotropic mixtures can be formed during the distillation. How it forms let us understand this.

If you talk about the water-n-butanol system, the azeotrope is quite heterogeneous. The temperature of zeotrope lies between 91.7 to 92.4 (°C) and the water content in azeotrope is 38%, it is quite a high amount. When you talk about the water-ethanol azeotrope it is a homogeneous mixture. The temperature is around 78.1 (°C) and water content is very less 4.4. Now this gives us an excellent information this table that was should be my recovery strategies.

Now, if you understand that you see here the water is more, so the target should be to the move the other component. So, here the water is very less, so if possible we can target that water has to be dehydrated so that other components 95-96% are anyway remains pure. So, then there are ways to deal with the azeotropes.

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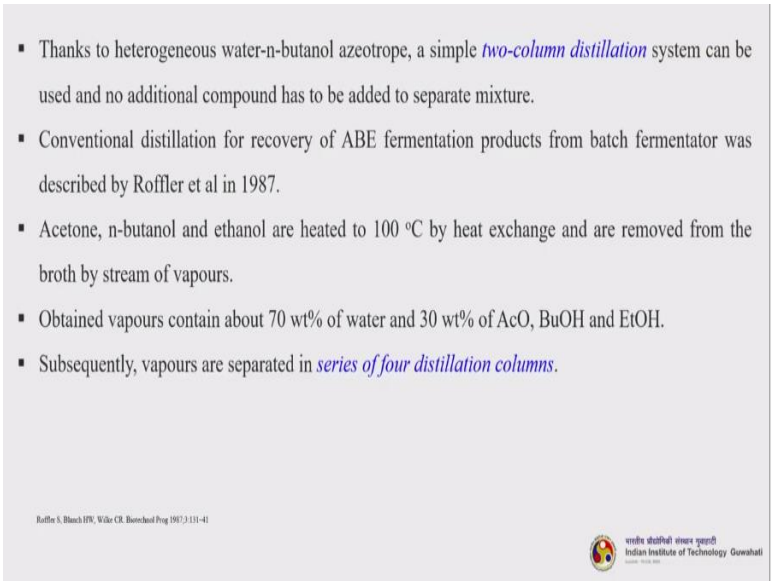

Thanks to the heterogeneous water-n-butanol azeotrope, a simple two-column distillation system can be used. This is what I was telling that the table gives us a lot of information about which type of process or unit operations has to be choosen and no additional compound has to be added to separate the mixture. Conventional distillation for recovery of the ABE fermentation products from batch fermentator was described by Roffler et al in 1987.

Now acetone, n-butanol and ethanol are heated to 100 degrees centigrade by heat exchange and are removed from broth by the stream of vapours. Obtained vapours contain about 70 weight percent of water and 30 weight percent of the acetone, butanol and ethanol. Subsequently, vapours are separated in the series of four distillation columns.

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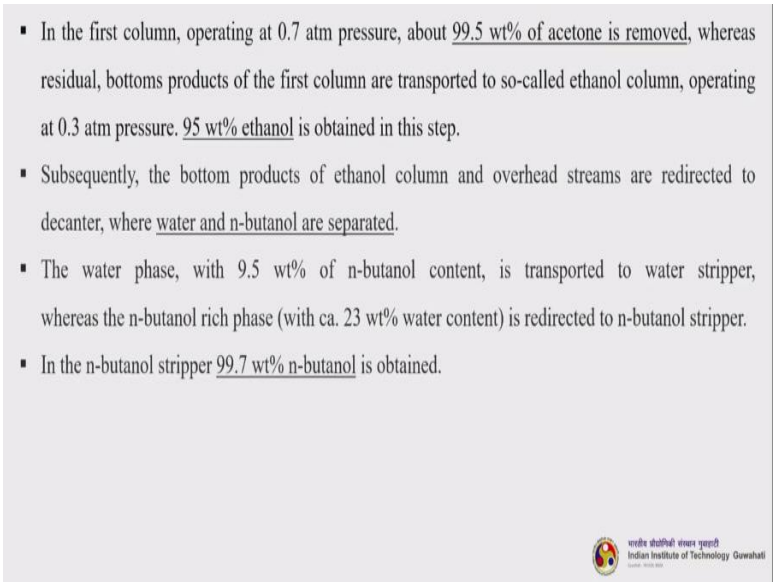

In the first column operating at 0.7 atmospheric pressure about 99.5 weight percent of acetone is removed, whereas residual bottom products of the first column are transported to so-called ethanol column operating to 0.3 atmospheric pressure a 95 weight percent ethanol is obtained in this step. Subsequently, the bottom products of ethanol column and overhead streams are redirected to the decanter where water and n-butanol are separated.

The water phase with 9.5 weight percent of n-butanol content is transported to water stripper, whereas n-butanol rich phase with approximately 23 weight percent water content is redirected to n-butanol stripper. In the n-butanol stripper 99.7 weight percent n-butanol is obtained. I hope you got a understanding of how this distillation can help us to remove and purify acetone, butanol and ethanol.

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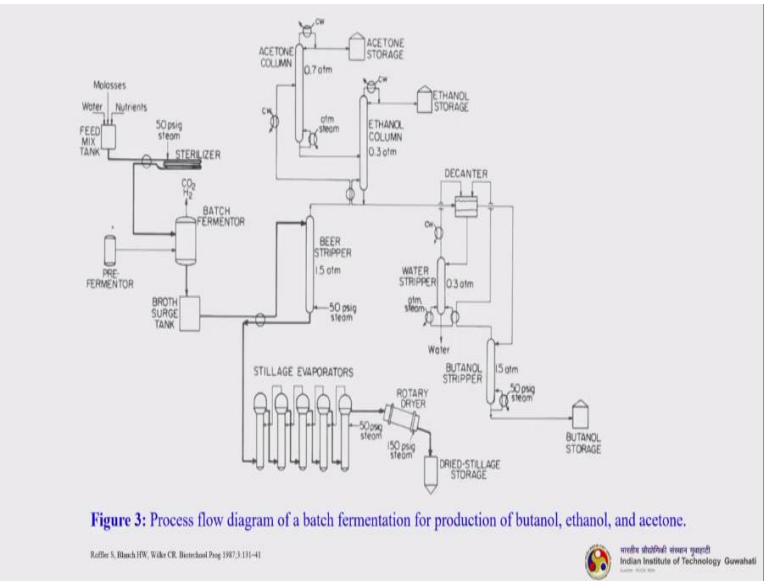

Figure 3: Process flow diagram of a batch fermentation for production of butanol, ethanol, and acetone.

Now, this is a process flow diagram of the batch fermentation for production of butanol, acetone and ethanol. So, the molasses that goes to the feed mixture tank and you supply the required amount of water and other nutrients which are much more important, some micronutrients for the fermentation. So, you need to do some sterilization, then it goes to the batch fermenter. From here, the broth surge tank, whatever it is there it is being fed into a stripper. Now from the stripper, this stillage, there are stillage evaporators, so it goes through a series of evaporators and finally we get dried stillage storage. It can be also a value added product if you go for further processing. Now, whatever is the top portion is being collected from the stripper, so this goes to different types of unit operation.

So, in the first water stripper it can be stripped and then whatever we get is the butanol rich phase that can go to another butanol stripper and you get the butanol, pure butanol. Similarly, the other fraction goes to the acetone and ethanol column from where you get ethanol and acetone, purified acetone and ethanol.

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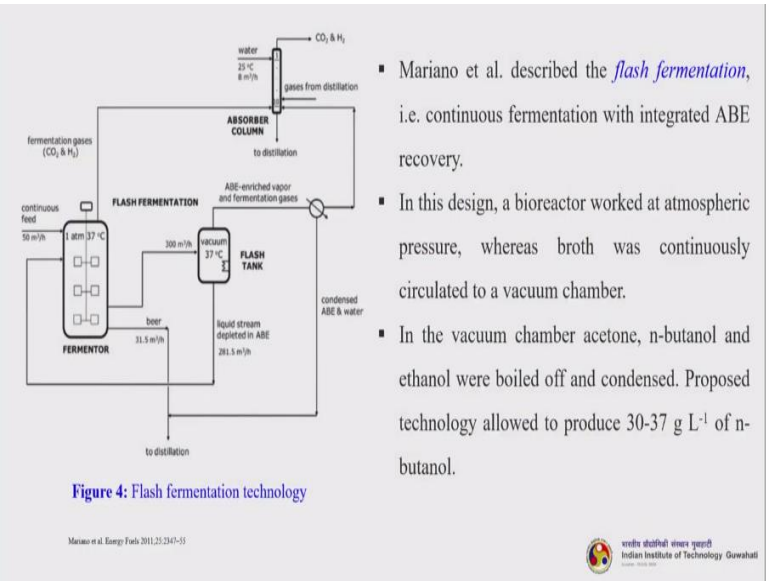

So, the next is flash fermentation. So, Mariano et al described the flash fermentation that is the continuous fermentation with the integrated ABE recovery. Now, in this particular design, a bioreactor worked at atmospheric pressure whereas broth was continuously circulated to a vacuum chamber. You can see this, this is a much simpler process than that of the what I have shown you the earlier slide.

See, this is your fermenter. Here continuous feed is there, continuous fermentation is happening. Then it goes to the vacuum section or vacuum chamber, where whatever we are getting that is in that top section is the ABE enriched vapor and fermentation gases. And whatever the liquid product that is getting condensed is the liquid steam depleted in ABE. It can be recirculated back to the fermenter and can be used while preparing for the fermentation media.

Now whatever the ABE enriched vapours, this goes to the absorber tower. Please understand that it is not a single absorber, there may be multiple absorbers. So, you get carbon dioxide and hydrogen from the top and whatever you are getting actually from the bottom product that goes to the series of distillation towers where you can purify your acetone, butanol and ethanol.

So, the bioreactor worked at atmospheric pressure whereas broth was continuously circulated to a vacuum chamber. In the vacuum chamber, acetone and butanol and ethanol were boiled up and condensed. Proposed technology allowed to produce 30 to 37 grams per liter of n-butanol which is a good yield.

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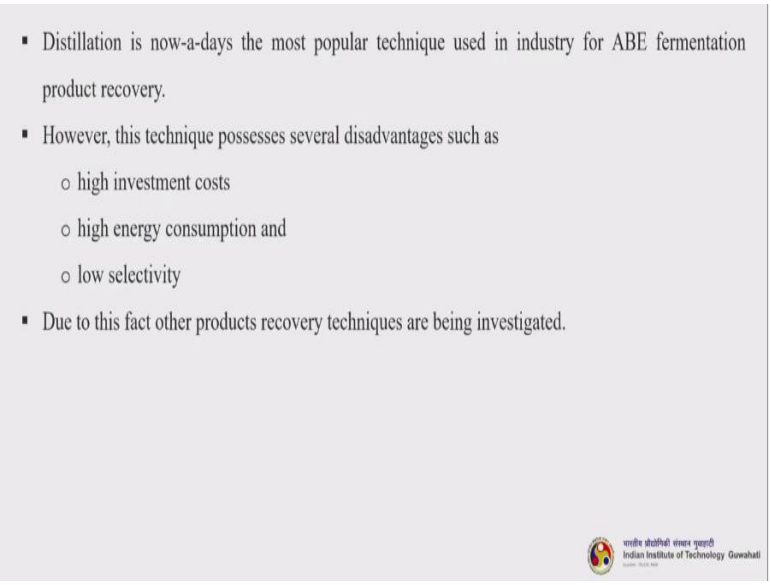

So, distillation is nowadays the most popular technique used in the industry for ABE fermentation product recovery. However, this technique possesses several disadvantages. The first and foremost important thing is high investment cost. The capital investment is very high. High energy consumption because you need to produce steam to do the distillation and of course the selectivity is low. Now due to this fact, other product recovery techniques are also being investigated.

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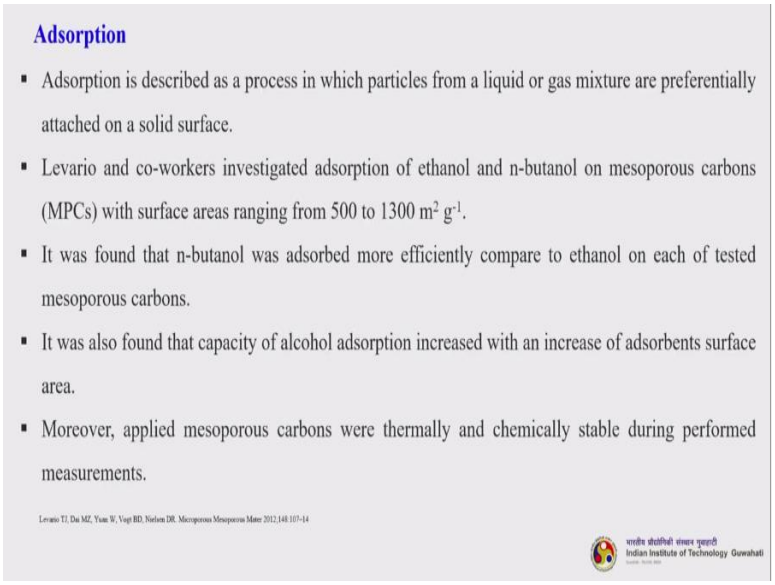

The next one is adsorption; it is also a very important unit operation. So, it is described as a process in which particles from a liquid or a gas mixture are preferentially attached to a solid surface. So Levario and co-workers investigated adsorption of ethanol and n-butanol on mesoporous carbons with surface areas ranging from 500 to 1300 meter square per gram.

It was found that n-butanol was adsorbed more efficiently compared to ethanol on each of the tested mesoporous carbons. It was also found that capacity of alcohol adsorption increased with an increase of the adsorbent surface area. Moreover, applied mesoporous carbons were thermally and chemically stable during perform measurements.

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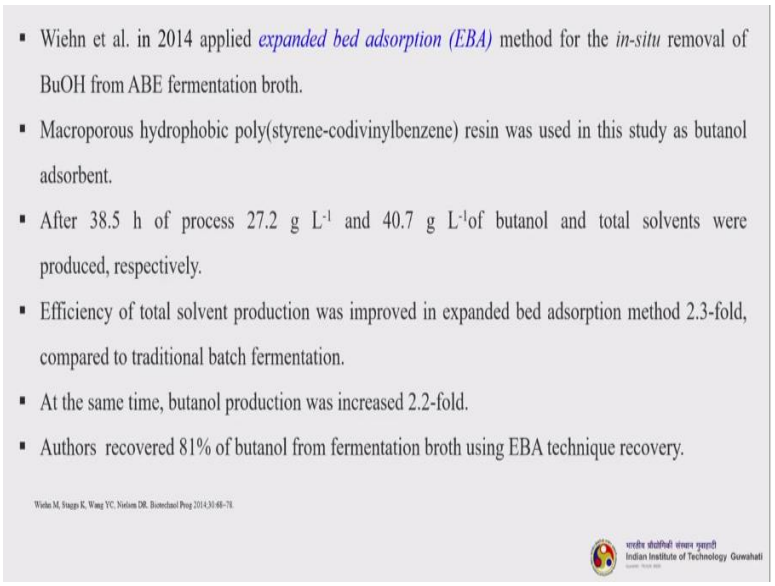

Wiehn et al in 2014 applied expanded bed adsorption method which is widely used in some of the biorefineries and refineries of course for the in-situ removal of the butanol from ABE

fermentation broth. If you recall, we just discussed that the process dynamics should be in such a way that if you continuously remove butanol, then butanol will not inhibit the fermentation pathway. So macroporous hydrophobic polystyrene-codivinylbenzene resin was used in this study as butanol adsorbent.

After that 38.5 hours of the process 27.2 grams per liter and 40.7 grams per liter of butanol and total solvents were produced, which is a little less compared to other one, but it is a good yield. Now, efficiency of the total solvent production was improved in expanded bed adsorption method almost 2.3 fold compared to the traditional batch fermentation. Now at the same time, butanol production was increased to 2.24. Authors recovered 81% of butanol from fermentation broth using the EBA technique recovery.

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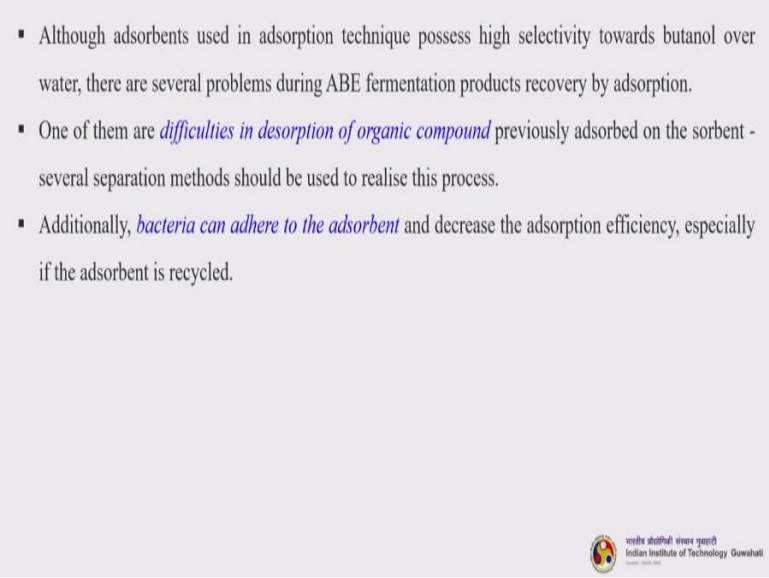

Now, although adsorbants used in adsorption technique possesses high selectivity towards butanol over water, there are several problems during ABE fermentation products recovery by adsorption. One of them are difficulties in desorption of organic compounds previously adsorbed on the sorbent.

Several separation methods should be used to realise this process. Additionally, bacteria can also adhere to the adsorbent and decrease the adsorption efficiency especially if the adsorbent is recycled. Anyway you have to regenerate the adsorbent and recycle it to take care of the entire process economics.

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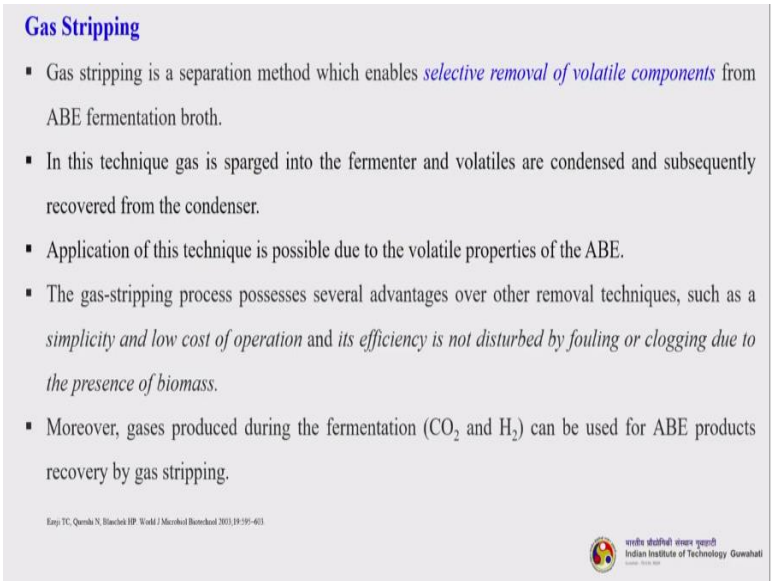

So, the next one is gas stripping. Now, gas stripping is a separation method which enables selective removal of volatile components from the ABE fermentation broth. Now, in this technique gas is passed into the fermenter and volatiles are condensed and subsequently recovered from the condenser. Application of this technique is possible due to the volatile properties of the ABE.

The gas stripping process possesses several advantages over other removal techniques such as the simplicity and low cost of operation and its efficiency is not disturbed by fouling or clogging due to the presence of biomass. Moreover, gases produced during the fermentation that is carbon dioxide and hydrogen can also be used for the ABE product recovery by the gas stripping.

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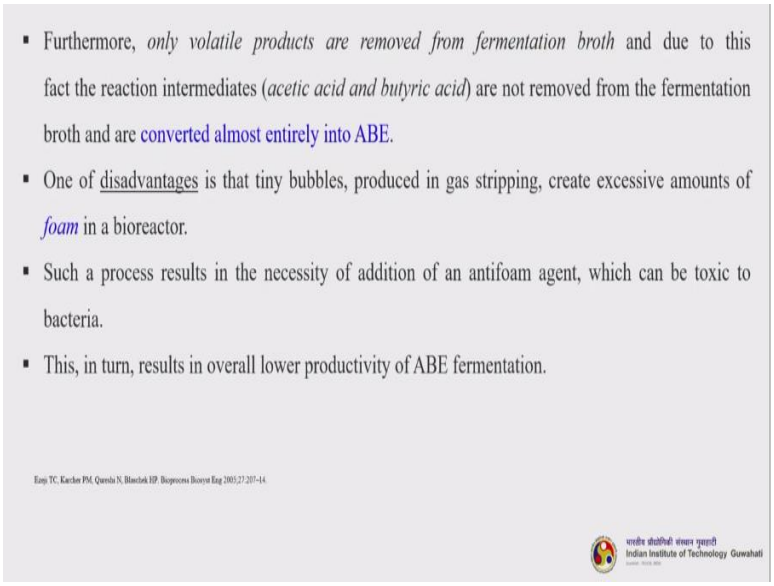

Only volatile products are removed from fermentation broth and due to this fact the reaction intermediates which is your acetate and butyrate are not removed from the fermentation broth and are converted almost entirely into ABE, which is a win-win situation actually. However, one of the disadvantages is that that tiny bubbles which are produced during the gas stripping create excessive amounts of foam in the bioreactor, so much of foaming takes place.

Now, such a process results in the necessity of addition of anti-foaming agent which can be toxic to bacteria. So, these are complicated issues. But you have to add the anti-foaming agent at a certain concentration so that it will not create a toxicity problem for the bacteria. Now, this in turn results in overall lower productivity of the ABE fermentation. These are the issues that needs to be taken care of.

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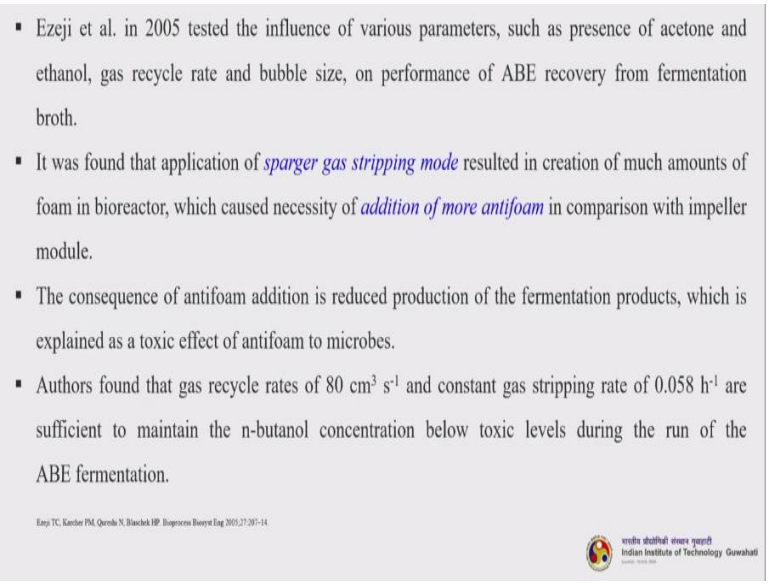

Now Ezeji et al in 2005 tested the influence of various parameters such as the presence of acetone and ethanol, gas recycle rate, bubble size on the performance of ABE recovery from the fermentation broth. It is an excellent work, reference is given in the bottom of the slide. It was found that the application of sparger gas stripping mode resulted in creation of much amounts of foam in the bioreactor, which caused the necessity of the addition of more anti-foaming agent in comparison with the impeller module. When you sparge the gas, you are sparging to create bubbles that is the reason why the foaming will be more, but when he used impellers which are mechanically driven impellers basically, then the foaming will be less. But when you talk about the gas stripping mode, it is always preferred mode when you do not have a, what you can say, a live system or fermentation.

If you are only carrying out some chemical reaction and all, so it is much more advantageous because you continuously generate bubbles, the bubbles squirls, then they break. Again they squirls, they break - in that way they create huge mass transfer area. So, that is the advantage actually, but not so good when you talk about fermentation. Now, the consequence of antifoam addition is reduced production of the fermenttation products, which is explained as a toxic effect of the anti-foaming agents to the microbes. Authors found that gas recycle rates of 80 centimeter cube per second at a constant gas stripping rate of 0.058 per hour are sufficient to maintain the n-butanol concentration below toxic levels during the run of the ABE fermentation.

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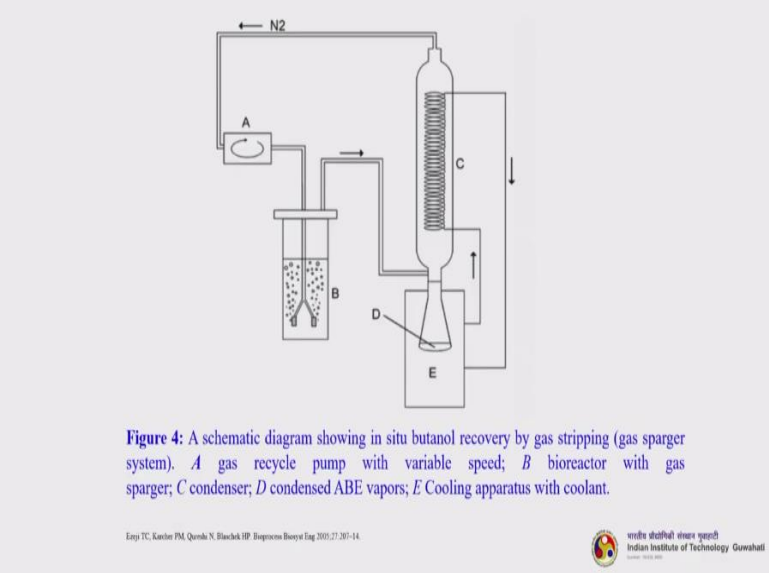

Figure 4: A schematic diagram showing in situ butanol recovery by gas stripping (gas sparger system). *A* gas recycle pump with variable speed; *B* bioreactor with gas sparger; *C* condenser; *D* condensed ABE vapors; *E* Cooling apparatus with coolant.

Ezeji TC, Karcher PM, Qureshi N, Blaschek HP. Bioprocess Biosyst Eng 2003;27:207–14.

This is a schematic diagram showing the in-situ butanol recovery. It is a simplified schematic just to make you understand that how the in-situ butanol recovery is happening using the gas stripping by the gas sparger system. So, this you can say the B is actually the bioreactor and A is the gas recycle pump with variable speed, you can control it. And the C is your condenser and D is the condensed vapour.

This is what we are collecting that is the condensed ABE vapours. Now please understand that it contains ABE, it further needs to be distilled out. And E is the cooling apparatus with the coolant. So this is simple system, can be practiced in the lab scale also, many people work like that. It has so many advantages, but at the same time you have to take care of this foaming issue.

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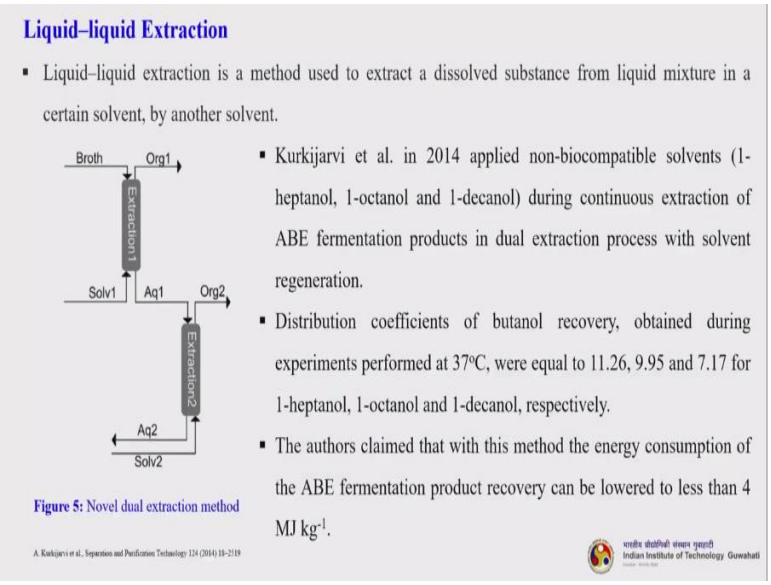

Now, let us talk about the liquid-liquid extraction, another most important product recovery technology. LLE is a method used to extract a dissolved substance from the liquid mixture in a certain solvent by using another solvent. So, Kurkijarvi et al in 2014 applied non-biocompatible solvents such as 1-heptanol, 1-octanol and 1-decanol during continuous extraction of the ABE fermentation products in dual extraction process with solvent generation.

So, you can see this there is a dual extraction system here as proposed by this particular authors. That means two different extracting units. So, distribution coefficients of butanol recovery obtained during the experiments performed at 37 degrees centigrade were equal to 11.26, 9.95 and 7.17 for 1-heptanol, 1-octanol and 1-decanol respectively. The authors claimed that with this method the energy consumption of the ABE fermenters and product recovery can be lowered to less than 4 mega joule per kg - it is a good process basically.

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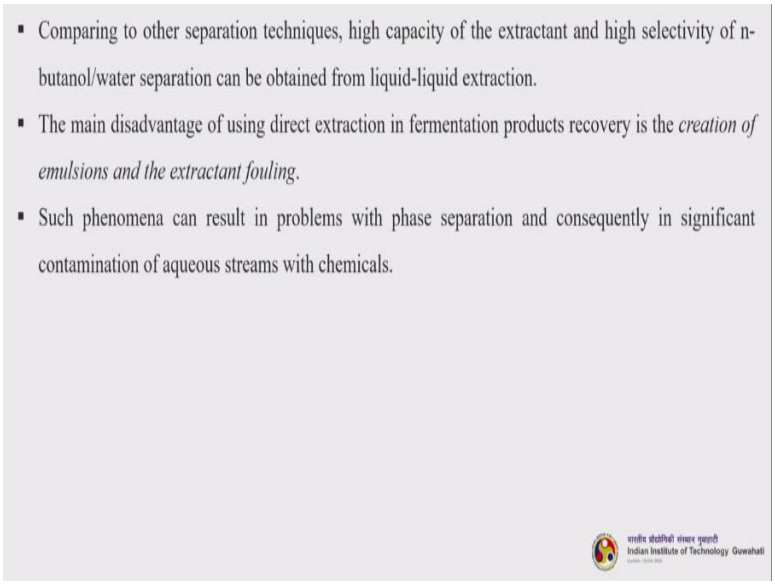

So, comparing to the other separation techniques high capacity of the extractant and and high selectivity of n-butanol water separation can be obtained from the liquid-liquid extraction. The main disadvantage of using direct extraction in fermentation products recovery is the creation of emulsions and the extractant fouling. So, such phenomena can result in problems with phase separation and consequently in significant contamination of aqueous streams with chemicals.

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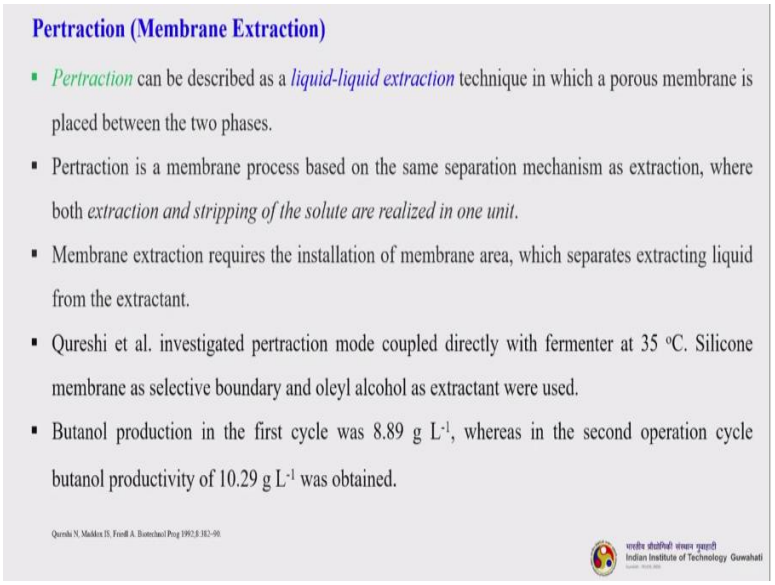

Now, from this slide onwards whatever we will be discussing these are all based on the membrane separation processes. So, first one is **pertraction**, the membrane extraction basically. So, it can be described as a liquid-liquid extraction technique in which a porous membrane is placed between the two phases. Now, pertraction is a membrane process based

on the same separation mechanism as extraction, where both extraction and stripping of the solute are realized in one unit.

Member extraction requires the installation of membrane area, which separates extracting liquid from the extractant. Qureshi et al investigated pertraction mode coupled directly with the fermenter at 35 degrees centigrade. Silicone membrane as selective boundary and oleyl alcohol as extractant were used. Butanol production in the first cycle was 8.89 grams per liter, whereas in the second operation cycle butanol productivity increased to 10.29 grams per liter.

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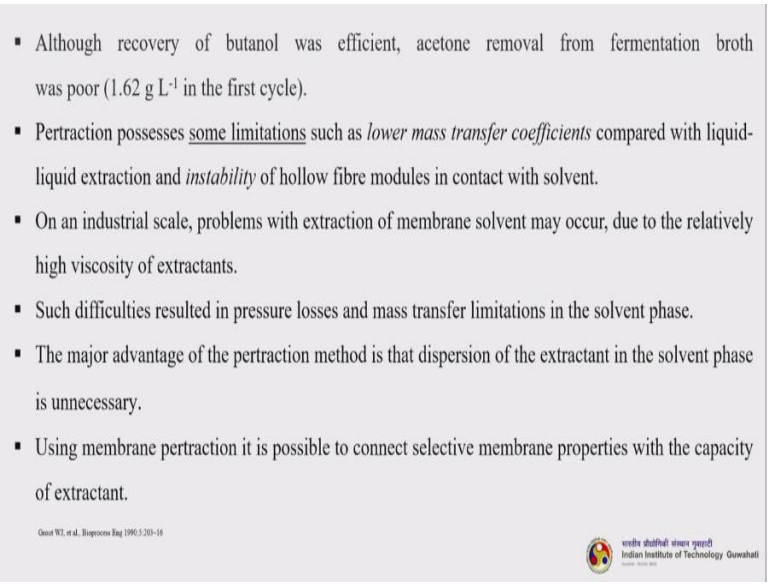

So, although recovery of butanol was efficient, acetone removal from fermentation broth was poor, almost close to 1.62 grams per liter in the first cycle. Now, pertraction possesses some limitations such as lower mass transfer coefficient compared with liquid-liquid extraction and the instability of the hollow fibre modules in contact with the solvent. Now, on an industrial scale problems with extraction of membrane solvent may occur due to the relatively high viscosity of extractants.

Such difficulties resulted in pressure losses and mass transfer limitations in the solvent phase. The major advantage of the pertraction method is that the dispersion of the extractant in the solvent phase is unnecessary. Using the membrane pertraction it is possible to connect selective membrane properties with the capacity of extractant.

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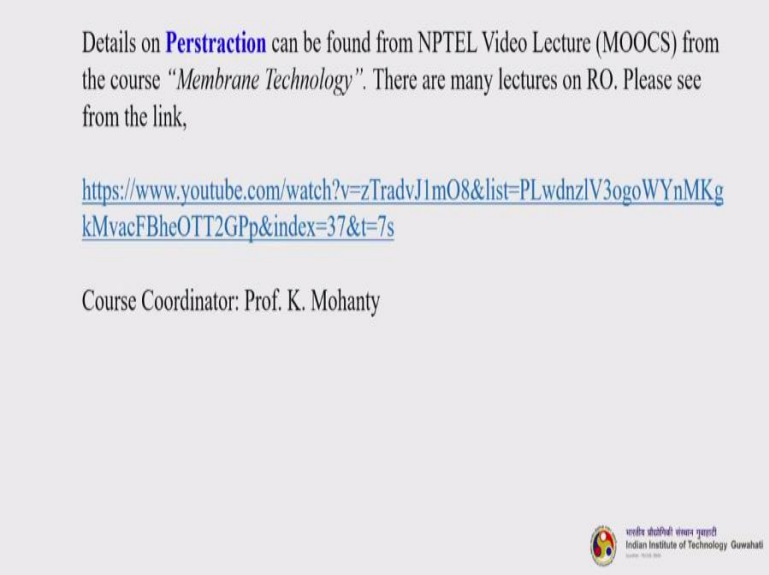

Now, I wish to say that I have also run a course last time actually last semester this NPTEL course, MOOCS course on membrane technology. Now under which I have discussed about all this what we are going to discuss like this pertraction in detail. Now, if anybody is interested to learn more on such processes whether it is pertraction, reverse osmosis, then let us say pervaporation or membrane distillation which we are going to discuss in our subsequent slides.

You can also refer to my course which is available now free in YouTube. And again, I also wish to say that this course will be redone in the January to May session in the 2021. If you wish you can also register for that course. Now, for this particular lecture on pertraction, you can see this link.

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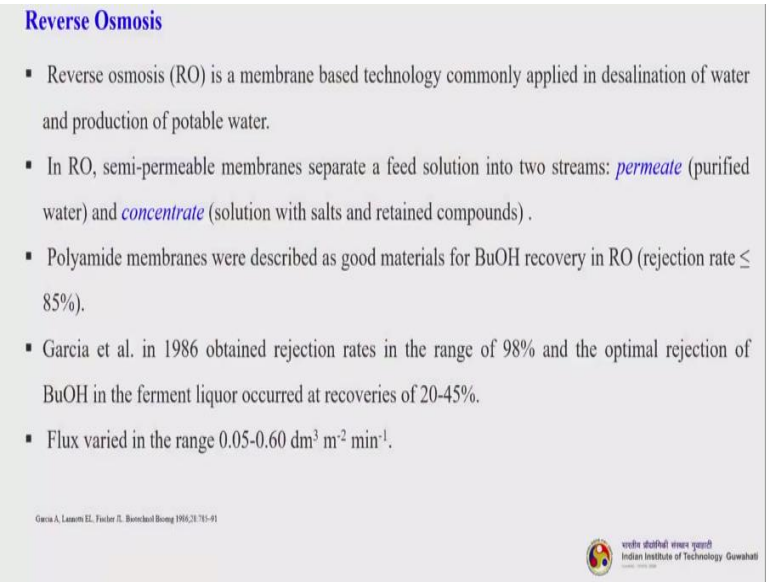

So, the next is RO, reverse osmosis. Now, RO is a membrane based technology commonly applied in desalination of water and production of potable water. In RO, semi-permeable membrane separate a feed solution into two streams: permeate - which is a purified water - and concentrate - solution with salts and retained compounds. Polyamide membranes were described as good materials for the butanol recovery in RO so with a rejection rate almost less than equivalent to 85%.

Garcia et al in 1986 obtained rejection rates in the range of 98% and the optimal rejection of butanol in the ferment liquor occurred at recoveries of 20 to 45%. Flux varied in the range of 0.05 to 0.6, dm cube meter square minute inverse.

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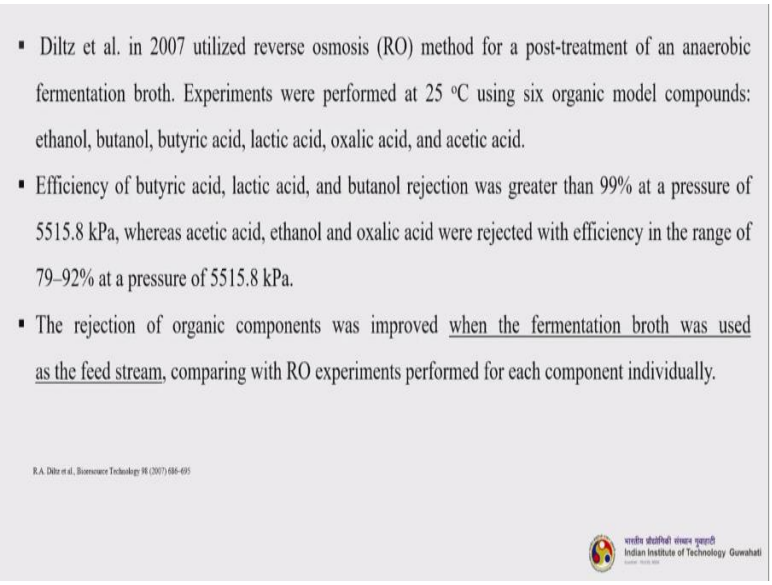

Diltz et al in 2007 utilized RO method for a post-treatment of an anaerobic fermentation broth. Experiments were performed at 25 degrees centigrade using six organic model compounds; ethanol, butanol, butyric acid, lactic acid, oxalic acid and acetic acid. Efficiency of the butyric acid, lactic acid and butanol rejection was greater than 99% at a pressure of 5515.8 kilo Pascal.

Whereas acetic acid, ethanol and oxalic acid were rejected with efficiency in the range of 79 to 92% at the same pressure, Now, the rejection of organic components was improved when the fermentation broth was used as a feed stream comparing with RO experiments performed for each component individually.

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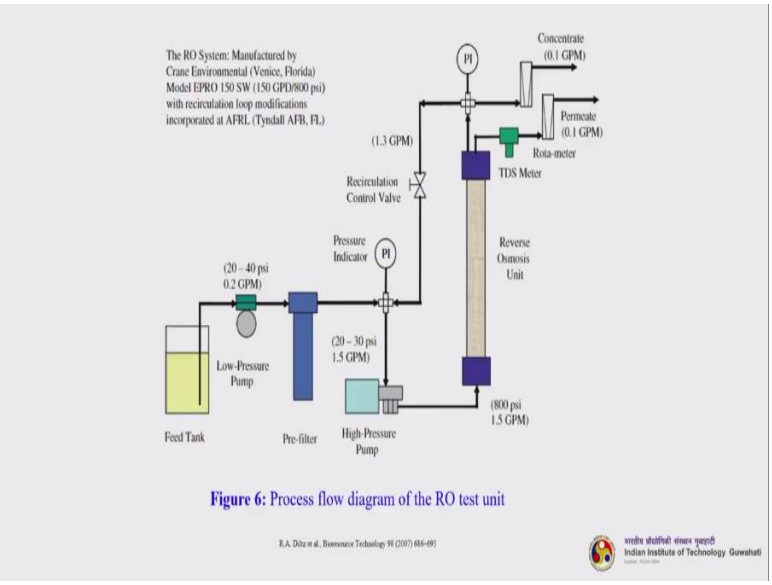

So, this is a process flow diagram for the RO test unit. So, you can see that this is the feed tank. From the feed tank the feed has been pumped to a pre-filter where you remove the suspended particles and other things whatever present and should not go to the RO system. Then a high pressure pump will pump it to the RO unit. Now, from where you get the permeate as well as the concentrate - permeate is the desired product. So, that is basically your purified water and the concentrate will have more salts and other components.

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So, RO has been discussed in detail in NPTEL course on membrane technology. The link is there. So, in this particular link the entire membrane technology course is there, so you can choose the respective RO because it is not a single lecture, there are many.

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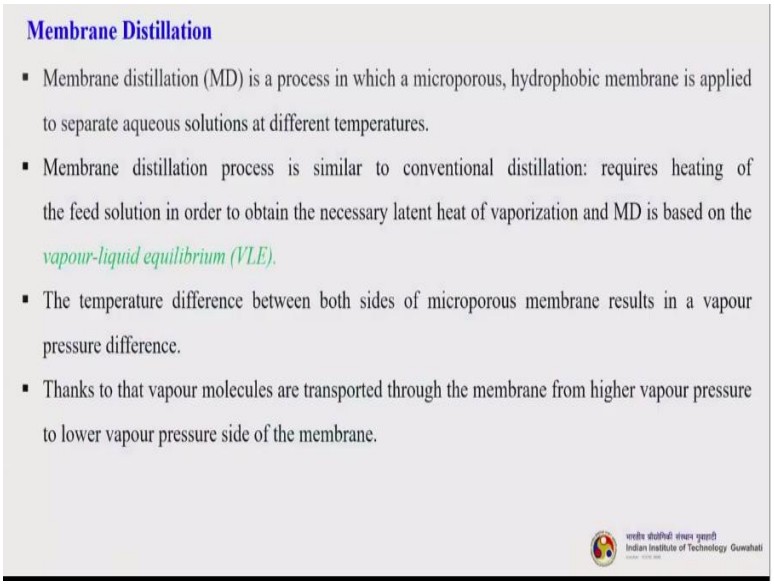

So, the next is membrane distillation. Now, membrane distillation is a process in which a microporous, hydrophobic membrane is applied to separate aqueous solutions at different temperatures. Membrane distillation process is similar to conventional distillation, requires heating of the feed solution in order to obtain the necessary latent heat of vaporization and MD is based on the vapor-liquid equilibrium just like the distillation.

Now, the temperature difference between both sides of the microporous membrane results in a vapour pressure difference. Thanks to that vapour molecules are transported through the membrane from the high vapour pressure to the lower vapour pressure side of the membrane.

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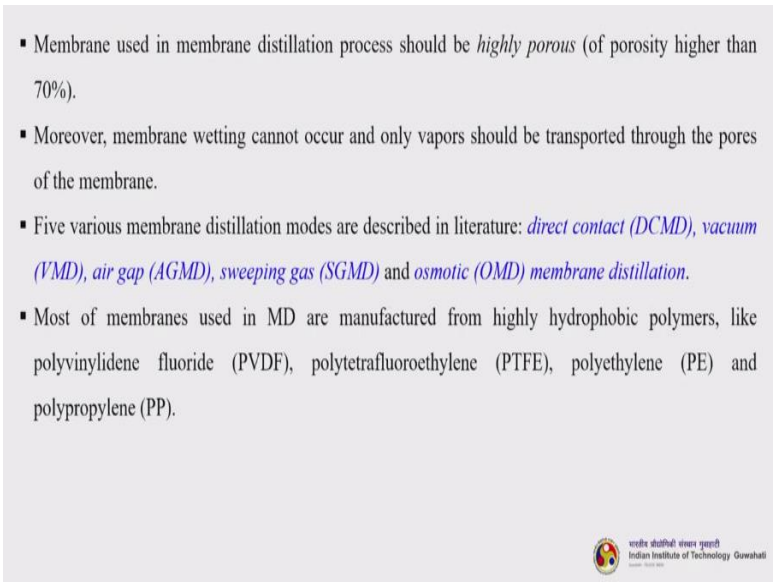

Membrane used in membrane distillation process should be highly porous, of course almost close to or more or greater than that of the 70%. Moreover, membrane wetting cannot occur

and only vapour should be transported through the pores of the membrane. Five various membrane distillation modes are described in literature and it is there in my lecture also. Those who are interested, I have given the link in the next slide you can refer.

First is called DCMD, the direct contact membrane distillation, then the vacuum membrane distillation, then air gap membrane distillation, sweeping gas membrane distillation and osmotic membrane distillation. Most of the membranes used in MD are manufactured from highly hydrophobic polymers such as PVDF, PTFE, then polyethylene and polypropylene.

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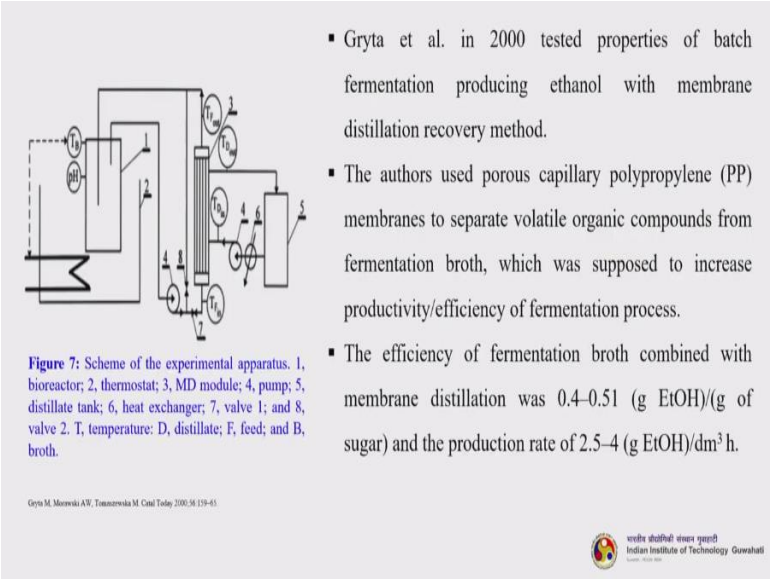

So, this is a schematic representation of the experimental apparatus of the membrane distillation. Now, Gryta et al in 2000 tested properties of the batch fermentation producing ethanol with membrane distillation recovery method. The authors used porous capillary polypropylene membranes to separate volatile organic compounds from fermentation broth, which was supposed to increase productivity or efficiency of the fermentation process.

The efficiency of fermentation broth combined with membrane distillation was 0.4 to 0.51 grams ethanol per gram of sugar and the production rate of 2.5 to 4 gram ethanol per dm cube hour.

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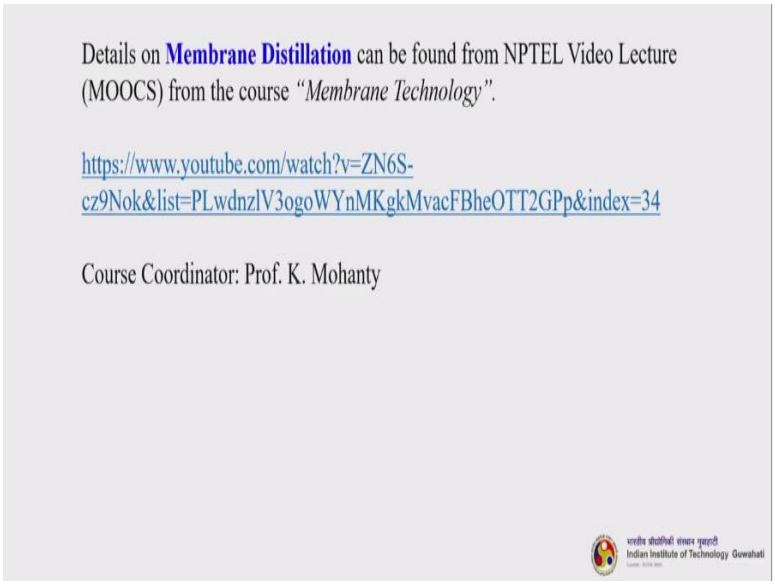

So, again I have given the link here of the membrane distillation. It is a single lecture.

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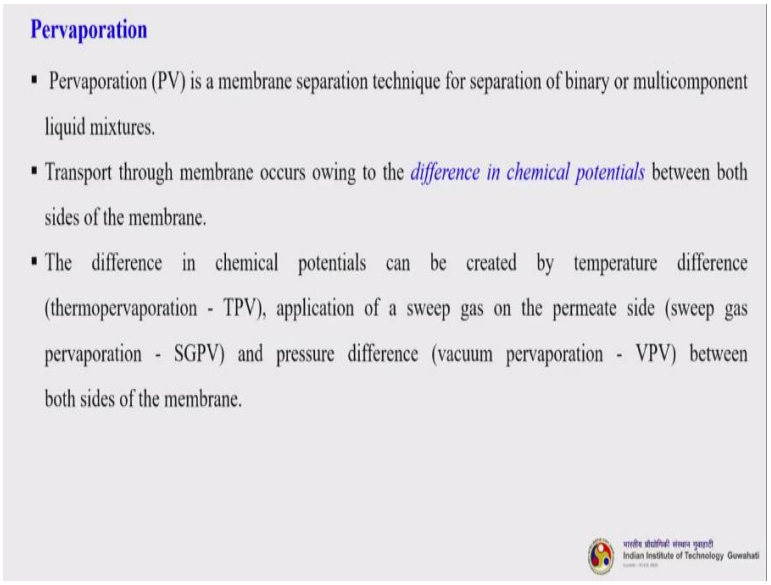

So, next is pervaporation. Pervaporation is a membrane separation technique for the separation of binary or multicomponent liquid mixtures, mostly organic components. And the transport through the membrane occurs owing to the difference in the chemical potentials between the both sides of the membrane. The difference in chemical potentials can be created by the temperature difference - which we can call the thermopervaporation, application of sweep gas on the permeate side - it becomes sweep gas pervaporation and the pressure difference - it becomes a vacuum pervaporation. So, basically between the both sides of the membrane, upstream and downstream side.

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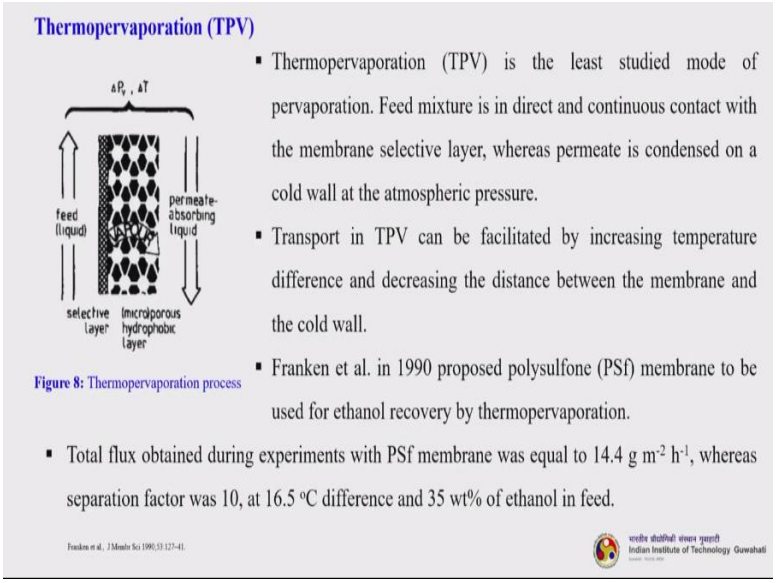

So, this is a schematic representation of a thermopervaporation process. So, thermopervaporation is the least studied mode of the pervaporation. Feed mixture is in direct and continuous contact with the membrane selective layer, whereas permeate is condensed on a cold wall at atmospheric pressure. Transport in the TPV can be facilitated by increasing temperature difference and decreasing the distance between the membrane and the cold wall.

Franken et al in 1990 proposed polysulfone membrane to be used for ethanol recovery by thermopervaporation. Total flux obtained during experiments with this polysulfone membrane was equal to 14.4 grams per meter square hour whereas separation factor was 10 and at 16.5 degrees centigrade difference and 35 weight percent of the ethanol in the feed.

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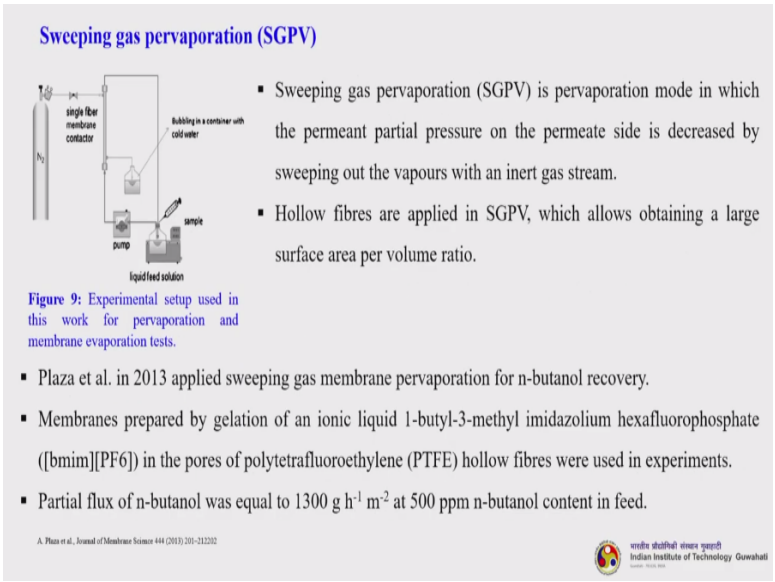

So, the next is the sweeping gas pervaporation. So, now this system in this way or this mode, the permeant partial pressure on the permeate side is decreased by the sweeping gas that takes out the vapours with an inert gas system. So, basically on the downstream side you use a sweeping gas. So, hollow fibres are applied in SGPV, which allows obtaining a large surface area per volume ratio.

Plaza et al in 2013 applied sweeping gas membrane pervaporation for n-butanol recovery. Membranes prepared by gelation of an ionic liquid 1-butyl-3methyl imidazolium hexafluorophosphate which is known as the bmim PF6 in the pores of the polytetrafluoroethylene hollow fibers were used in the experiment. Partial flux of n-butanol was equal to 1300 grams per hour meter square at 500 ppm n-butanol content in the feed.

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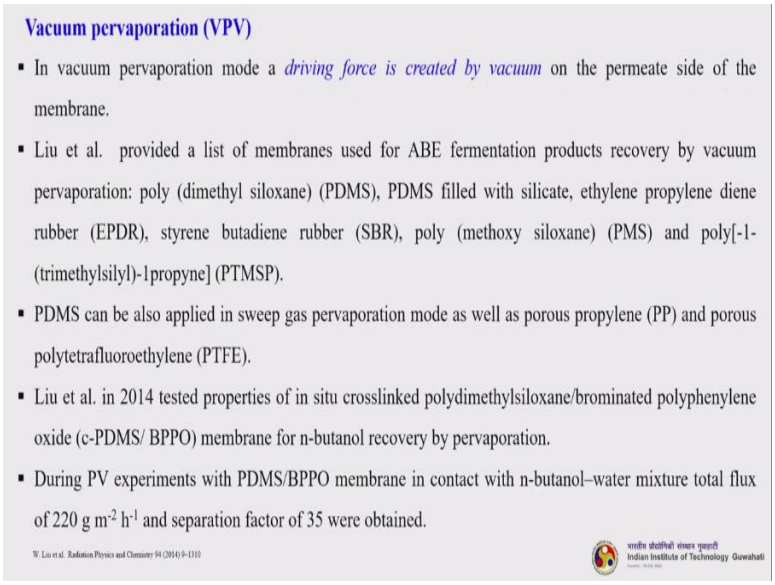

Next is vacuum pervaporation. In vacuum pervaporation mode a driving force is created by vacuum on the permeate side of the membrane. So, basically the permeate side or the downstream side of the membrane module is being connected to a vacuum pump. So, Liu et al provided a list of membrane used for ABE fermentation products recovery by vacuum pervaporation.

So, PDMS, PDMS filled with silicate, ethylene propylene diene rubber, styrene butadiene rubber, PMS and PTMSP were used. Now PDMS can also be applied in sweep gas pervaporation mode as well as porous polypropylene and porous polytetrafluoroethylene membranes. Liu et al in 2014 tested properties of in-situ crosslinked polydimethylsiloxane/ brominated polypropylene oxide membrane for n-butanol recovery by pervaporation.

During PV experiments with PDMS/BPPO membrane in contact with n-butanol-water mixture, total flux of 220 grams per meter square hour and separation factor of 35 were obtained.

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So, again details of the pervaporation can be found from this link.

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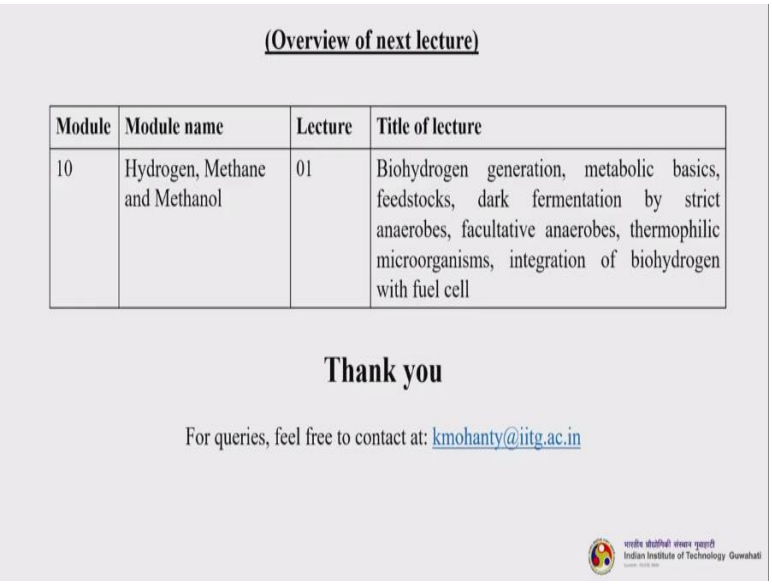

So, with this I conclude today's lecture. So, next class we will be starting our module 10 which is dedicated to hydrogen, methane and methanol. So if you have any query, please register your queries in the Swayam portal or you can also write a mail to me at kmohanty@iitg.ac.in. Thank you.