Applied Environmental Microbiology
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Lecture – 35
Bioremediation III

Dear students, in this lecture will continue from about bioremediation from the previous lecture, we will start again from the hydrocarbon degradation and then move on to degradation of other xenobiotics. So, let us get started. So, last time we talked about hydrocarbon oxidation and I when underwent the process from how hydrocarbon turns into an alcohol to aldehyde acid, then acid in a under goes beta oxidation.

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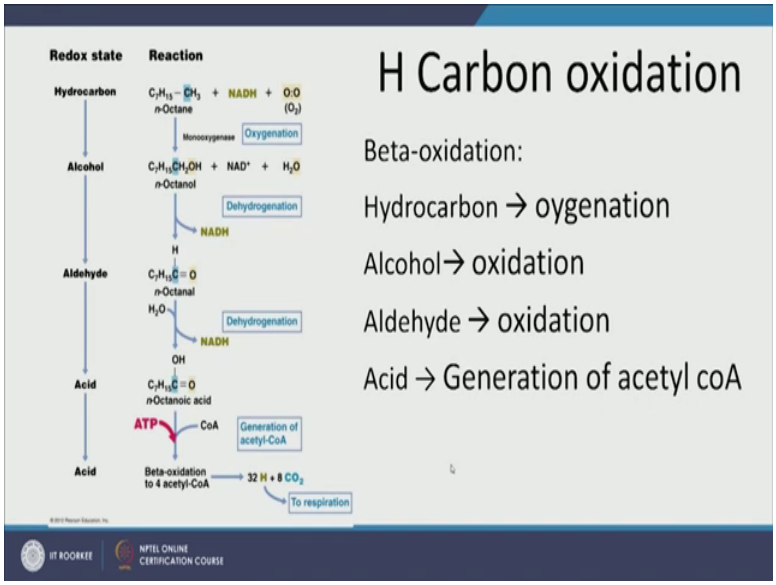

So, what is beta oxidation? Beta oxidation takes hydro its oxygenation of hydrocarbon to alcohol, and then to aldehyde and then to acid a finally, to generation of acetyl c o A.

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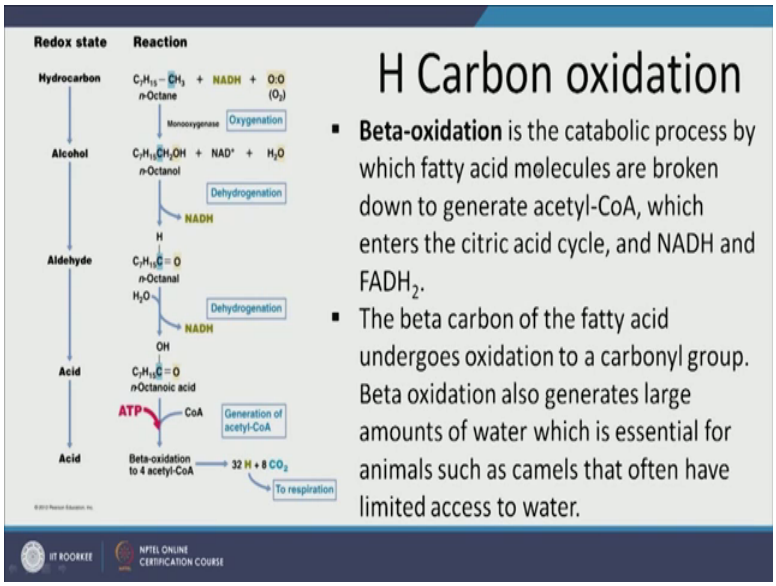

All right; now little bit more information about beta oxidation it is the breakdown process, in which the fatty acids are broken to generate acetyl CoA, which entered and citric acid cycle; obviously, and the beta oxidation works in this way that we process, we proceed to from alcohol to aldehyde and it generates lot of water in each step, and that is when like here and that is very beneficial for live living beings that need water in our a in water scared conditions.

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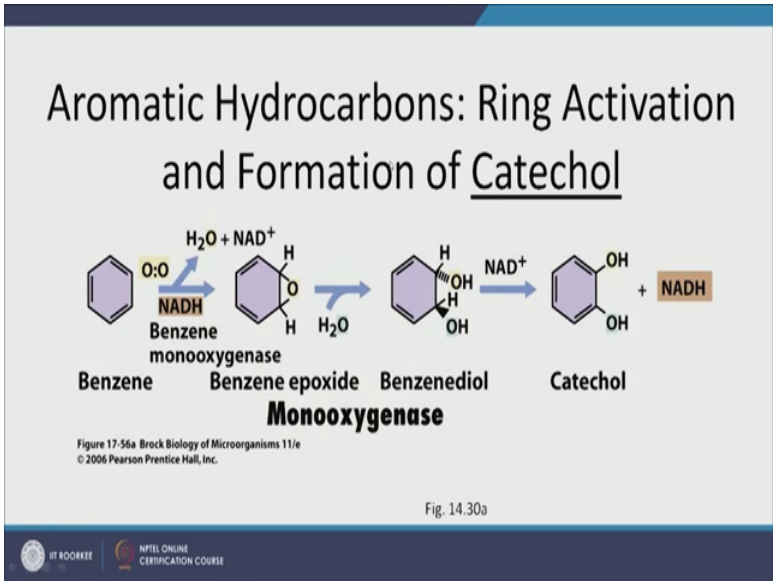

Ok, now let us look at aromatic hydrocarbons; I have mentioned this before that aromatic hydrocarbons are extremely stable and that makes it really difficult to degrade them, because the energy barrier of degrading them is very high. So, there are certain enzymes that specialise in degradation of aromatic hydrocarbon, and many a times it goes through the catechol pathway. So, it makes catechol.

So, let us explore what catechol is, because compared to an aromatic compounds such as benzene catechol is easier to degrade. So, here we have benzene, now we have benzene monooxygenase. So, monoooxygenase you can tell add one oxygen how will you add one oxygen? You will break the bonds here and one double bond will be broken and oxygen attached to both of them.

Now, you can see that this is not a various stable position for oxygen to be in. So, it will except one hand water molecule and then it will have OH, OH here now these lines and this triangle represent this studio chemistry, that they are pointing in different direction because it gets too congested if you have OH in the neighbouring carbon atoms in a benzene.

Now, what will happen is it will turn into catechol, this is catechol which is easier to degrade. So, this is the monooxygenase pathway.

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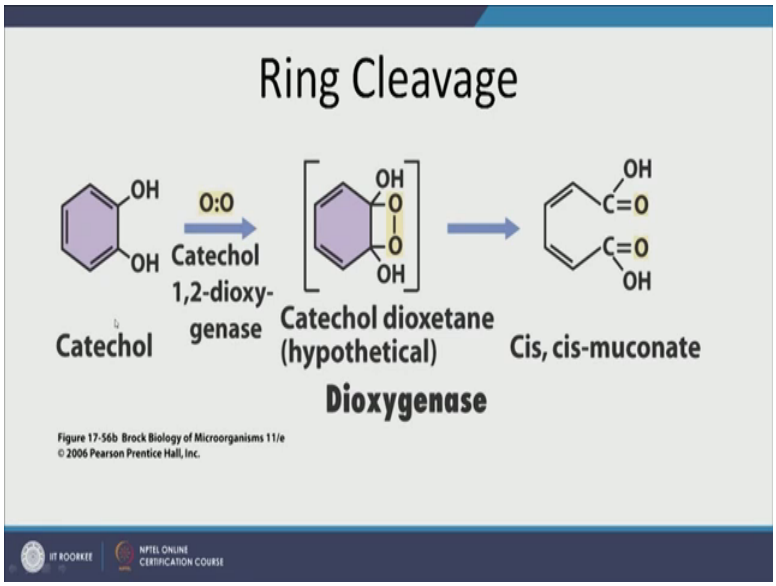

Now, the catechol will use the catechol 1, 2 dioxygenase. Now dioxygenase you can say little at two oxygens again here, one oxygen here one oxygen and these bonds should be broken. So, here we have this Catechol dioxetane; now we do not know about this it is still hypothetical we believes how it proceed, but we do know that the bond this bond breaks and then we have Cis, cis-muconate which is very easy to degrade.

Now, catechol 1, 2 dioxygenase enzyme has some very specific genetic signatures. This genetic signatures can do they do serve as genetic biomarkers to detect the aromatic ring cleavage all right. Now let us look at the dioxygenase path way. So, hear you had the monooxygenase path way when let us look at the dioxygenase path way. So, you have to lean not lean means benzene with methane mythel radical here.

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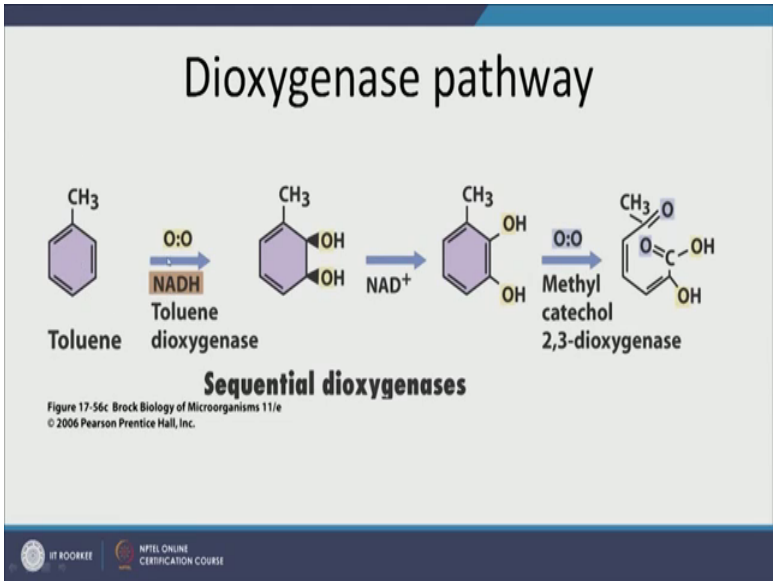

Now because its dioxene is you will have one bond breaking and o o attaching to each carbon. So, you have this, and then eventually what happens is that they initially this is what happens and then they make a bond again and you have this compound, and then it uses the enzyme call methyl catechol 2,3 dioxygenase. So, basically this is a catechol this is a methyl. So, this is a methyl catechol, and then it is methyl catechol 2,3 dioxygenase. Because its 2,3 dioxygenase the bone breaks there and the ring has cleaved. Once the ring has cleaved the aromaticity has been lost and this is very easy to degrade. So, this is your dioxygenase pathway.

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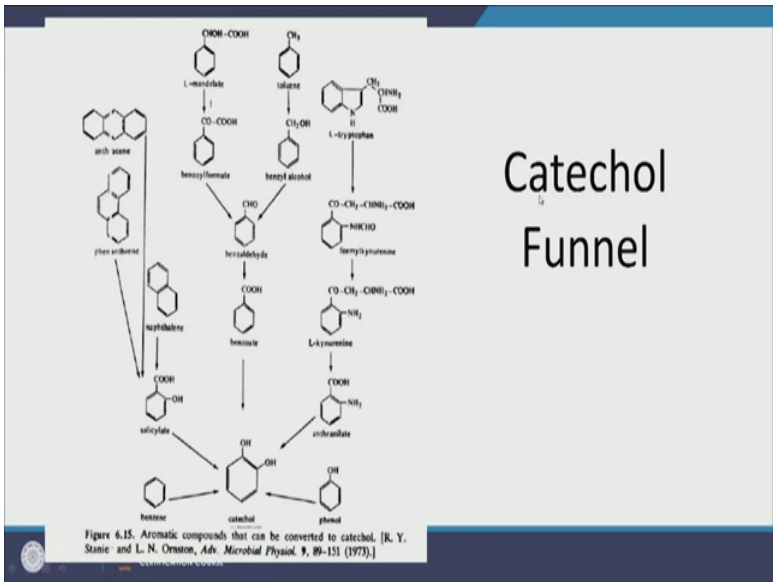

Now let us look at catechol funnel. So, picture from a very old book. So, here we have different aromatic compounds and all of them. So, this is a funnel. So, we are starting with at this anthracene,we are starting with tryptophane with toluene with mandelate. So, no matter way of benzaldehyde, whenever we are starting from naphthalene, they all benzene and phenol all undergo the catechol pathway.

So, this is where the benzene ring is most vulnerable to cleavage. So, that is why microbes want, no matter which I have benzene form and starting with aromatic compound, I am starting with phenanthrene, anthracene, naphthalene, benzyle dehyde we all want to catechol, because once we have reach catechol its easy to break it.

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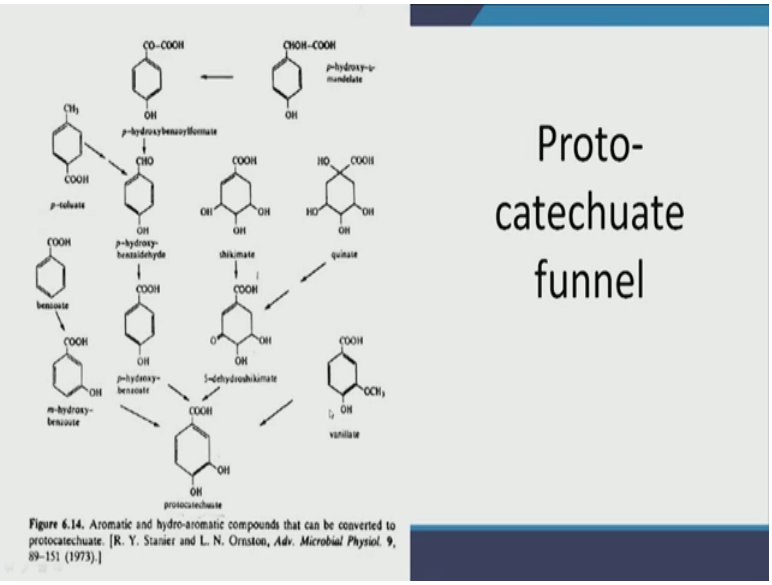

Now, there another which is protocatechuate funnel, instead of catechol I mean this is catechol if you hide this COOH here, but if this this is called protocatechuate. So, catechol funnel is not the only path way for arome degredation aromatic compounds, but we also have protocatechuate and some aromatic compound, they goes undergo the protocatechuate path way this is also very easy to degrade.

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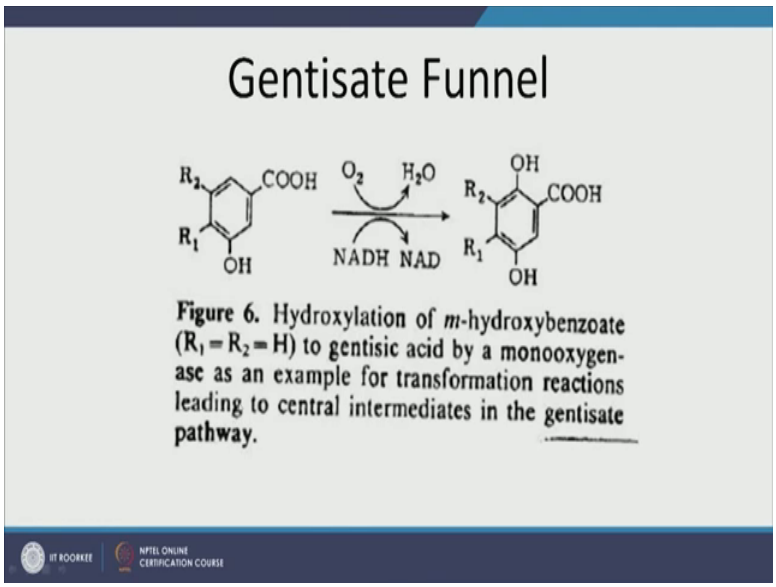

Now, the other pathway is gentisate funnel. So, many other aromatic compounds undergo genti sate funnel, and let us say this is this and then what happens in the dividend OH here enzymatically; obviously, and this is this will lead them to the gentisate pathway.

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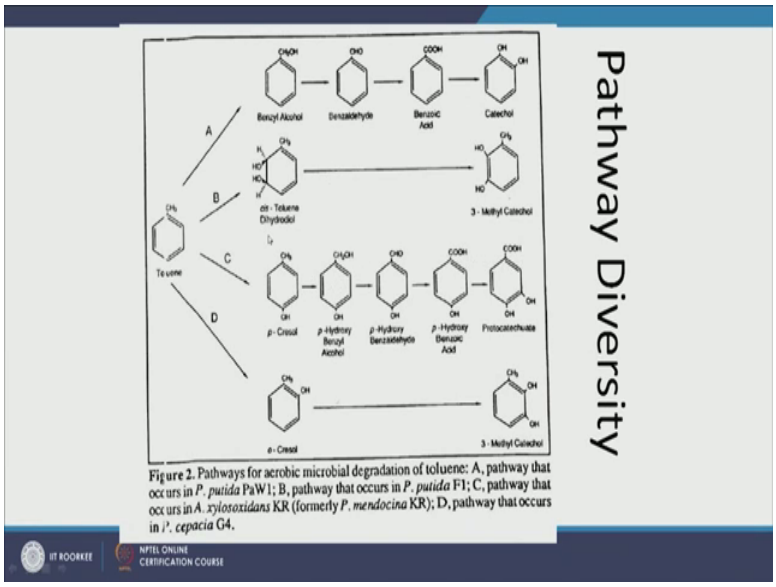

Figure 2. Pathways for aerobic microbial degradation of toluene: A, pathway that occurs in *P. putida* PaW1; B, pathway that occurs in *P. putida* F1; C, pathway that occurs in *A. xylosoxidans* KR (formerly *P. mendocina* KR); D, pathway that occurs in *P. cepacia* G4.

So, note that different kind of aromatic compounds can either go catechol pathway or protocatechuate pathway or gentisate pathway.

Similarly, the compound can undergo different pathways. So, for example, toluene is very interesting because it exhibits degradation through different pathways, it can undergo catechol pathway where it first makes benzyl alcohol then the benzyl alcohol is degraded in to aldehyde. So, it is a beta oxidation benzaldehyde, and then into acid benzoic acid and then finally, into catechol. And then you know catechol we can de cleave there we can cleave easily. The other thing that toluene can undergo is that instead of oxidation of its methyl radical, it can have a breakdown of the bond here and it makes Cis toluene in the dihydrodial and then this undergo, this becomes three methyl catechol and then this is also very easy to degrade.

Basically a catechol with a methyl ridiculer attached to it. And then toluene the third pathway that toluene can undergo for clean cleavage, which is the protocatechuate pathway,where it adds in which here in the para position and it this is cresol para cresol, and once the para cresol has made the made then the methyl radicals can undergo oxidation. So, eventually final at the difference between first pathway the third pathway

is that, even though and the methyl radicals gets oxidized here we have catechol forming near the way methyl radicals use to be, but this will be opposite for methyl radical. The OH that will attach to (Refer Time: 07:30) protocate this is protocatechuate pathway will the OH that will attach attached near the para OH.

Now, the other is instead of making para sresol it might be ortho cresol. So, the OH might attached to in the ortho position, and once the OH attaches in the ortho position it again under go catechol different kind of catechol pathway. So, we had three methyl catechol. So, again catechol, but the methyl radical is adjacent to one of the OH. So, the same toluene can undergo a path degredation through at least four different pathway that are vales that worldwide established rarely in nineteen rarely increase 1970s and 1980s.

So, notice that there is not just one sure shot way of degrading an contaminant, the multiple ways each step are enzymatically catalysed, and their different microbes that might be expert in undergoing different kinds of pathways. So, if I am talking about that I have toluene contamination and toluene is undergoing degradation, I might not only sees benzoic acid benzaldehyde, but I might see different forms which I might find para cresol, ortho cresol, three metyl catechol. So, does not mean that the microbial communities doing weird things, it just means are there multiple pathways of degradation.

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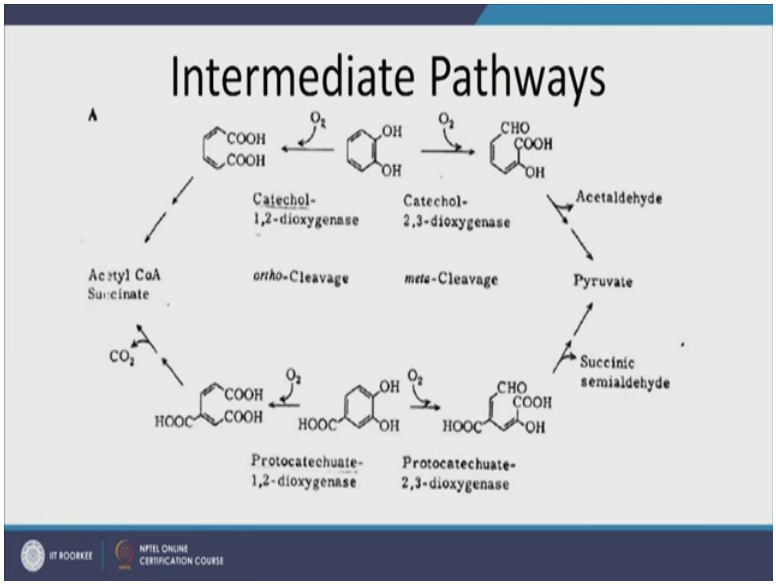

Okay then there are intermediate pathways let us look at them, we are let us say starting here with catechol. Now how the catechol will degrade the two ways can catechol degrade, it can either undergo catechol 1, 2 dioxygenase. So, it will add the oxygen in 1, 2 position or it can undergo catechol 2,3 dioxygenase. So, in 2,3 position.

So, if it is 1, 2 dioxygenase then it will add to 1, 2 dio position and 1, 2 position means, the it will these OH radicals you get oxidise the bond will break. Now here you have a dia acetic acid. So, this is break into acetyl C o A succinate, and this will go into citric acid cycle. The other possibility is there the catechol 2,3 dioxygenase enzyme might add oxygen in different positions here and here, instead of in these two and as a result we have this the per the bond is broken the ring held in broken, this will turn into acetaldehyde if you give a pyruvate, which will further undergo degradation very easy we have talked about, citric acid cycle and pyruvate degredation earlier. So, you must know about this.

Now, let us look at protocatechuates. So, we have protocatechuate here. So, basically this is catechol, but with the COOH part as here. Now this might undergo again 1, 2 dioxygenase or it might undergo 2, 3 dioxygenase. If its undergo the 1, 2 dioxygenase pathway the enzyme catalyzed its called protocatechuate 1, 2 dioxygenase enzyme. So, this enzyme will it again 1, 2 position. So, these OH radicals will be oxidised in to COOH and then they will break down into carbon dioxide acetyl C o A succinate and undergo citric acid cycle and the other hand the protocatechuate might undergo protocatechuate 2, 3 deoxygenase pathway, in which the oxygen dioxygen means oxygen gas that oxygen that would be consumed will attached 2, 3 position and this is how the (Refer Time: 10:30) it will make succinate semialdehyde and pyruvate and it will further undergo degradation.

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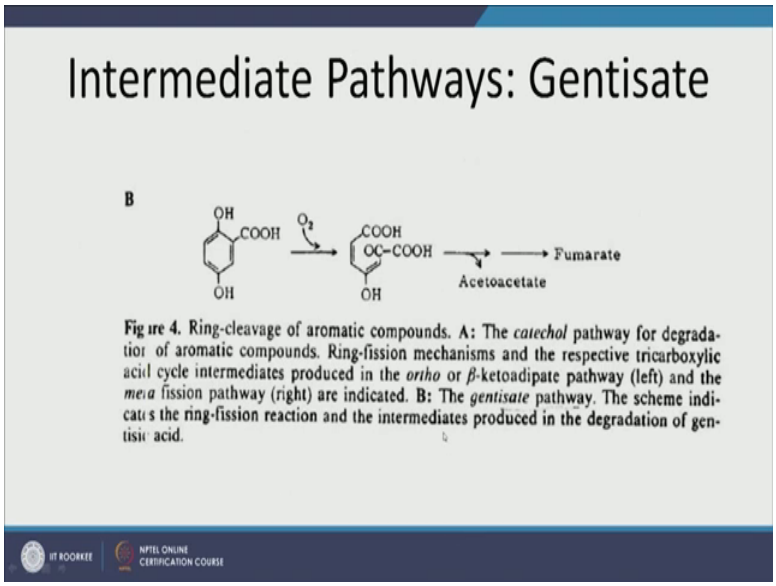

Now, let us look at gentisate path funnel in gentisate pathway how the degradation happens. So, in this the this is your starting aromatic compound and then it breaks here and add OC-COOH and here COOH it may removes acito acitate and then makes fumarate and fumarate is very easy to degrade.

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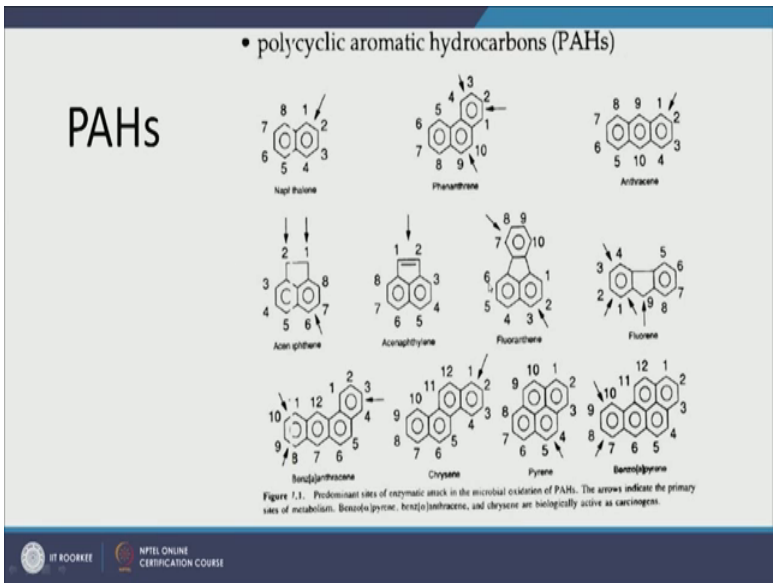

Now, these are different PAHs. PAHs are very commonly used abbreviations I would like you to be familiar without PAHs are. PAHs stands for ploy aromatic hydrocarbons.

So, one benzene ring is hard enough to degrade correct, we are talking about catechol pathway, protocatechuate pathway, the gentisate pathway and even once cate once catechol has been made the catechol can undergo 1, 2 dioxygenase or 2, 3 dio dioxygenase in enzyme pathway, but now added multiple aromatic rings and the they are resonating together. So, the electron cloud for naphthalene for example, for naphthalene the electron clouds move like this. It is much much more stable than a single benzene now degrading this is going to be more tricky.

How are you going to degrade this and look these are even more difficult to degrade pyrene, chysene, fluoranthene and most of pesticides most of our insecticides and many other xenobiotics that are popularly use in our country are PAHs. So, the arrows in the diagram are actually showing you the most common sites in which enzymes attack PAH s through Arabic degradation. Note here until now we have been talking only about aerobic degradation of aromatic compounds. So, anaerobic degradation of aromatic compounds looks slightly different, but if you look at the arrows carefully for example, in naphtheline, it is this bond to be attached by the enzyme enzymetic activity for oxidation.

In phenarthrene we have three sides, in anthracene we have one side this is very stable and the same by the way here we have two sides; obviously, the less resonant one here also we use this bond here it breaks this bond, because this part of the less has less resonance than this part. In flouorene it can degrade it can attack this nine position, it can attach here these bonds in benzaanthracene it attacks these points, in crysene these only one point where it can attack, in pyrene it attacks here and benzopyrene attacka the the isolated benzene ring.

Now, in one thing you want to note is that the benzopyrene, then the anthracene and chrysene their carcinogens. So, we want them and most of them are mutrozens, taratozens and they affect human health environment health, but benzaanthracene, chyscene and benzapyrene they carcinogens if a human being is exposed to these compounds we are very likely to get cancelled.

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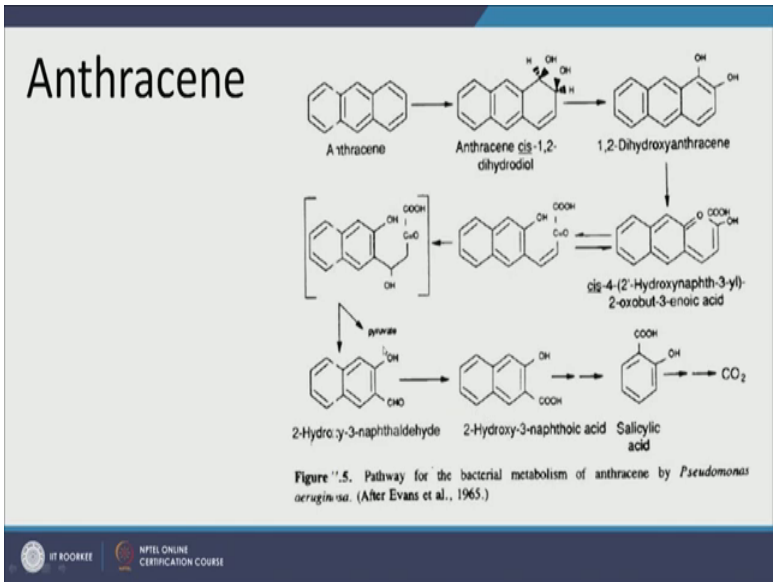

Now, let us look at anthracene. So, among these let us choose anthracene. So, anthrecene is here three benzene ring. So, anthresene whenas a arrow suggests one to this part is most likely to attacked by enzymatic oxidation. So, this bond gets broken when the this bond breaks, the OH attached here, one OH attached here and we have anthracene cis 1, 2 dihydrodiol and once we have this the aromaticity changes, and it makes double bond here and yeah. So, this is what we get.

Then this further oxidized and it is this bond cleaves and this becomes in acetone, and we have an acidic acid here and OH here and then this down let us see here we have a equilibrium. So, what basically they are saying is that at times this OH radical will give it hydrogen to this acetone here, and then this will become OH alcohol and this will become acetone. So, this is an equilibrium. So, sometimes its in this form, some times its in this form.

So, basically we can write it like this, and it eventually it will break down it will make two pirate, it will make pirate once it will make pirate it will realise a pirate and it will make this. So, this bond breaks down releasing pirate, and then this and has to undergo further oxidation. Now look here you have two aromatic rings attached to OH into CHO so; obviously, beta oxidation CHO will undergo degradation makes COOH. So, this is naphthaldehyde this will makes naphthoic acid, and then this breaks down further and makes salicylic acid.

This is also the starting point for making a anthracene, and then this is degraded very easily into carbon di oxide. So, this is how bacteria they catalyzed metabolism of anthracene. Now this voucher study happened very early in 1965 Evans et al, and the micro that they this was done in pure cultures. So, I 1965, 1950s, 70s, 80s, 90s maybe even up to 2000 most of the biodegradation studies for focused on pure cultures.

So, sea dominos aerogenes have one particular microbe in lab, how does degrade anthracene. Now again if you remember I have mention this in past that an isolated microorganism working in lab, might not will show different behaviour once it is given one it is once it put in environment, for various reasons because not interacting with the other microbes its undergo computation and its regulation of its expression genetic expression, but also change. So, its behaviour will change and then it may not be the only microbe that is successfully degrading anthracene. So, we might see different pathways of anthracene degradation, but this is the pathway that was first established.

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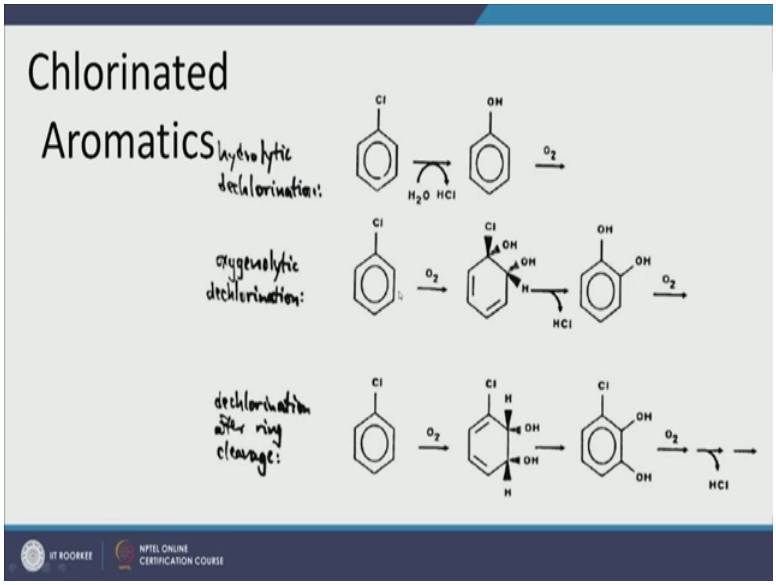

Now let us look at chlorinated aromatics. So, chlorinated aromatics are again very very difficult to degrade, and until recent passed they were the major environmental challenge when we talked about PHA degradation. So, here you have simple benzene ring chlorinatize. So, what we want to do because the chlorine is very strong electron acceptor, and benzene cloud is very rich in electrons, because their the pi electrons are happily resonate here.

So, this makes a very strong bond between chlorine and benzene. So, where you want to do is you want to do hydrolysis and you want to remove the chlorine. So, in hydrolytic dechlorination the chlorine is removed by hydrolysis HC listed innovated and OH attaches here, and then this phenol is very easy to oxidize. The other is oxygenolytic dechlorination where we directly oxygenate this and then one OH attaches here, one OH wants to attach here and then one OH wants to attach here this H and this C l escape as H C l and we are left with this, and this is also again very easy to degrade.

Now, the other thing is we can first break the ring and then dechlorinated. So, if you are breaking the ring the oxygen may instead of attaching here and here might attach in this position and this position. So, in when meta and ortho position, when atta attaches there the this bond breaks and we have this which is again very easy to degrade and once this has created the H C l will escape later ok.

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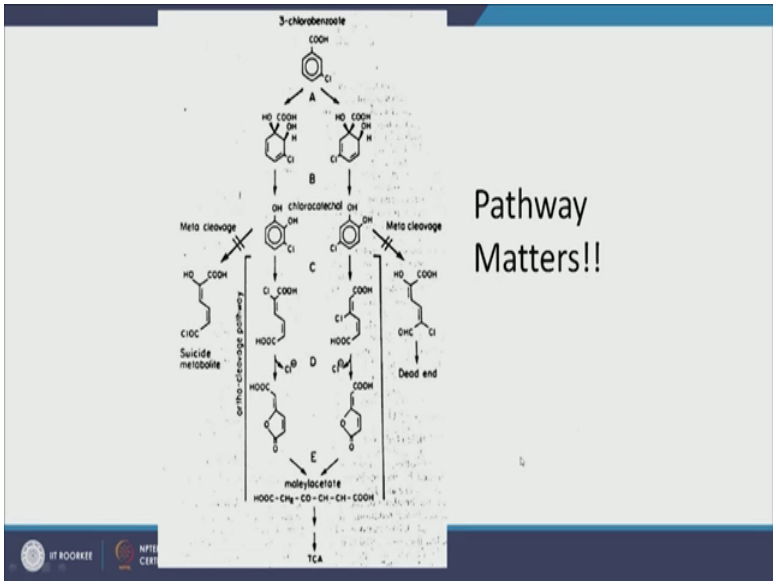

Now, in this slide what I am trying to tell you is that, the pathway of degradation matters. So, we have learnt earlier that same compound can be degraded by multiple pathways right we talked about aline we also talked about how different compounds can undergo different pathways same compound can undergo different pathways now pathways matter.

For example let us see we are starting with three chlorobenzoate. So, this is benzoate with the chlorine and third position, it might undergo cleavage from here and here or it

might undergo cleavage like this, which is from here and here, here and here. So, depending on whether the OH is attaching here or attaching here, its daughter product would look different.

Obviously if it OH attaches here then this is what will have if OH attach attaches here instead and will have a very different structure eventually they end up making same compound and then they go to TCA cycle. However, once it has reached here in this place it, instead of going through this cycle it may undergo meta cleavage and it might make a suicide metabolite. This is called suicide metabolite because this will inhabit further degradation.

So, it ends everything the cell will die micro metabolism would end. So, if instead of letting it go through this pathway, it we alive to go through undergo this pathway then this is the end of the story. On the other hand here also if it undergoes meta cleavage then this is the dead end this cannot be degraded for that is why this is very stable.

So, the pathway matters if my mother four pathways, two of them very good degradation one of them is suicide and other is dead end. So, pathway matters.

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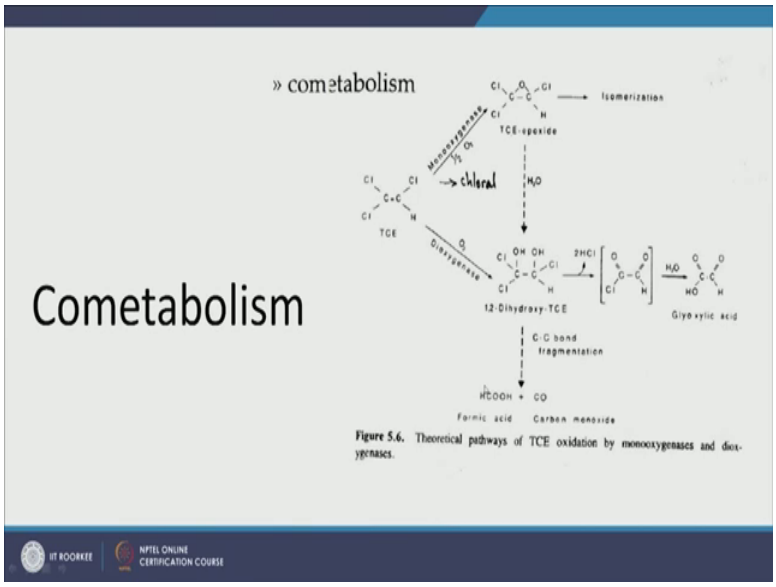

Figure 5.6. Theoretical pathways of TCE oxidation by monooxygenases and dioxygenases.

I have talked about cometabolism before. So, let us look at cometabolism again and I have talked about cometabolism in context of uranium. So, we have uranium and we

want to reduce it. So, that the ready because reduce from less mobile less soluble, but no there is not much energetic advantage for any microbe to reduce uranium.

So, we look at microbes that reduce other metals extracellular we like r n reducing microbes and then the enzymes they use for reducing iron; they can use a same enzymes for reducing uranium. So, now, we have uranium reduction happening and this is in the way coreduction of uranium. Similarly there are compounds that microbes do not want to degrade because its energetically not favourable and they would prefer doing other things then degrading that contaminant, but if we add some electron donor electron acceptor, that can be utilise from microbes, that can be degraded by microbes are transformed by microbes and the original contaminant its possible undergoes cometabolism.

So, let us take an example. So, these are the TCE is again a very very contentious and con contaminant in environment and some many decades scientist across the global very hard on hard to degrade TCE there are two pathway for TCE degradation in theory one is monooxygenase.

So, this is one oxygen that breaks this double bond attaches here, it makes epoxide it may undergo isomerisation or it might convert into this which is dioxygen which is basically one OH attaches here one attaches here. So, instead of one oxygen we have two oxygen. So, it might become this and then this can undergo degradation makes glycoli glyx oxylic acid which is very easy to degrade or if C C bond might fragment and it will one might fragment, and we get formic acid and carbon oxide. So, theoretically this is how TCE degrade, but TCE degradation was a big challenge. So, we switched for cometabolism.

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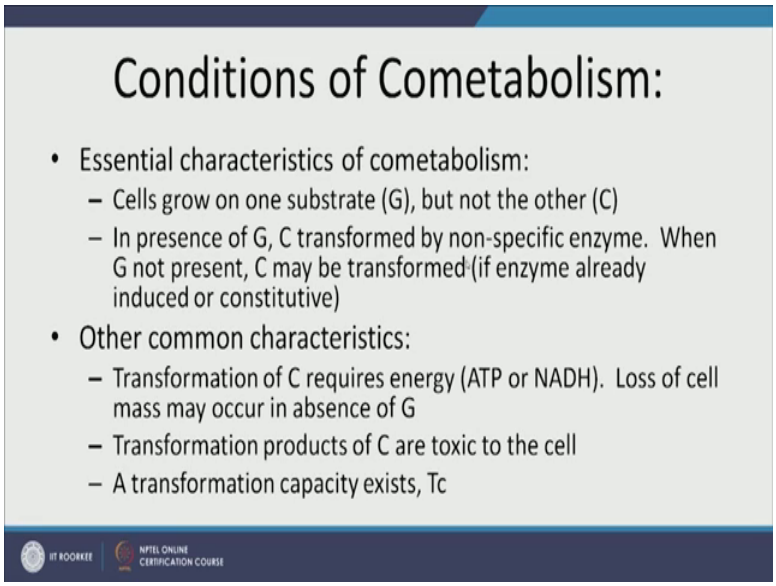

Now, how do we do cometabolism, the essential characteristics of cometabolism are that the cells will grow on the one substrate, but not the other. So, C is the what will be cometabolize in presence of G C is transformed by nonspecific enzyme. So, much like iron reduction and iron reducing enzymes are nonspecific, they will reduce any metal that is there in the vicinity thatis why they reduce uranium.

So, in presence of G, c will also be transformed by nonspecific enzyme produced for metabolism of G. When G is not present if enzyme is present let us say the substrate is gone this is substrate G is acetate. Acetate is gone, but the enzymes that are metabolizing acetate are present, then the C might still undergo transformation. Other thing is that transformation of C requires energy; transformation of G will give energy. So, G is not present in cells are trying to transform C because they have enzymes or whatever reason then we will loss cell if G is not present.

The other possibility is that when C is oxidized or C is transformed the daughter products are toxic for the cells. So, first do not want to do this, in that case cometabolism is helpful and the other thing is that the capacity to get transform should be there its not be reconsidering.

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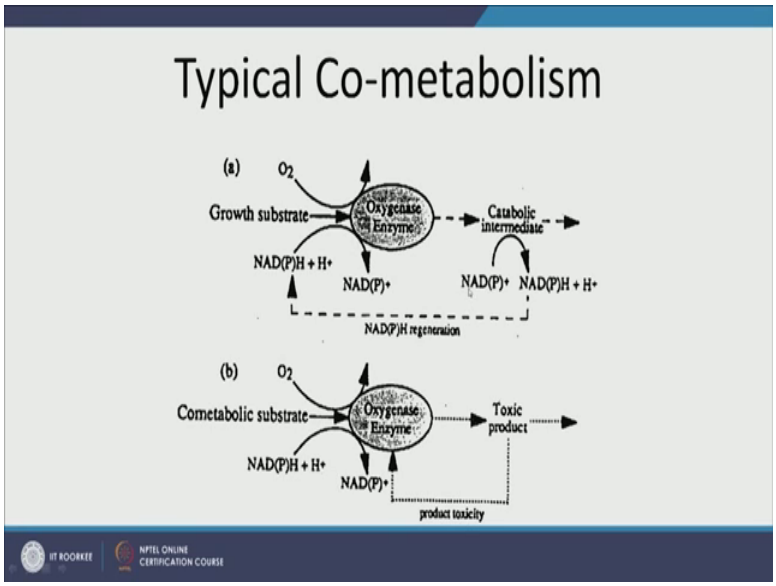

So, typical cometabolism looks like this, you have your growth substrate this is oxygenase enzyme and you will get your catabolic intermediate and your NAD P H gets regenerated you get energy out of it.

Now, this your growth substrate, substrate that gives you food gives you energy now this is cometabolic substrate does not give anything. So, your cometabolic substrate also undergoes oxygenation, it makes a toxic product let us see the last point describe it makes a toxic product and it toxics. So, at least some of the cometabolic substrate got degraded for after a point the, this will stop ok.

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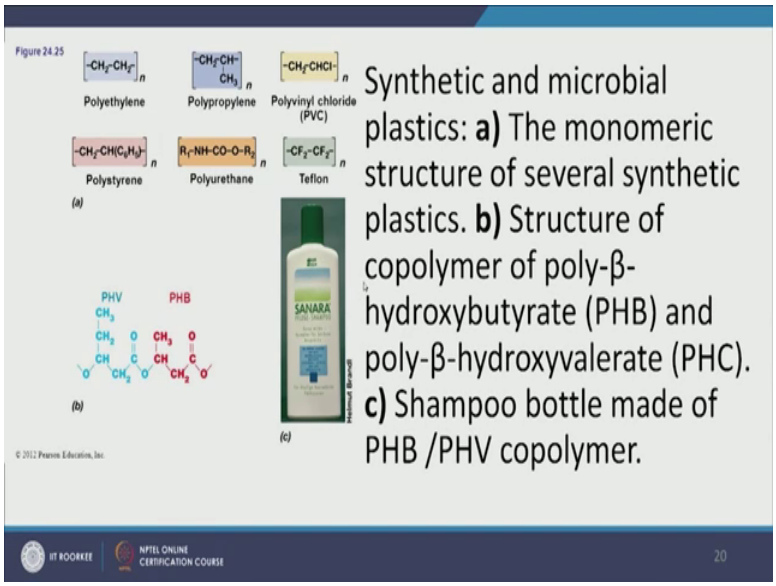


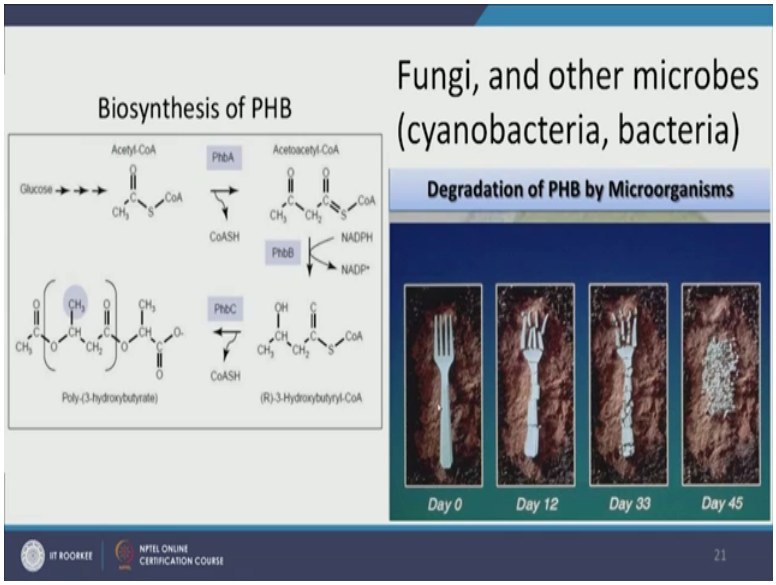
Now, having talked about biodegradation, I want to talk to you about bioplastic. So, we want contaminate should be degrade, but we have certain contaminants that are very hard to degrade like plastic. Now even the plastic is basically polymer of petroleum hydrocarbons, its very very hard from microbes approach into degraded. So, now, what scientist and previous few years what they have done is, they have noticed that there are certain bacteria that actually make plastic, where actually make p h assign PHVs, p h bs and p h vs which make polymers with each other, and the polymers are plastic they behave like plastic.

Now, these bacterial plastics are also biodegradable they degrade (Refer Time: 24:35). So, in in the conventional approach we have polythene. So, polythene and these are just ethyl radicals that are attached to each other and then we have proli polypropylene. So, this is one CH 3 here and then we have PVC polyvinyl chloride. So, the plastic that we use is mostly ether polystyrene use for making cloths polyethylene, polyethylene use for making polythenes, polypropylene PVC used for making fire PVC pipes use for different containers you have Teflon, that is used in our cooking ware also and you have polyurethane which has multiple uses.

So, these are not degradable on the other hand the bioplastic will have a structure like this. So, this is your PHV and this is your PHB, they attach with each other they make a PHV PHB polymer, and this is one of the shampoo that that whose container is made by

PHV PHB the bio plastic polymer. So, its looks like normal pros behaves like normal plastic but put it into the environment and degrades like this.

(Refer Slide Time: 25:31)



So, this is the PHB fork after 12 days, the microbes have decomposed with this much and after 33days, after for45days are just (Refer Time: 25:41) and going to decay.

So, that is why this there is lot of. So, this lot of expectation and hope from the bioplastic they will helps us our plastic problem. So, synthetic and microbial plastic these are the monomers of several synthetic plastic these are poly beta hydroxybutyrate and poly bêta hydroxyvalerate and they make polymers together and this is the shampoo bottle.

Now, how I PHB is made, how I poly beta hydroxybutyrate made. So, basically the microbes are use glucose we use acetyle-CoA, we use different enzymes and then they make monomers of hydroxybutyrate, which make polyhydroxybutyrate beta hydroxybutyrate. Similarly hydroxy validate for monomer is made and PHB, PHV polymer is very nice plastic. So, this is one example of degrading.

And who can degraded we know that fungi can degraded many other microbes bacteria can degraded. So, this is not a problem, the problem is which is want to make it economically feasible all right dear students this is all for today. In the next lecture we will explore microbial communities from other relevant environments, and continue our

journey on the applied environmental challenges and how we apply microbiological techniques to solve them.

Thank you very much.