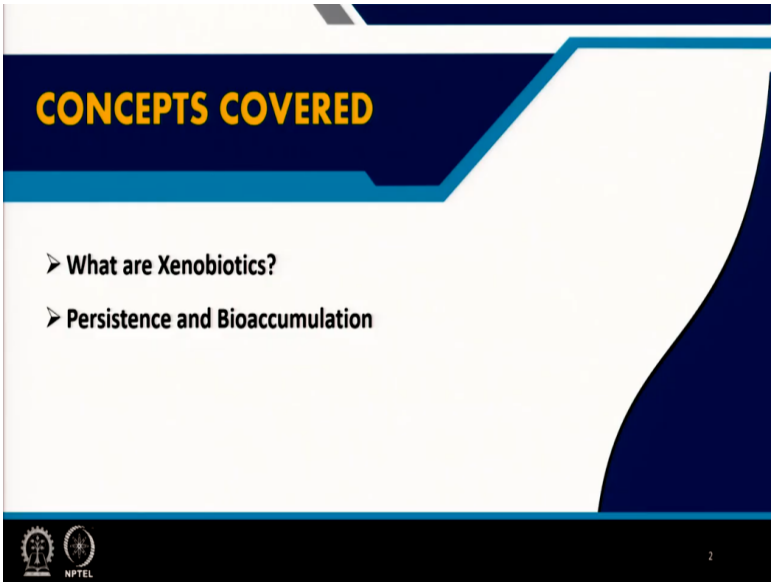
Environmental Chemistry and Microbiology
Dr. Anjali Pal
Dr. Sudha Goel
Department of Civil Engineering
Indian Institute of Technology - Kharagpur

Module - 9
Lecture - 47
Xenobiotics - I

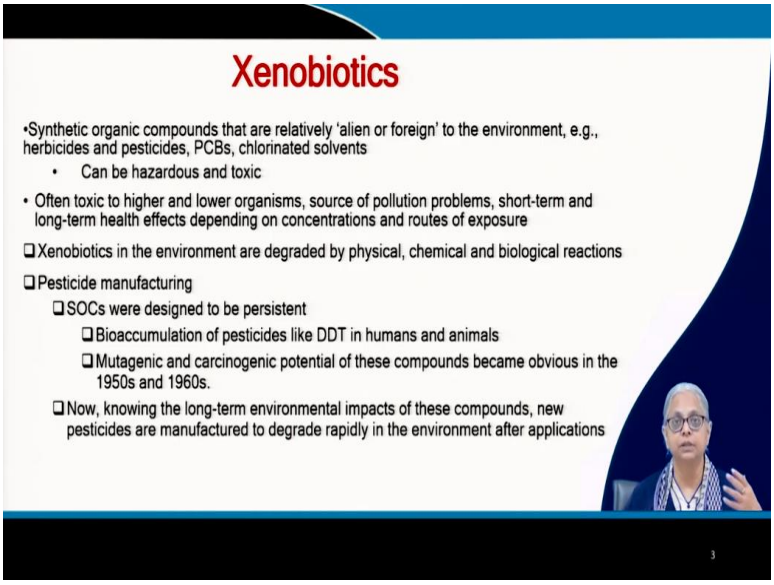
Welcome everyone to the next topic in module 9. We are starting with a new topic called Xenobiotics. It is divided into 2 parts. So, lectures 47 and 48 will cover xenobiotics, and this is part 1.

(Refer Slide Time: 00:43)



What are xenobiotics? is our first subtopic. We will look at what these compounds are and where they come from and why they are persistent; why do they bioaccumulate in the environment. These are some of the things that we are going to look at in part 1.

(Refer Slide Time: 00:59)



So, let us come to the definition of xenobiotics. What are xenobiotics? Xeno means alien or foreign. So, any organic compound that is made in the lab; whether it is an industry lab or a research lab, it does not matter. Synthetic organic compounds that are manufactured, that are anthropogenic in nature, are therefore by definition alien or foreign to the environment. And this has an impact on the biota of the environment where these synthetic organic compounds are released.

So, we know that examples include herbicides, pesticides, some of the polychlorinated biphenyls, chlorinated solvents. All of these are examples of synthetic organic chemicals that are constantly being released into the environment. So, many of them are hazardous as well as toxic. And any chemical that is toxic to either higher or lower organisms. If you are using a pesticide, it is going to be toxic, yes, to the pest that you are trying to eliminate, but there will be some impact on higher organisms as well.

I would like to mention one little point over here, and that is that their toxicity is related to body weight. So, the smaller, let us say you have 2 organisms, one has a very high body weight, and the other has a very small body weight. So, let us say you are dealing with a mosquito or a cockroach or some other pests like that, and you are comparing yourself and your exposure to the same chemical and the same concentration. So, because of the higher body weight, we are taking in a dose that is far smaller in amount compared to the smaller organism and that is why the toxicity to the smaller organisms is visually evident to all of us. But for the higher organisms, like human beings and other animals, their toxicity is apparent only over the long term or short-term exposure, depending on the concentration that they are exposed to. So, when a chemical is toxic, it is likely to be toxic to both higher as well as lower organisms. What matters is the dose, which I do not have time to go into at this point.

But that is a very important part of exposure to xenobiotics. It is a source of pollution problems. Today, we are worried about the pesticides in water; we are worried about many of these compounds of synthetic nature that are now part of the streams and lakes and so on; and therefore, they become part of our drinking water, and many other problems are associated with them.

They can have short-term and long-term health effects, not just on us, but on other species, and that depends on their concentration and routes of exposure. What is the reason for all these problems? The biggest reason for these problems is that these are new to the environment, which means that the microbial flora in a particular area, that is already existing in that area, is not adapted to the presence of these alien chemicals.

So, in a sense, many of these chemicals are recalcitrant or persistent, and they do not degrade easily. They can be degraded by physical, chemical, and biological reactions. The biological reactions are a little more difficult compared to the physical and chemical reactions. And I can spend a lot of time on this, but I have limited the scope of this lecture to certain ideas. So, there is a lot more to this topic, but I would not be able to address all of it. I am spending just about an hour on it. And if there is interest, we can think about something else. So, let us come to the idea of pesticides. We are all concerned, we know every; I think even little kids who go to school have been told that pesticides can be toxic, they can be hazardous, you should not let them go into your mouth, into your eyes; all these things, I think are well-known.

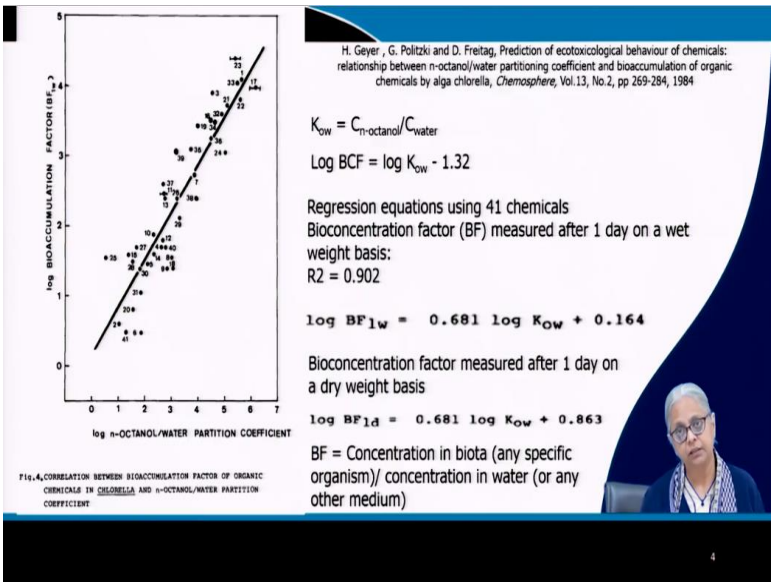
These SOCs or synthetic organic compounds were designed to be persistent. So, let us say, in the 1950s, when it became big to manufacture all these compounds, the idea that a compound will last for the entire plant growing season; remember that most of the pesticides are used by farmers in their agriculture. And the idea of using a particular chemical, allowing it to be in the field, and it would remain there for at least one growing season, was a good idea.

It sounded like a good idea; until in the 50s, 60s, 70s, it became more and more apparent that these pesticides were bioaccumulating in the tissue of human beings as well as animals and birds. And DDT is one of the best-documented examples of a pesticide that bioaccumulates. Many other compounds, not just DDT, but many other pesticidal compounds have mutagenic and carcinogenic potential, which became obvious, like I said, through the 50s, 60s, 70s; and people are still working on these compounds.

Once it was recognized that these compounds are likely to cause long-term health effects, it has now become necessary for pesticide manufacturing companies to prove that whatever

they are manufacturing and selling will degrade as rapidly in the environment as possible. Now, what does this all have to do with microbiology? Remember, the whole idea starts with this point. These are alien to the biota of the environment. So, if we have microbes that can be acclimated to degrading these kinds of compounds, then we can solve the problem with relative ease.

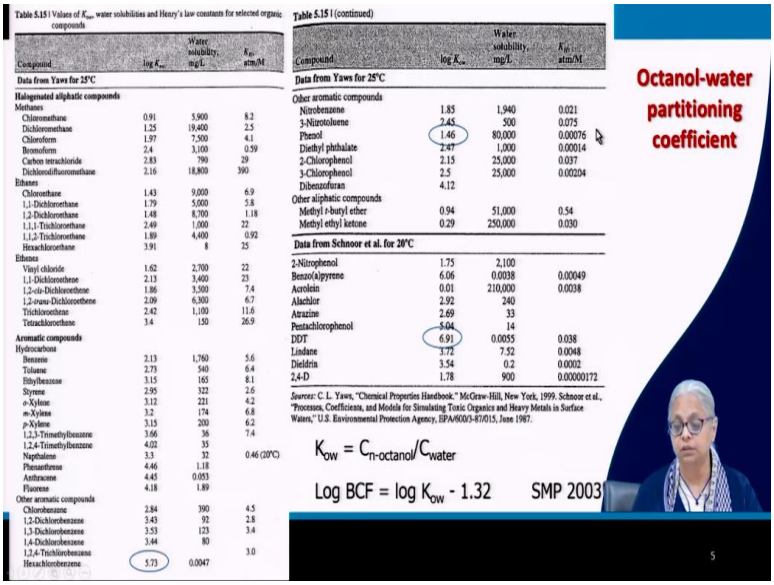
(Refer Slide Time: 07:42)



Before I go into all the details, let me give you an example of bioaccumulation and what it is related to. So, here on this graph, you see 2 factors. On the x-axis, you have log n-octanol divided by water partitioning coefficient. That is log K_{ow}. K_{ow} stands for the octanol-water partitioning coefficient. So, you have $C_{n\text{-octanol}}/C_{water}$. Now, C stands for concentration in octanol versus concentration in water.

Now, if a compound is hydrophobic, it will prefer to be dissolved in octanol and not in water. If it is hydrophilic, the concentration in water will be higher than in octanol. So, let me just show you a few examples of that.

(Refer Slide Time: 08:38)



So, here we have, let us say phenol. Let us take phenol. It is something that we see around us in our environment. It is called carbolic acid. If you go to the shop and ask for carbolic acid, they will give you phenol. So, this is the aqueous solubility. It is highly soluble. It has a low K_{ow}; it is very close to 1. And the last column is Henry's constant.

Let us take DDT. Look at the water solubility of DDT. It is 5.5 micrograms per liter. So, it is not very soluble in water. It is a well-known hydrophobic compound. And the log K_{ow} value is extremely high, 6.9. So, 10 to the power close to 7 is how much of DDT will dissolve in octanol compared to water. So, this is the log octanol-water partitioning coefficient. And on the y-axis, we have the log bioaccumulation factor.

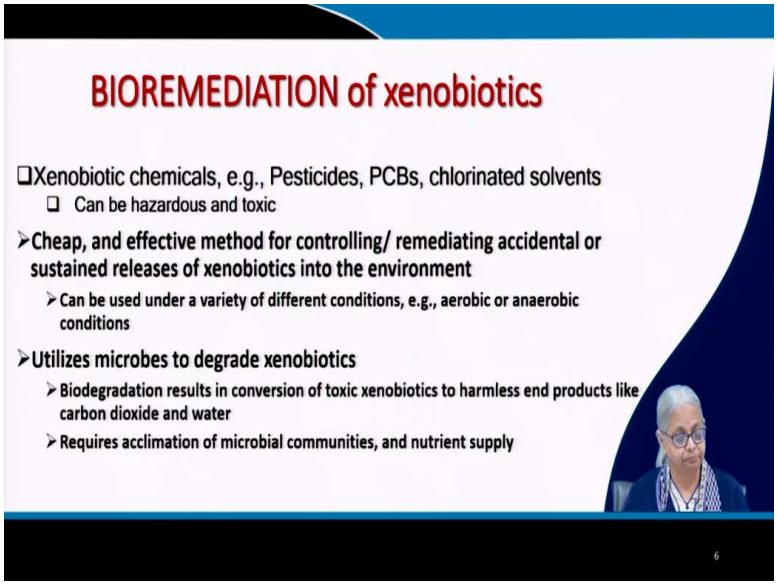
This is a set of data from a particular paper where the (octanol)- water partitioning coefficient and the bioaccumulation of various chemicals; I think there are 41 different chemicals that have been tested using one particular species of algae chlorella. So, this chlorella species which is an algal species have been exposed to 41 different chemicals; now, when you add a particular chemical to water, and then you try to grow a particular species, whether it is algae, whether it is bacteria, whatever it may be, you can harvest those cells and then determine how much of that chemical is still present in the tissue of those cells.

And that is, I think, what they have done here. And this is a clear and simple correlation. So, we have K_{ow}, which is a chemical factor; it has nothing to do with biology. And then, we have the bioconcentration or the bioaccumulation factor, which is correlated to the chemical factor. So, this is the best evidence we have. And there are several papers; this is one of the earlier papers, it is 1984.

So, as far back as that; and there are many studies. This is just an example. But there are thousands of papers out there that will give you similar data. My point here is that you have a clear correlation between the chemical factor which is K_{ow} and the bioaccumulation factor. Now, chemical factors are much easier to determine; or you can just open a reference book and look at the chemical factor.

And knowing this kind of relationship exists, you know that there is a high potential for bioaccumulation of these compounds, based on their K_{ow} values. So, when you have a high log K_{ow} value, it means that the compound is going to bioaccumulate in humans as well as the tissue of other higher organisms. So, there is a certain health impact associated with these chemicals which can be readily looked at knowing these ideas, knowing these concepts. What is the bioconcentration factor or the bioaccumulation factor? The concentration in the biota of a particular organism is divided by the concentration in water.

(Refer Slide Time: 12:21)



So, that is about xenobiotics. So, that is a characteristic of xenobiotics, that they are difficult to biodegrade; one, because they are hydrophobic; remember, most biological organisms, most microbes live in water, they want water. So, if something is hydrophobic, it becomes difficult to biodegrade. That is one reason. The second reason is that these are chemically stable compounds. They are very persistent. They are very recalcitrant.

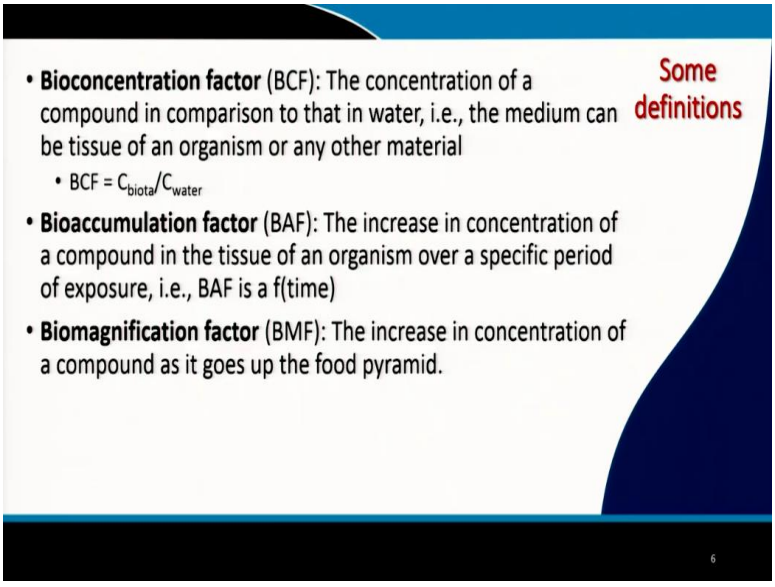
And I will come to more details about this in the next few slides. So, if I want to use my knowledge of microbiology for the bioremediation of xenobiotics, can it be done? And the answer is yes. It is cheap and effective. So, we have xenobiotic compounds like pesticides, polychlorinated biphenyls, chlorinated solvents. They are all hazardous and toxic. let us say you have large areas that have been contaminated by these synthetic organic compounds.

Is it possible to use biological processes to clean up the mess? And the answer is yes. So, they are cheap, they are effective methods for remediating either accidental releases or sustained releases of these xenobiotics in the environment. It can be done under aerobic conditions as well as anaerobic conditions. You might be wondering how that is possible; because, when I say xenobiotics, it means that you need microbial biota to be acclimated to the presence of these compounds. And once you can acclimate them and find out if they have any particular nutrient requirement and so on, or what is missing in the environment, then you can design your bioremediation strategies. So, you can use microbes to degrade these xenobiotics. The biodegradation can go the full length, which means to the endpoint, harmless end products like carbon dioxide and water; or in many cases, what does happen if you leave nature to itself, what you get is toxic intermediate products.

So, DDT, when it was sprayed in the fields, resulted in compounds like DDD and DDE. And DDD and DDE are considered to be more toxic than the parent compound. The parent compound is toxic, but the intermediates are even more toxic. So, these are some of the problems associated with leaving nature to itself. So, remember we are introducing something into nature that does not belong over there; and therefore, it creates a bigger problem.

So, what do you need for bioremediation? You need acclimated microbial communities. You can have pure species, which means single species, or you can have consortia. And then you need adequate nutrient supply.

(Refer Slide Time: 15:27)



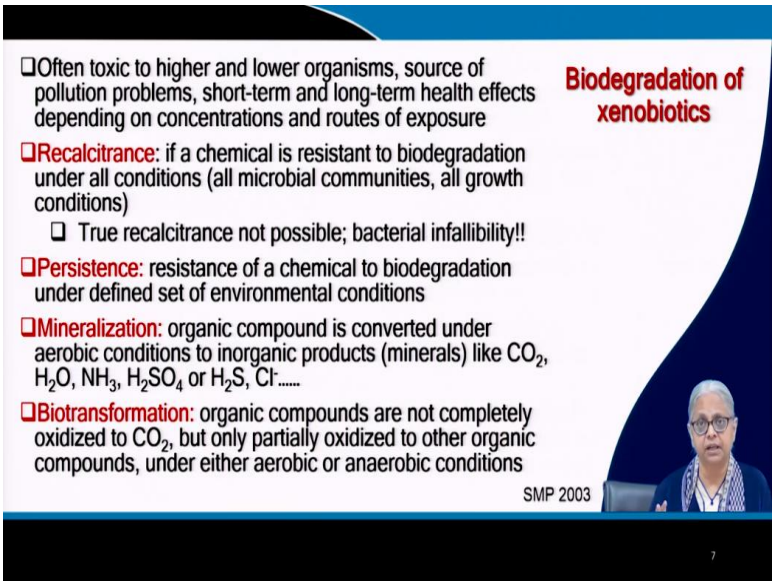
So, having talked about bioconcentration and bioaccumulation, I would like to add some definitions here. What is a bioconcentration factor? The bioconcentration factor has been

defined as the concentration of a compound in comparison to that in water. So, the medium, in comparison, can be the tissue of any organism or any other material. So, in an equation form, that would be the C_{biota}/C_{water}.

Then we have another very common word, and that is the bioaccumulation factor. This is the increase in the concentration of a compound in the tissue of an organism over a specified period of exposure. So, if a particular organism has been exposed to a particular compound for either months or years and so on, then it is accumulating that compound in its tissue and therefore, it is a function of time. So, that is the bioaccumulation factor.

And the last one is the biomagnification factor. The biomagnification factor tells us about the increase in the concentration of any compound as it goes up the food pyramid. So, assuming that water is below the lowest level, below the lowest trophic level, whatever concentration there is in the water, that will increase in the tissue of the phytoplankton which is at the bottom of the pyramid. And from the phytoplankton, right up to the top of the pyramid, you will get an increased concentration of the compound in the tissue of the organisms.

(Refer Slide Time: 17:13)



Before I go into further details, let me define a few terms that are commonly used in the literature regarding the biodegradation of xenobiotics. So, the first term that is very common is recalcitrance. So, when I say a particular compound is recalcitrant, it means the chemical is resistant to biodegradation under all conditions. No microbe will grow on it and under no-growth conditions.

No matter what environmental conditions you provide, no microbe will be able to grow. Now, true recalcitrance has never been observed; it is not possible. I already said it in my first

lecture, there is this concept of bacterial infallibility. That means, if you provide sufficient conditions, sufficient time for the microbial community to acclimate itself, they will find a way to degrade it. So, that is part of bacterial infallibility. They can never be wrong. They are always capable of adapting themselves to new environmental conditions including the presence of a new chemical.

Persistence is very common. When we look at DDT, when we look at phenol, lindane or endosulfan; there are so many pesticides out there. When we look at these compounds, and we find that they have half-lives that are more than a year; so, that means that these chemicals are resistant to biodegradation under certain environmental conditions. So, there is a long list of compounds which are considered to be persistent.

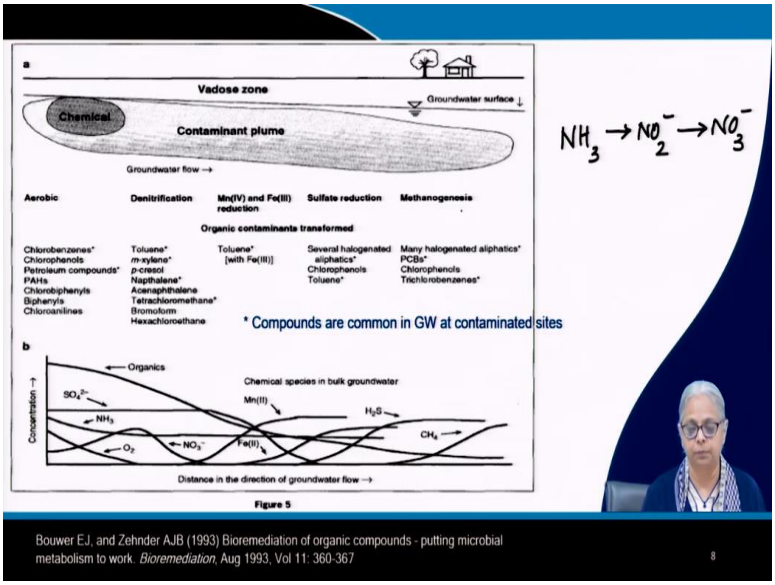
Then we use another term called mineralization. Mineralization is when the organic compound is converted under aerobic conditions to inorganic products like CO2, water, ammonia, sulphuric acid, hydrogen sulfide, chloride, and the list goes on. That is what we want. Ideally, we want an organic compound to be converted to these harmless end products; but it is not that easy.

We do not always get complete oxidation of the organic compound to these harmless end products. The last one is biotransformation. I will also mention here, something that is not mentioned on the slide. The use of the word biotransformation has been applied to both organic compounds as well as to heavy metals. Now, let us go through organic compounds, before I talk about heavy metals.

So, here we have organic compounds that are not completely oxidised to CO_2. So, if I want complete oxidation, the organic compound has to be converted to CO_2. If it is partially converted, like I said, DDT going to DDD or DDE, that is partial oxidation. Two other organic compounds, whether it is aerobic or anaerobic, that is possible. So, we call that biotransformation or biodegradation, but it is not mineralization.

So, that is one major point. The second issue that we have is that heavy metals can be utilised by microbes. They can be biotransformed. They can never be biodegraded. A heavy metal will remain a heavy metal. You cannot change its nature. So, it can be biotransformed, because you are changing the oxidation state; you may be creating organo-metal complexes; all those kinds of things are possible. But you cannot biodegrade a metal; you can only biotransform a metal.

(Refer Slide Time: 21:15)



So, let us come to the next point, and knowing what you know about the sequence of electron acceptors. So, I have shown you in the previous few slides; and in the last lecture, I have shown you the different terminal electron acceptors that may be present in the environment, and they can pair up with electron donors that are either utilizing organic carbon or they are utilizing inorganic donors, electron donors.

Now, here we have an interesting situation. So, you may have, let us say, I always talk about leaking underground storage tanks which contain petroleum products. So, next time you go to a petrol pump, just look around where the petrol is being stored. In general, most petrol pumps have underground storage tanks. So, these storage tanks over a long period of time will probably start leaking at some point.

If the groundwater table is fairly close to the bottom of the leaking tank; in the area that I am in, in West Bengal, the water table is often at the surface during the monsoon season, and it drops about 6 to 10 feet after that. So, here the water table is very high. And when the water table is very high, these leaking underground storage tanks will impact, they will contaminate the water, the groundwater in this area.

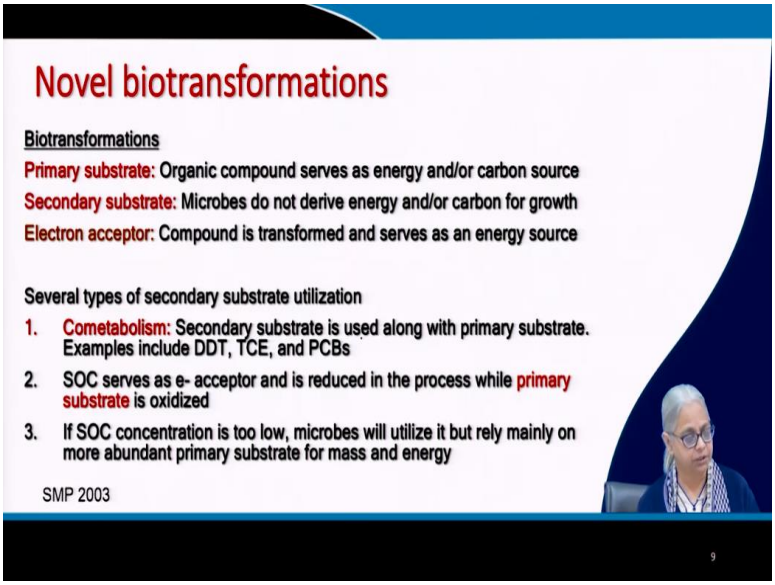
So, here you have the leakage of a chemical from an underground storage tank; it is leaking into the subsurface. These chemicals will flow along with the water and you will get a contaminant plume. Now the subsurface, it is not visible to the eye, but it is not dead, it is not sterile. There are microbes that live in the subsurface. Now, what are they going to do when they are exposed to these compounds, whether they are petroleum compounds, whether they are from an industrial site.

So, you may have an industrial site where they are manufacturing certain chemicals. And if they are dumping the chemicals on the site, on the land or in the neighboring areas, in streams and so on; all these compounds will end up either in the subsurface or in the surface water bodies. So, here we are looking at subsurface contamination by different chemicals. I have already shown you the sequence of electron acceptors.

So, here we have oxygen; denitrification, where nitrate is converted to nitrogen gas; Mn(IV), Fe(III), these are reduced. Then you have sulfate reduction and you have methanogenesis. What I want to show you over here is that as long as oxygen is available; remember our electron tower, the best electron acceptor, the best terminal electron acceptor is oxygen. So, the bacteria as long as there is oxygen will utilize some of the organic compounds that are present in groundwater and along with oxygen, and you can see the oxygen level going down. Ammonia, if it is present, will also go down. If oxygen is present, ammonia will be converted to nitrite and nitrate. You will get an increase in nitrate because nitrate is part of the end (point); let me see if I can write that. So, this is the conversion, if there is ammonia to begin withm this ammonia will be converted to nitrite and nitrate. So, what you see over here is reduction in ammonia and an increase in nitrate concentration.

Now, this nitrate can serve as a terminal electron acceptor in these denitrification reactions. Then you have Mn (IV) and Fe (III). They can serve as terminal electron acceptors. And you can see that Fe^{2+} concentration; here is the Fe^{2+}, yeah. And Mn^{2+} is going up as Mn (IV) is being converted to Mn (II) and Fe (III) is being converted to Fe (II). So, these are studies; and this is again a fairly old paper. And these studies have been published and it has been shown that these particular compounds that are listed under each terminal electron acceptor, that they have been degraded with this combination. So, you have these compounds as the electron donors and the various electron acceptors are listed at the top.

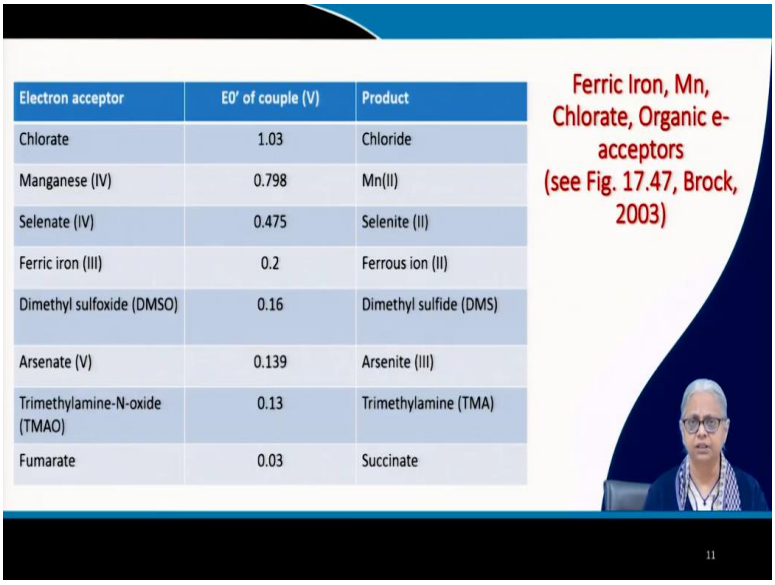
(Refer Slide Time: 26:36)



Novel biotransformations: So, when we talk about synthetic organic compounds, we have novel biotransformations and novel in the sense that they are new, new for the environment. So, there can be 2 possibilities that the organic compound can serve as the primary substrate. If it is a primary substrate, the organic compound serves as an energy or carbon source. And if it is a secondary substrate, the microbes are not going to get either carbon or energy from this particular compound, but what they will do is that because the enzymes for utilizing the primary substrate are already there, so, there is an automatic conversion, a natural conversion of the secondary substrate, even though it serves no real purpose for the bacteria.

And the electron acceptor is the compound that is transformed and serves as the energy source. So, there are 3 types of secondary utilization. The first is cometabolism. Cometabolism is when this secondary substrate is used along with the primary substrate. So, you can have DDT, TCE, trichloroethylene, and polychlorinated biphenyls.

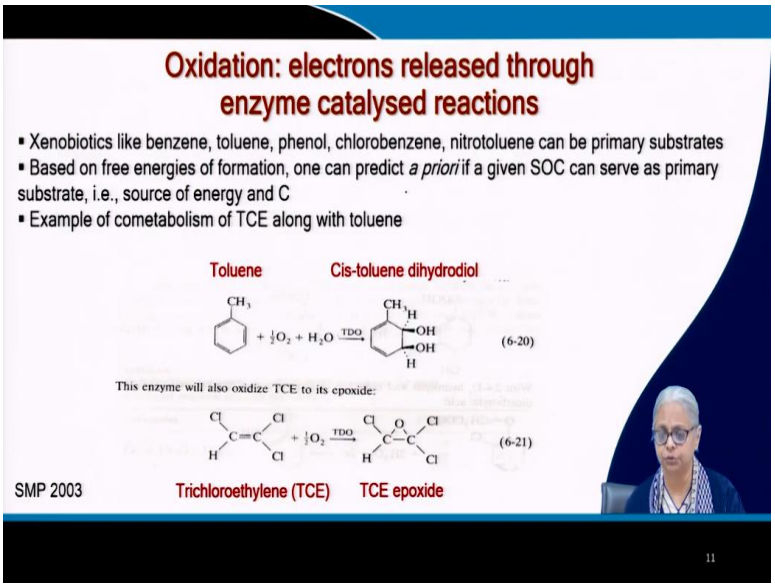
(Refer Slide Time: 27:55)



Before I come to that example, this is a list of several terminal electron acceptors that are kind of novel in that sense. So, you have chlorate. Chlorate is not something you are going to see in the environment. It is formed when you add chlorine to drinking water. Then you have manganic iron. So, you have Mn 4+ being reduced to Mn 2+. Selenate, which is selenium(VI) that is being reduced to selenite. Fe(III) being reduced to Fe(II).

DMSO is a compound that is often found in water, especially seawater and so on; and TMAO as well. And then you have arsenate. Arsenate 5 is being reduced to arsenate 3; and fumarate and succinate. So, these are some; I do not want to call them novel electron acceptors, that is the wrong word, but these are very different; they are not the usual electron acceptors. Then we come to the next one.

(Refer Slide Time: 29:08)



Now, petroleum compounds contain benzene, toluene, phenol, xylene and so many, ethylbenzene; all these are compounds that are present in petroleum, the petrol that you buy when you go to the petrol pump. So, here you have compounds like benzene, toluene, phenol, chlorobenzene, nitrotoluene. These are compounds that are known to serve as primary substrates.

So, what I want to show you here is that let us say you have toluene. In the presence of oxygen, one particular enzyme called toluene dioxygenase is capable of converting this very stable aromatic ring to cis-toluene dihydrodiol. So, the first thing I want to say is that it is very difficult to break an aromatic ring. So, any aromatic compound is much more difficult to biodegrade compared to an aliphatic compound. So, when you have aromatic compounds like polychlorinated biphenyls, DDT, naphthalene, phenol, toluene, all these; on a relative scale, they are difficult to biodegrade.

So, the first step itself is quite difficult. So, toluene dioxygenase is doing that. It is converting it to a dihydrodiol, which is easier to biodegrade. That will break the ring and allow biodegradation to happen. Once this enzyme is there in the system, and if you have trichloroethylene, this trichloroethylene in the presence of oxygen and in the presence of this enzyme is going to be converted to TCE epoxide.

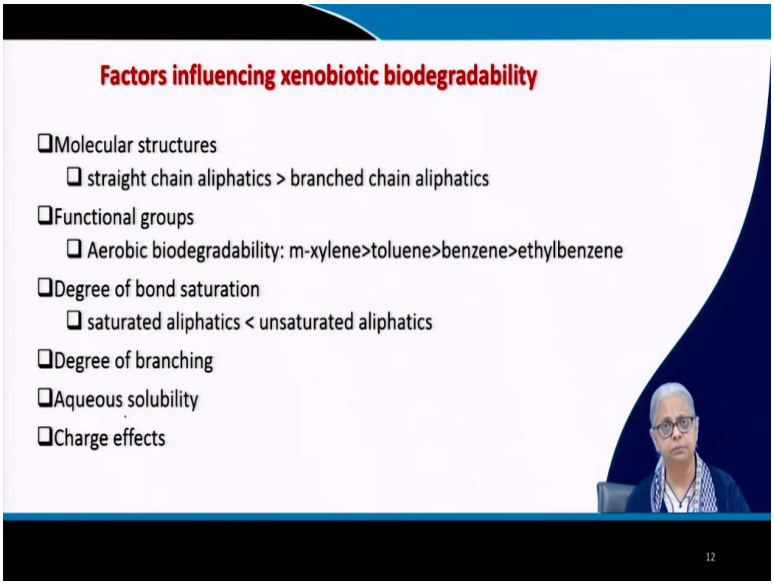
Now, I think there are 2 references here. One says it is C-O-C. This double bond is converted to a single bond, or there is C-O-O-C. So, in either case, this compound I think is easier to biodegrade compared to the first one. So, having gone through this first step, it becomes easier.

Now, here is another very important point. No matter what the SOC is, whether it is of the anthropogenic origin or whether it is in petrol or in any other mixture of industrial chemicals, whatever it is, if you know the chemical formula for a compound, you can use that information to determine the free energy of formation and then come up with half-reactions as shown in that table, table 6.4. You can create those half-reactions on your own, based on your understanding of thermodynamics and what we have gone through. So, once you know your electron acceptor, once you know the free energy of formation of your SOC, you can write the half-reactions, make oxidation-reduction reactions, and then answer the question, is it thermodynamically favorable for this compound to be utilized as a primary substrate by bacteria?

Once the answer is thermodynamically favorable, yes, then it is worth trying experimentally. But if the answer is no, thermodynamically it is not favorable, then it is unlikely that there will be any bacterial species that can survive under those conditions. So, that is just one way

of utilizing this information. So, that was one example that I showed you. The SOC can serve as an electron acceptor and it will be reduced in the process while the primary substrate is oxidized. If the concentration is too low, the microbes will utilize it, maybe as a primary substrate; but it is going to be called a secondary substrate because they are not really utilizing it for creating biomass and energy. So, it will get utilized, but as a secondary substrate.

(Refer Slide Time: 33:24)



What are the factors that influence xenobiotic biodegradability? The first thing is the molecular structure. So, if you have a straight-chain aliphatic compound compared to a branched-chain aliphatic; remember, I showed you examples of starch, cellulose, and glycogen. Starch and cellulose are straight-chain aliphatics and they are much easier to degrade.

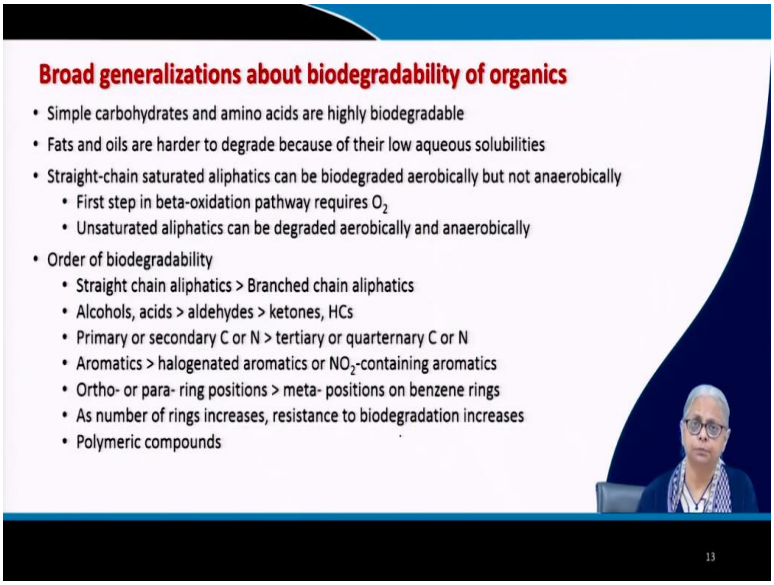
Starch is the easiest; cellulose, slightly more difficult; and the branched-chain compound, even more difficult. Functional groups: under aerobic conditions, you have these ring structures. So, you have benzene, you have toluene, xylene, ethylbenzene. So, it has been known that meta-xylene is more degradable compared to toluene, more degradable compared to benzene; and ethylbenzene is the least degradable.

So, it all depends on the nature of the functional group that is attached to the aromatic ring. I have not mentioned anything about aromatics here. But aromatic compounds, benzene-based compounds, whether they are 1 ring or more than 1 ring-like naphthalene which has 2 rings; aromatic compounds are considered to be more difficult to biodegrade compared to aliphatics. Within aliphatics again, degree of saturation.

At some point in the past, I have also mentioned that saturated aliphatics are more difficult to biodegrade compared to unsaturated aliphatics. I am not going to go into any details; you can go through the literature. And then, saturated aliphatics, in general, are more difficult to degrade compared to unsaturated aliphatics. I have already mentioned the degree of branching. The greater the degree of branching, the more difficult it is to degrade.

Aqueous solubility is a huge factor. If it is a hydrophobic compound, it is very difficult to biodegrade. If it is hydrophilic, it is going to remain in solution; and therefore, it is easier to degrade. And then, charge effects. So, depending on the charge carried by the compound, let us say at pH 7; and if those are the conditions under which it has, let us say a negative charge, the cell is negatively charged, and the outer surface of the cell is generally considered to be negatively charged. So, if you have a negatively charged compound, it may be difficult to degrade it, depending on the other environmental conditions and vice versa. So, that is it.

(Refer Slide Time: 35:58)



Let us come to some more generalizations. Simple carbohydrates and amino acids are highly biodegradable. So we all know that if you add sugar to water and put in some bacteria as well, they will have a great time because these sugars are highly biodegradable. If you want proof, take some fruit sugar, leave it; and it will pick up some microbes from the air and they will start degrading. It becomes a real mess. It will start stinking very easily.

Fats and oils are harder to degrade because of their low aqueous solubility. Straight chain saturated aliphatics can be biodegraded aerobically but not anaerobically. The first step in the beta-oxidation pathway requires oxygen; and the second step, it can be done under both aerobic as well as anaerobic conditions. Unsaturated aliphatics can be degraded both under aerobic as well as anaerobic conditions.

I have already mentioned all of this. A straight chain is easier than branched. Alcohols and acids, easier than aldehydes, easier than ketones and hydrocarbons. Hydrocarbons are what you see in petrol. Primary and secondary carbon and nitrogen are easier to degrade than tertiary or quaternary carbon and nitrogen. Aromatics greater than halogenated aromatics or nitrate-containing aromatics.

Ortho and para ring positions are easier to degrade than meta ring positions on the benzene ring. As the number of rings increases, resistance to biodegradation increases. The simplest example, benzene versus naphthalene. Naphthalene has 2 rings, benzene has 1 ring. Naphthalene is much harder to degrade compared to benzene. And then, polymeric compounds.

Thank you for your attention, and we will continue with this particular topic in the next lecture. Thank you.