Applied Environmental Microbiology
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Lecture - 38
Built Microbiology

Dear students welcome to this lecture of environment applied environmental microbiology where we will be focusing on built environment. The built environment in its of consists of everything that has been built and thus is very any civil infrastructure that has been built and thus it is very diverse in its scope and it was different kinds of microbiological processes that happen in built environments.

For example, the perhaps they do not get that you are sitting in they do not that we eat and in the room in which I am recording all of them include built environment. And because of the different distinct characteristics and local environment will have very different microbiology. Built environment also includes things such as wastewater treatment water treatment because we have basically built them; it also includes the pipes and the buildings that we create. And since all of them are included in built environment we will first start with advance treatment and wastewater treatment. And then move on to what happens in our concrete pipes, in our pipes and I have already talked about corrosion in waste in water distribution system.

So, that is also typical example of microbiology in the built environment; so, let us get on with the built microbiology. Now in waste water treatment plant a typical primary treatment process will remove 50 percent of BOD will get rid of lot of COD will get rid of lot of suspended solids and the big material. And the secondary treatment will get rid of most of the BOD, COD and nutrients.

However, at times the end product of secondary treatment which is bio chemical degradation of waste in wastewater is not sufficient for us to meet the standards. And also at times we might know become aware of contaminants that our waste water treatment plant was actually built for. In this case we need to add a tertiary treatment step or advanced waste water treatment steps. For example, nitrogen and phosphorous are typical nutrients that we want to remove and are not effectively removed in the secondary treatment processes and for to remove them we often need to add advanced wastewater treatment steps.

Now, nitrogen and phosphorous if you recollect a very important because they contribute to (Refer Time: 02:44) growth and (Refer Time: 02:46) blooms in our lakes and result the nitrification accelerated nitrification of the lakes. So, secondary treatment is often not sufficient for achieving desired standards; now this is your typical secondary treatment process.

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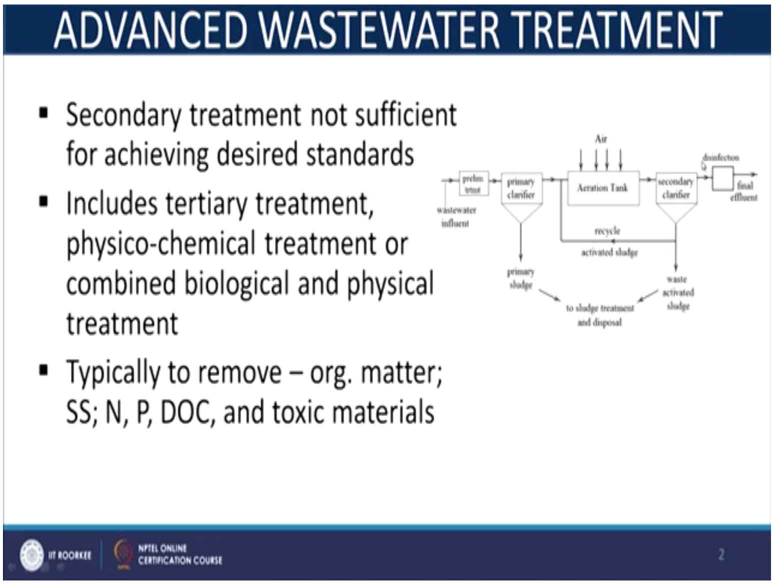

So, therefore, we include the advance wastewater treatment which includes tertiary treatment, physico chemical treatment or combined biological and physical treatment. For example, if I want to remove phosphorous I have two approaches I can either have physical removal or purely biological or I can combined them. Thus all of them would still be my advanced wastewater treatment process. Typically they are used to remove organic matter that is still not removed from over secondary treatment process suspended solids, nitrogen, phosphorous, dissolved organic carbon and toxic materials.

Interestingly the toxic materials might include and often do include emerging contaminants. So, the thing in emerging contaminant is that they are persistently released to the environment and because they always continually being released in the environment they are (Refer Time: 03:51) does not really make a difference how long they persist in the environment does not make a difference because there always a continuous inflects of emerging contaminants.

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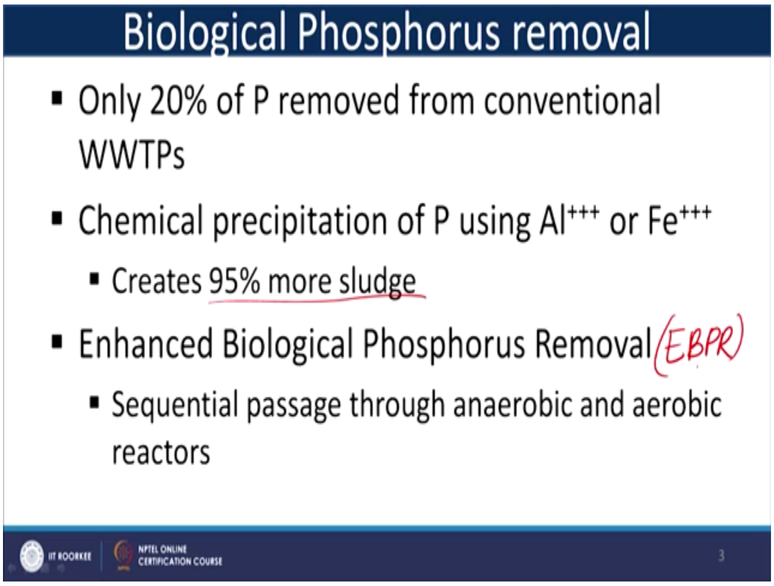

Now, here is a thing only 20 percent of phosphorous that comes into the in front wastewater treatment plant is actually removed from primary and secondary treatment. So, we still have plenty of phosphorous that we need to get rid of before disposing our treated wastewater into environment.

Now, if I typically the chemical processes involve coagulation flocculation using aluminium or iron of an iron is used. And iron precipitate of phosphates and other phosphorous based compounds results and removal of phosphorous; however, there is a major limitation of this which is that chemical precipitation of phosphorous makes up to 95 percent more sludge than simple secondary treatment would. So, this and sludge processing, sludge removal, sludge management is quite an expensive affair for any wastewater treatment plant.

So, definitely we do not want to double the volume of sludge; if there were an easier way to get rid of the phosphorous without getting more sludge it would be very preferable and there to our rescue comes enhanced biological phosphorous removal. So, here we have EBPR Enhanced Biological Phosphorous Removal.

Now the weight works is that we have sequential passage of our water that has been treated initially through a fast anaerobic chamber and then trough an anoxic or aerobic chamber.

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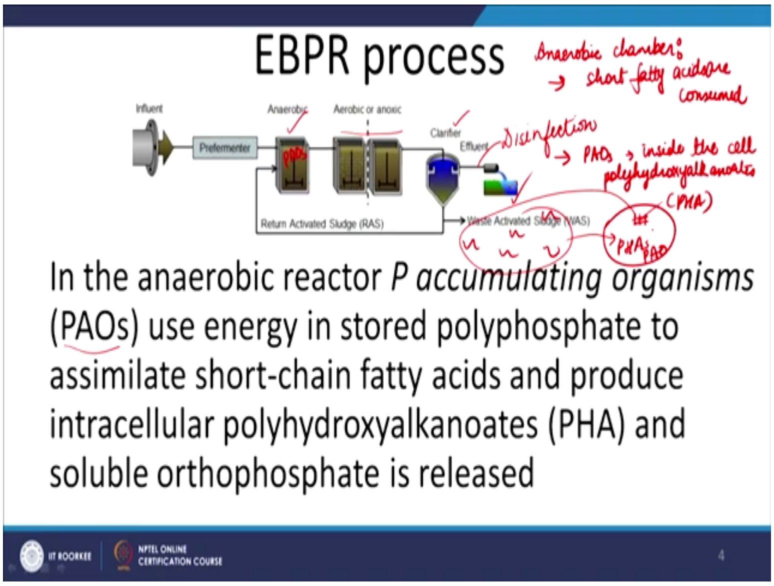

Now, this is what your typical EBPR process would blow you have first passing it through an aerobic chamber and then through an aerobic or anoxic chamber. And then you have a clarifier which is secondary settlement tank and then a part of it is returned activated sludge and a part of the it is wasted and then there is affluent that you send for disinfection. So, there is a disinfection step here and then you dispose it in the environment.

Now in the anaerobic reactor what happens is that the phosphorous is accumulated by phosphorous accumulating or organisms. So, they are short in PAO; so, there are lot of PAOs present here what they do is they use the energy that is present in poly phosphate in the water and they assimilate short chain fatty acids. So, in the anaerobic chamber; short chain fatty acids are being consumed. So, their energy is stored in this short chain fatty acid is being consumed and the next step is the microbes which are PAOs, they store the phosphorous in inside themselves.

So, this is intra cellular; so, inside the cell they store in form of poly hydroxyl alkanets and they often written as PHAs. So, we have first polyphosphate lying outside in wastewater now these PAOs phosphate accumulating microbes they will burn they have lot of short chain fatty acids in them, they will burn the energy and they reduces energy to accumulate them the phosphates out poly phosphates outside extra cellular phosphates and make intra cellular PHAs. So, now, the phosphates it has produced intra cellular PHAs.

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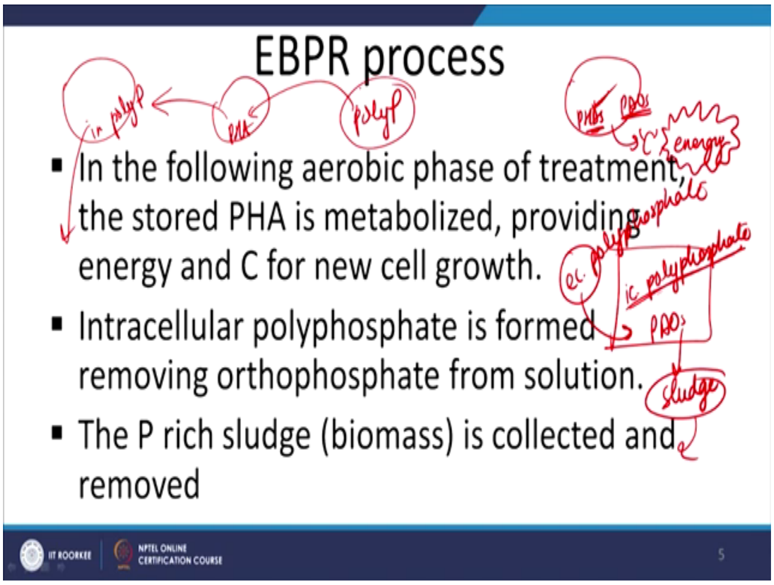

In the next step after anaerobic treatment becomes the aerobic face where now the PHAs that has been stored is burnt. So, now, the microbes are rich in PHAs and these are your PAOs by the way phosphate accumulating organism they are rich in PHA, but they are low in their short chain fatty acid. So, they burnt the PHAs and they give then they burn they release lot of energy. So, they have and carbon; so, there are lot of carbon and they have lot of energy to increase their biomass. Now when this has been done what they do is they convert the available phosphorous, they convert the available phosphorous into poly intra cellular polyphosphate. So, remember initially we get extra cellular polyphosphate; now we have intra cellular polyphosphate and thus the phosphorous that was lying outside the extra cellular polyphosphate is now, inside the microbe; this is your PAO by the way.

I have made the PAO rectangular in this case, but yeah. So, now, what happens is that the biomass that has been produced using this energy and by burning PHAs and converting it into extra intra cellular polyphosphate is if it makes sludge. And this sludge is removed and now you have removed the phosphorous; so, the basic principle is the polyphosphate that is outside the cell.

So, if your cell is circular again and you have polyphosphate outside the cell it is taken in converted into PHAs and then in the aerobic zone; the microbes convert this into intra cellular polyphosphate. And now you can remove the biomass and thus you have remove the phosphate present in the wastewater.

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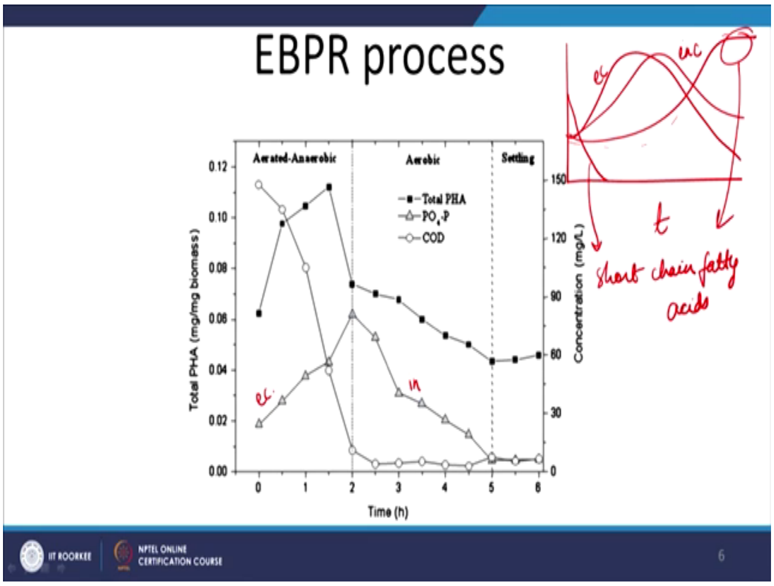

So, a typical EBPR process would look like this the total PHAs would first increase in the anaerobic zone. And then in the aerobic zone as they are getting degraded and intra cellular poly phosphorous phosphate is being accumulated they would decrease and in the settling they would remain constant.

Now, your COD in array in aerated zone will act in the anaerobic zone, it will drop because the short chain fatty acids are being consumed and then it will not rise again. Your polyphosphate in the anaerobic zone would increase and then in the aerobic zone this is your extra cellular; this is your intra cellular because its intra cellular in the water it decreases. Now the cool thing that you need to notice here is that the time how things would change in the typical EBPR process the short chain fatty acid would do a quick decline.

So, this is your short chain fatty acids your extra cellular polyphosphate would be first raised and then it would decline. And then your intra cellular polyphosphate would raise and then decline. So, this is the extra cellular, this is intra cellular and then the intra cellular polyphosphate would raise and a first shallow and then it will raise and then this is what is removed as your biomass alrighty.

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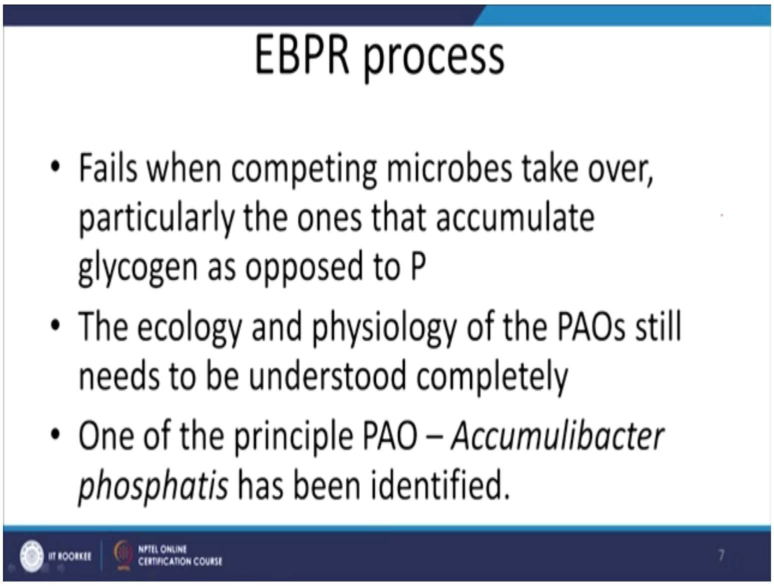

Now, there is there is some limitations with EBPR process one of them is that it fails when it is competing with the other microbes. So, if we have lot of energy released when the PHAs are converted into polyphosphate and then this is your extra cellular polyphosphate and then microbes will assimilate them in the first anaerobic process and then your intra cellular PHAs would increase and then in the aerobic zone they will decline too.

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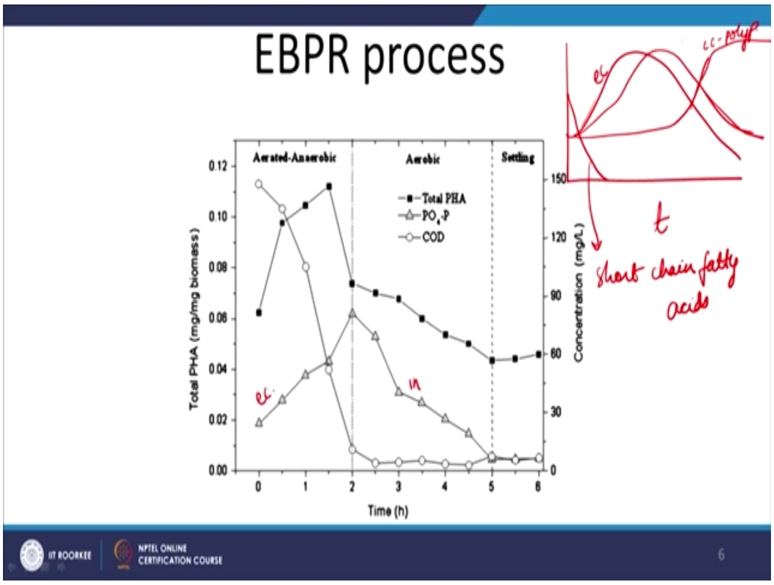

And what will happen is that your intra cellular polyphosphate; once the PHAs start degrading; they would increase and the phosphate that you had as polyphosphate extra cellularly would be stored here as intra cellular poly phosphate ok.

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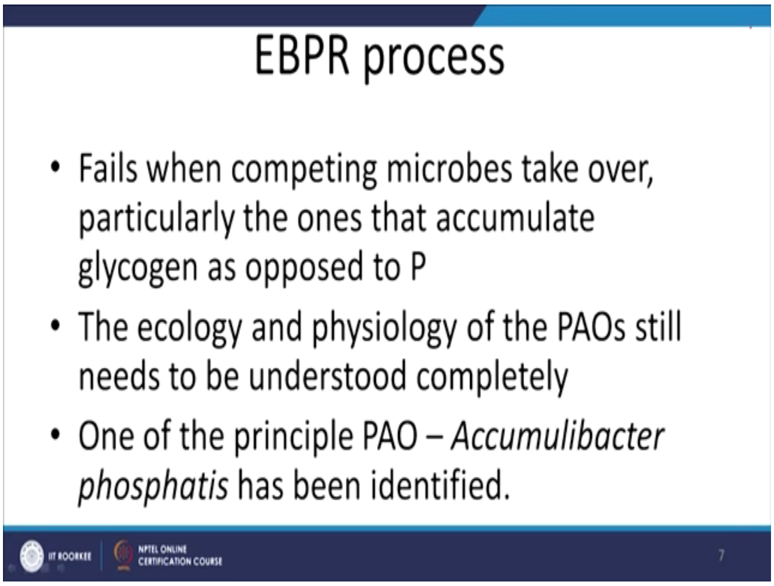

There is a major limitation with EBPR process which is that it fails when the competing microbes take over. So, in this case when the PHAs are degrading lot of energies being produced out of carbon is being released. So, biomass is increasing and if instead of our poly PAOs which are phosphate accumulating organisms.

Other microbes increase then they will be very easily out competed; they are not very quick to thrive in diverse competing microbial communities. And there are particularly out competed microbes that store glycogen; so, the carbon and energy they have released will be used by this is glycogens storing microbes. And the EBPR key microbes PAOs will be out competed ok.

Important thing about this PAOs is phosphate accumulating organisms is that their ecology and their microbial community structure their functions have not been completely understood, they are still matter of great research, but; however, we have identified one of the principle PAO which is accumuly battered phosphates.

So, very relevantly named and we have also managed to culture one of the PAO, but then we still need to identify. And hopefully the fourth generation, the third generation sequencing techniques would throw lot of light on PAOs alrighty.

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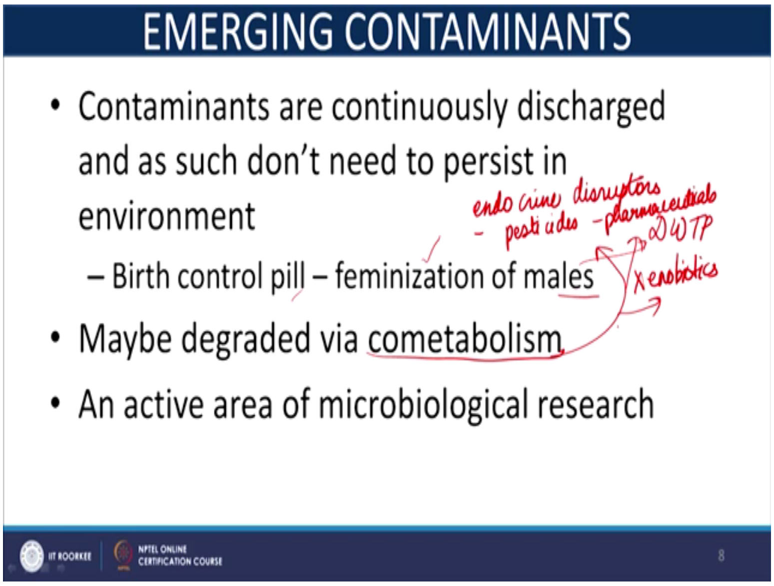

The next thing that is very important in built environment is emerging contaminants. So, the emerging contaminants they are released in our environment and in their then microbes in the environment are expose to them. And then, they transform it, affect it or degrade it or just stay a loaf to the emerging contaminant.

Now as such the emerging contaminants they are continuously I have mentioned this earlier in the lecture, they are continuously discharged and do not need to persist environment to have an effect. For example, the birth control pill that is taken by women worldwide now this pill when excreted through urine and released into wastewater treatment plant it is it is not degraded in wastewater treatment plant.

And when it reaches are water systems it results in feminization of frogs and other aquatic lives the male ones. And there is the jury is still out that how it when this water is now taken up by drinking water treatment plant in the next city downstream; how it will affect the people the presence of these indo crane disruptors will affect the health of people who drink this water.

Now, in wastewater treatment plant how do we get rid of this emerging contaminants we do not want that; we do not want these endo crane disruptors which might include pesticides or pharmaceuticals we do not want him to last into wastewater treatment plant. So, it is very important that we want to get rid of them in wastewater treatment plant.

Many of these are xenobiotics some are not or many of them happen to be re calls it trend they are not easy degraded. And even if they can be degraded easily microbes do not really get an energy trick kick or lot of energy take profit out of them to amount to degrade them readily. So, what we often notice is cometabolism; a microbes trying to degrade another compound it gives lot of energy and the same ensigns because they are extra cellular functioning extra cellularly, they also happened to degrade these endo crane disreceptors emerging emerging an emerging contaminants.

And this is an active area of current microbiological research; so, again for students who are very interested in research this is very interesting and I hope you look into it all right. The next thing when we talk about built environment it is very important is how do microbes or microbial activity destroy the built environment that we have made. Now as human beings we first focus on how they hurt us and then we focus on how they help us.

A good example would be pathogens were understood very before pro biotic approach to treatment was understood which is still being understood right now atleast scientifically. So, microbial we are going to talk about microbially influenced or induced corrosion of metals concrete and other surfaces.

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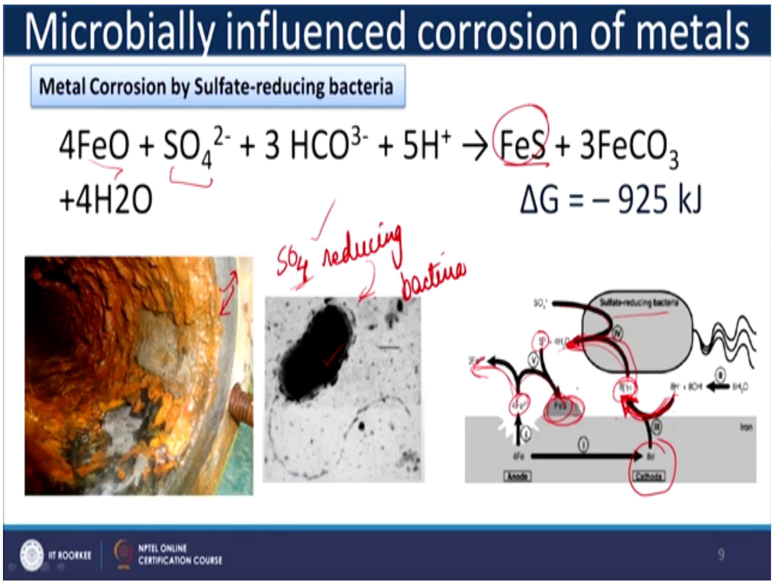

So, here you have a heavily corroded pipe and this is a sulphate reducing bacteria.

Now, if the water is carrying if the pipe is carrying water such as marine salty water or its carrying sewage; then it will be probably be very rich in sulphate and then the sulphate reducing bacteria would thrive they will use whatever organics are present in water and they start reducing sulphate. Now here is a thing the sulphate that is reduced when sulphate gets reduced the F e O also gets reduced to ferric sulphide.

So, this reaction results in destruction of iron pipes and causes serious corrosion now recently it has been found that certain sulphate reducing microbes and they do not need to go through a long process to reduce the iron pipes and corrode them. But they can actually directly the sulphate reducing microbes they can directly receive electron from iron or give electron from the metal.

Now here is another picture where we noticing the galvanic corrosion couple with sulphate reduction by bacteria. So, here we have a sulphate reducing bacteria must like this one and it will reducing sulphate into sulphide the sulphide is forming iron sulphide and also there is galvanic corrosion going on.

So, we have a node and cathode and then the F e 2 plus is converting into reduced FeS and releasing a some of them is just escaping into the water, some of them is getting reduced and here we have a corrosion of the metal iron. And because of this corrosion the weight works is that we have hydrogen present and this hydrogen then encourages the hydrogen is reduce nascent hydrogen which encourages the reduction of sulphate by sulphate reducing bacteria.

So, all the three cycles here the cathode in galvanic corrosion the cathode and reduction of hydrogen iron; the consumption of hydrogen iron to reduce the sulphate I am quicker. And the using the N product of sulphate reduction for corroding the iron all these three things are happening together and in goods energy with each other.

Thus sulphate reduction of metal happens really quickly and this is the major this has been a major challenge for many decades for communities that built of scientific community that and the industrial community that is working on undersea pipes, undersea pillars undersea posts ok. The next thing is which is very important is bio deteration of stone and concrete. So, microbes do not only survive in sediment in air hardly any nutrient very little atleast to human experience very little master survive on.

But they also survive in the rocks in the stones and concrete and this is a picture of biodeterioration of different concrete structures. So, you can notice there is some black thing you can call it oh it is almost it is all algae, but it is usually a very divorce microbial community.

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And here we have algae and mass kind of microbial community doing this is concrete degrading. And look here is it not just concrete degradation not just colonisation microbe, but the quality of your structure also degrades that is we notice that concrete degrades. So, this is actually degrading the con concrete and stone and not just colonising it.

Now, the microbes that live inside the rocks, inside the stones and inside the concrete they are called as endolithic community. Now this is a very classical picture there is used to give an example of endolithic community that detoriate stone here we have a very beautiful picture of a statue of an angel. And now after microbial degradation it looks under colonisable nearly lot of it has degraded.

Now these microbes will live in the small crevasses and (Refer Time: 20:00) inside the in the stone and concrete structure and thus they are called as endolithic com microbial communities now indo is inside the ethic is rock. So, the ones that lives inside the rock now they because a living inside of the rock and there is not much nutrition available in the rock they are called this is then considered to be oligotrophic environment for them.

This is considered to be two considered to be an oligotrophic environment and they are they are colonisation of these structures depends on many factors such as sulphate roughness, the amount of pores that are available to it the attachment sulphates that is available and the mineral composition of the stone or concrete. Now often what happens is that again sulphate reduction and oxidation also at times and that destroys the line in the concrete and thus destroys the concrete structure.

Another thing is in case of fungal colonisation of stone and concrete; the fungus will produce oxalic acid which being acidic in nature does the same effect that acid rain would do; it destroys the surface of stone and concrete structures. Now here is list or different kinds of destruction that we can accept when it comes to biodeterioration of rocks.

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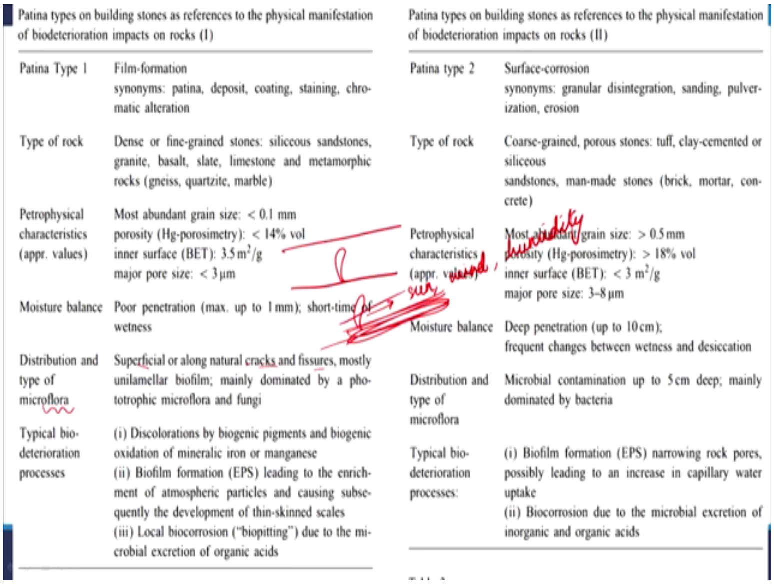

Patina types on building stones as references to the physical manifestation of biodeterioration impacts on rocks (I)

Patina Type 1	Film-formation synonyms: patina, deposit, coating, staining, chromatic alteration
Type of rock	Dense or fine-grained stones: siliceous sandstones, granite, basalt, slate, limestone and metamorphic rocks (gneiss, quartzite, marble)
Petrophysical characteristics (appr. values)	Most abundant grain size: < 0.1 mm porosity (Hg-porosimetry): < 14% vol inner surface (BET): $3.5\ m^2/g$ major pore size: < 3 μm
Moisture balance	Poor penetration (max. up to 1 mm); short-time of wetness
Distribution and type of microflora	Superficial or along natural cracks and fissures, mostly unilamellar biofilm; mainly dominated by a phototrophic microflora and fungi
Typical bio-deterioration processes	(i) Discolorations by biogenic pigments and biogenic oxidation of mineralic iron or manganese (ii) Biofilm formation (EPS) leading to the enrichment of atmospheric particles and causing subsequently the development of thin-skinned scales (iii) Local biocorrosion ("biopitting") due to the microbial excretion of organic acids

Patina types on building stones as references to the physical manifestation of biodeterioration impacts on rocks (II)

Patina type 2	Surface-corrosion synonyms: granular disintegration, sanding, pulverization, erosion
Type of rock	Coarse-grained, porous stones: tuff, clay-cemented or siliceous sandstones, man-made stones (brick, mortar, concrete)
Petrophysical characteristics (appr. values)	Most abundant grain size: > 0.5 mm porosity (Hg-porosimetry): > 18% vol inner surface (BET): $< 3\ m^2/g$ major pore size: 3–8 μm
Moisture balance	Deep penetration (up to 10 cm); frequent changes between wetness and desiccation
Distribution and type of microflora	Microbial contamination up to 5 cm deep; mainly dominated by bacteria
Typical bio-deterioration processes:	(i) Biofilm formation (EPS) narrowing rock pores, possibly leading to an increase in capillary water uptake (ii) Biocorrosion due to the microbial excretion of inorganic and organic acids

So, we can either have film formation or we can have the and this film formation can happen on dense of fine, grain stones, silicatia sand stones different kinds of stones. And then there is some information about the kind of microbial community and the kind of mineral there is. So, the kind of microbe flora they are using a very nice term flora because not all living members in this community which cause biodeterioration of stone and concrete are microbial.

So, then actually a visible like mass there usually superficial or inside the cracks they do not they are not found inside of nowhere in the concrete so, inside. So, on the surface inside the cracks and fissures and mostly uni lamellar bio films. So, they are not like water distribution system where if this is your pipe. So, you have bio films going like this it will not be going like this instead it would be very uni lamellar. So, they would why lumina lumina uni lamellar because now they are exposed to different extreme condition such as sun, wind, different changes in the humidity, desiccation or rain.

So, because of that any structure like this it wants to grow will be removed due to weather ok. Now type of biodeterioration process would include discoloration bio film formation and local bio corrosion. We have talked about bio film formation in water treatment water distribution system; we have talked about corrosion briefly.

And now, discoloration is that the biogenic pigments they will oxidise iron or manganese and thus would change the colour. The other kind of corrosion that happens is surface corrosion and this happens to course this is not fine grain will undergo film formation course grain will undergo surface corrosion. In case of surface corrosion often we notice that there is a deep penetration of the of the moisture; moisture goes deep and thus the microbial community also goes deep upto 5 centimetre and this is mostly bacteria.

So, the bio film is formed this is extra polymer extra cellular polymer exception that is released and this actually allows microbes when they found just bio EPS bio films or in the rock pores, they actually increase the capillary water obtained they release in they result in bio corrosion by production organic and inorganic acid due to microbial activities.

So, dear students, now when it comes to built environment microbiology and how microbes destroy; very relevant to India is destruction of concrete severe pipes and actually elsewhere in the world through because of biological activity.

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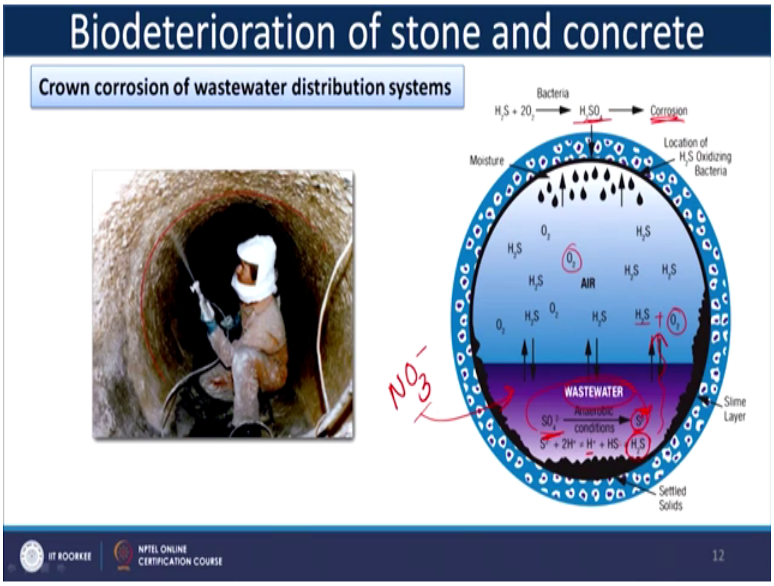

Now, remember the wastewater that is carried in our severe pipes is very very rich in nutrients, very rich in carbon and bio degradable organics. So, now, this wastewater undergoes very quick degradation organics and thus, the condition get anaerobic very quickly. In fact when wastewater is collected very soon you can see it turning into black because of production of sulphite the sulphite is very quickly reduced to sulphite.

And this changes the colour of wastewater to black and this is anaerobic condition and when this sulphite interacts with water it produces it might produce H 2 S gas and then this H 2 S gas is released up into the portion of the severe pipe that has air. So, because your pipes are rarely completely filled there is no pressurised collection system for sever. So, the air component when H 2 is released now there is oxygen present in the air unlike what was in the liquid portion.

And now, in the oxygen component what happens is the H 2 S gets oxidised when H 2 S gets oxidised what does it form? It forms H 2 S O4 which is highly acidic gas; it is very very acidic it has creates very low PH. And that very quickly corrodes the surface of your pipes and thus the surface of your concrete sever pipes undergoes corrosion very quickly. And in fact, across the world many many concrete sever pipes have collapsed and completely work in down because of this biodeterioration based on.

For sulphate reduction in the water and then its re oxidation ones it goes into the air and formation of sulphuric acid. To prevent that one of the things that people do is that they coat the sever concrete sever pipes with special compounds to protect them for the corrosive effect of the very strong acid sulphuric acid. The other thing that our marine businesses do is that they add nitrate; so, they add the smart microbiologist they will add nitrate.

Now, we know that nitrate is a better electron accept to than sulphate. So, instead of reducing the sulphate microbes would like to reduce nitrate and provided there is sufficient nitrate; they might not even ever get to reducing sulphate into sulphide until the wastewater reaches the wastewater treatment plant. And this way we can prolong the longevity of our concrete carry concrete sever carrying wastewater pipelines. Dear students this is all for today; in the next lecture we will go to other built environments and then move on to public health.

Thank you very much.