Applied Environmental Microbiology
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Lecture – 47
Solid Waste Microbiology II

Dear students, in the previous lecture I gave you a brief introduction of landfills, what they are used for, what are the typical problems in the country like India and definitely I shared about a very sad incident, the Ghaziabad landfill slide. So, today what we are going to do is, we are going to talk about the leach ate composition, what are the kind of microbes, what are their processes and how do they degrade the leach ate, because it is a big trouble and then, hopefully we will get to a portion where I, will be talking about at least three different kinds of research that have happened on solid waste management focusing on landfills. So, let us get started. So, in the last class I talked about the leach ate and I mentioned that leach ate is the liquid that collects in a landfill site over time.

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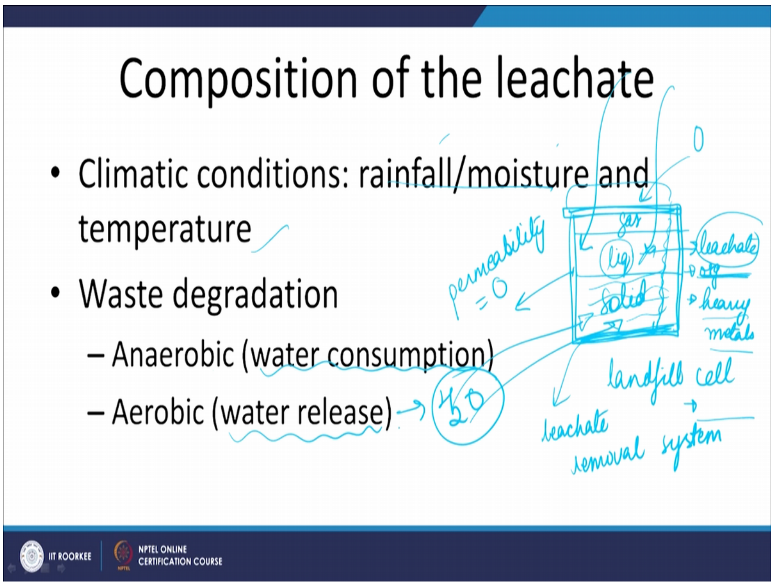

Now, if we have a landfill is such as this one, let us say this is a typical landfill itself. Now, in this landfill cell, over time, will have a certain solid portion. Now obviously, the ratio of solid - to liquid - to gas depends upon the conceived composition of the waste that was put in and also what along the climate. So, then will have a liquid portion and then we will have a gaseous portion.

Now, the liquid portion is what is referred to as leach ate. Typically, leach ate consists of all depending on its age, it depends on the organic materials in there, which are very hard to degrade. So, it might have heavy metals in it, depending upon the kind of mechanism happening in the landfill, its health and its age and it might have other compounds in it; most of them are not good for its health. Whether it is a heavy very complex organic material, whether it is a heavy metal, or it is the other compounds. Leach ate degradation is still a hot topic of research. So, let us get started about leach ate.

Now, composition of leach ate depends on the climatic condition, on the rain moisture and temperature. So for example, after we have sealed the landfill, ideally, the liner here is completely impermeable, which means that its permeability of liner is 0 and when we seal the landfill from the top, we add other liners and we typically add mud over it. Now, its permeability is also 0. So, ideally this is a complete and perfect batch reactor, completely sealed; however, there is always some drain that percolates into it overtime or maybe if there is some flooding and then the groundwater can also percolate in from imperfect liner and when this happens, the liquid content increases.

So, this rainfall that percolates in or the ground water that percolates from side depending on changes in the groundwater flow, then, this liquid acts at like a washout liquid and it percolates from top to bottom, dissolving whatever can be dissolved - heavy metals, organics, humic acids, whatever can be dissolved, it dissolves and then it pools, here at the bottom at different heights as leach ate.

Now, this leach ate builds up over time; obviously, as every season, the rainfall is new and the as a bio - degradation of the waste also continues, we have more water that is generated and thus more leach ate is generated, thus most landfills will have a proper leach ate removal system. Typical leach ate removal system would include pipes from which the leach ate is pumped out regularly.

So, basically the formation of leach ate and the composition of leach ate depends upon the rainfall, the moisture, the temperature, because the temperature governs the multiple activity, also the degradation of ways whether it is anaerobic or aerobic, if it is aerobic then the water is consumed, and if it is aerobic, then water is released. Obviously, if it is aerobic then oxygen will take electrons from hydrogen.

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Now, once the leach ate has been pumped out from the land, then these are the landfills and leach ate has been pumped out, they typically end up in a leach ate collection system; and this is a picture, not India, but this is your leach ate collection point, then this leach ate is taken for further treatment. Further treatment would include evaporation of the water content. So, we want to reduce the volume of the leach ate. It might include different kinds of purification process, to get rid of heavy metals, to get rid of other contaminants and then, eventually either we might evaporate the leach ate or we might incinerate remaining components.

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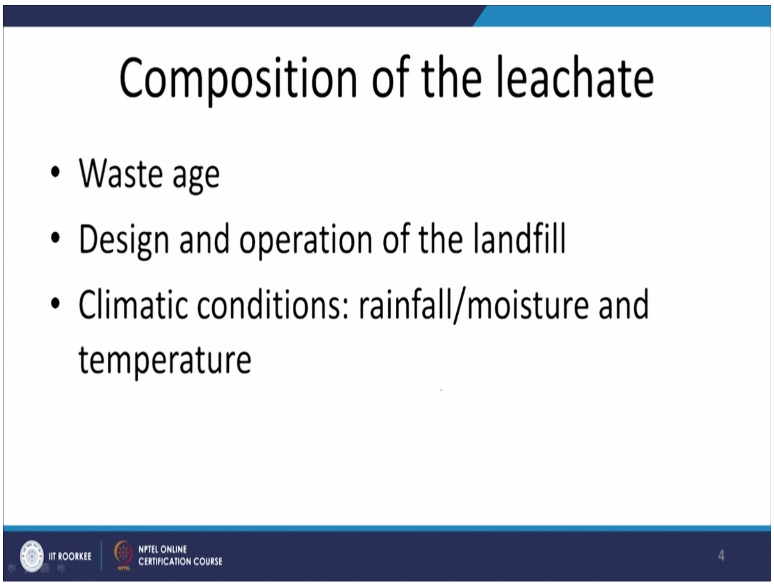

I have mentioned this that the composition of wastes depends upon the temperature, the climate and the rainfall and the kind of degradation. So, quickly I hope you gather, that the degradation happens over time and since degradation happens over time, at different time, we in the previous lecture about 5 phases of degradation of waste.

Now, these 5 phases progress over time depending on the age of the waste, the kind of leach ate produced would change. For example, when we have the aerobic; the first phase of degradation and we will have more water produced, and thus, leach ate will be low in concentration of recalcitrant compounds and they will be plenty of water, but over time leach ate will get more and more concentrated. Then it also depends on design and operation of landfill.

Depending on the design of landfill, the amount of waste it receives and it is filled into it, the way it is operated will also determine the concentration and the composition of leach ate and then climatic conditions that we had all talked about.

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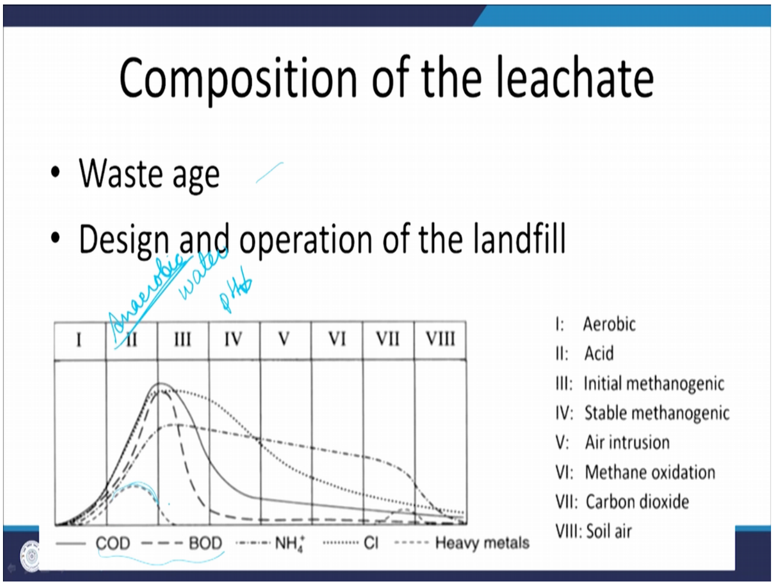

So as you know, treating of waste age undergoes different kinds of degradation. We will notice that different kinds of contaminants are found in the leach ate. In the first phase, where we have aerobic, we will have a little of COD, BOD, ammonia, chloride and heavy metals, but as it enters the acidic phase, where the pH drops, we will see that we have lot of heavy metals.

So, the heavy metals will start increasing. This is only the time when heavy metals peak and we will also see that the COD and the BOD will increase, because the way, the waste has been created to a level, that has been packed to a enough sufficient level that is D butyric reasoning, we will also see that the ammonia would increase, the chloride and the salts would increase.

So now, because this is an anaerobic process, one of the major reasons, why everything is increasing in concentration is, because the amount of water that was present in leach ate, in the landfill earlier, is now being consumed to carry out the anaerobic acidic degradation, and because the pH falls in this acidic degradation, we will notice that the high - heavy metals get dissolved at a higher level. Now, when it enters the third phase, which is the initial methanogenic phase, this is where the BOD undergoes a rapid drop. So, this is where the BOD remains minimal, also this are where the COD starts dropping.

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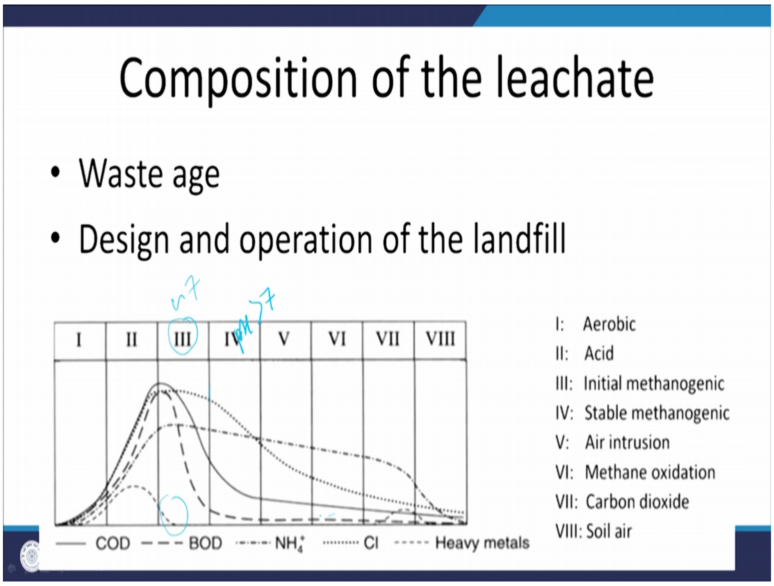

So, this is where the real BOD, COD of leach ate start dropping. Another thing to notice is that the salinity undergoes a slight drop and even ammonia undergoes a stride drop. The metals have reduced because now, the pH is coming back to nearly 7. Now here, on the stable methanogenic phase, on the pH might actually go above 7. So definitely, we are not expecting heavy metals. Also interestingly, the BOD has reduced completely and the COD has reduced considerably.

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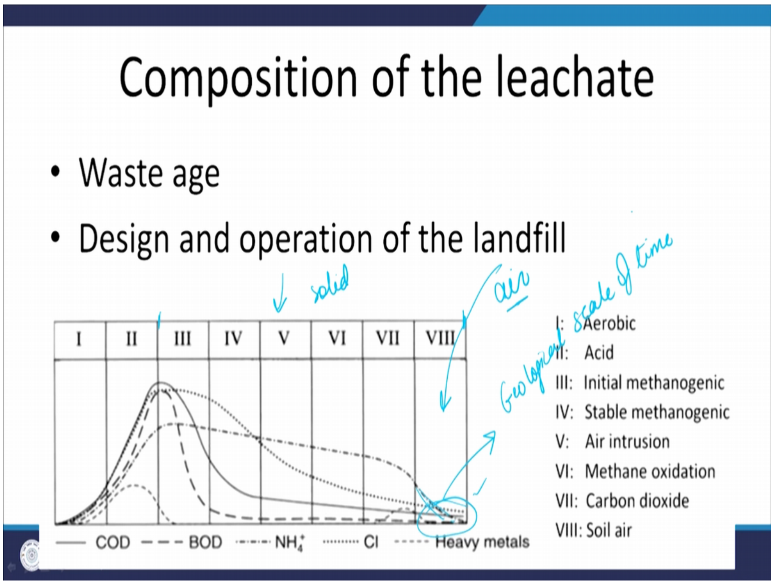

It is in these phases, from the third phase to the fourth phase that we see considerable decline in the solid content. Now, similarly in the fourth, fifth, sixth and seventh phase, there will be a continual decrease. And in the eighth phase, when there is re - infiltration of air, then, we notice that finally, our ammonia gets oxidized and we have perfectly stable landfill. Now, why is there re -infiltration of air? This is very important to oxidize ammonia and other reduced form of compounds that are products of all the other degradation and are awaiting the degradation. Now, reaching this stable phase might take again as mentioned earlier, geological scale of time and we might not see it in our lifetime, but this is what we expect.

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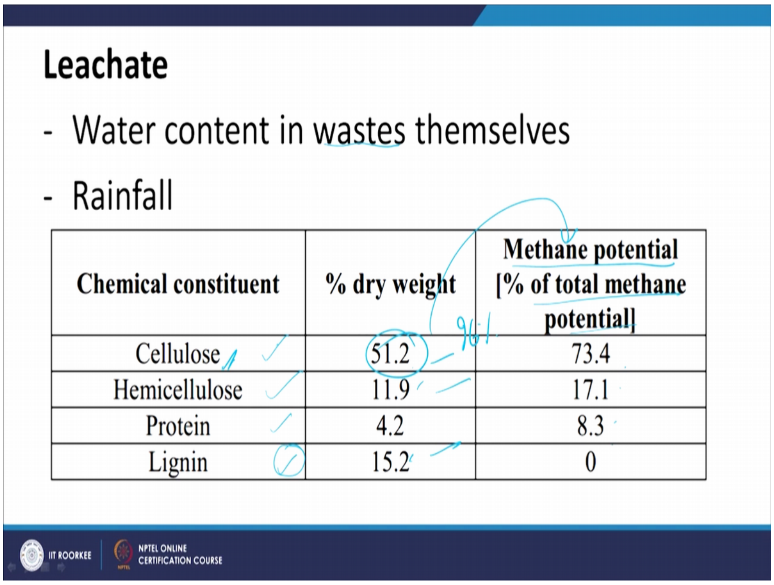

So, what does leach ate constitute of? Definitely there is water, because it is liquid, there is definitely water and the water comes from two sources. One is the waste itself and the other is rainfall. If we remove the water that is dehydrated, then we are left with some cellulose, hemicelluloses, protein and lignin. Please note that cellulose, hemicelluloses and lignin are very recalcitrant. Lignin particularly, is not amenable for degradation, as it takes forever long to degrade.

Most of it is cellulose, which undergoes anaerobic cellulose degradation and we will talk briefly about it, and then there is hemicelluloses, which differs from cellulose; in its structure and morphology. Now, each of these dry components should eventually be converted into methane. So, let us look at the total methane potential, its highest in cellulose followed by hemi cellulose, protein and lignin.

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	Microbial respiration pathways					
	Aerobic (electron acceptor: O2)		Fermentation (no alternative electron acceptor available)		Anaerobic Respiration (electron acceptor: CO_2)	
Oxygen conditions	aerobic		anaerobic		anoxic	
	ATP	Net Energy [kJ/mol glucose]	ATP	Energy [kJ/mol glucose]	ATP	Energy [kJ/mol]
Glycolysis	2	61.2	2-4	200-300	--	--
Citric Acid cycle	2	61.2	--	--	--	--
Respiratory chain	28	856.8	--	--	--	--
Methanogenesis	--	--	--	--	<1	131 kJ/mol CO_2 27.5 kJ/mol CH_3COOH
Total	32	979.20	2-4	200-300	<1	131 kJ/mol CO2 27.5 kJ/mol CH^3COOH

Now, what are the different kinds of degradation pathways that microbes can take when they are degrading waste in the landfill, they can undergo the aerobic degradation making undergo fermentation, they can undergo anaerobic respiration. We have quite talked about these processes in the earlier lecture. So, if you are not clear about aerobic degradation, fermentation or anaerobic respiration, please go ahead and review some of the early lectures.

In aerobic degradation, obviously, oxygen is your electron acceptor and we have a very high generation of energy, depending on the cycle. It can undergo glycolysis, it can undergo citric acid cycle respiratory chain and there is no scope of methanogenesis in fermentation, or there is no other electron acceptor available. In methanogenesis, we have carbon dioxide that serves as electron acceptor.

But in fermentation, we have no electron acceptor available. So, everything is getting reduced. We have no scope of citric acid cycle and respiratory cycle or methanogenesis and we have fermentation happening and the total energy output, from air compared to aerobic falls to nearly less than one third. Then in anaerobic respiration, carbon dioxide services as electron acceptor, it gets converted into methane and thus, this process is also called as methanogenesis, and we have methanogenesis undergoing and depending upon how much CO2 and acetate is present.

We will generate some energy.

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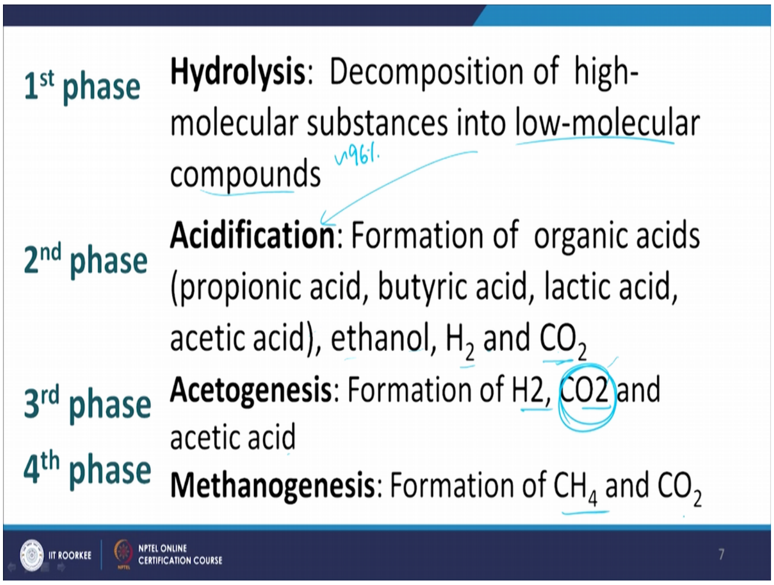

We have briefly talked about this before, but let us go through this again. The landfill undergoes 4 distinct phases and then there is a fifth phase of stabilization of microbial processes. So, the first microbial process is hydrolysis. So, if you remember, nearly 51 percent of waste is lignin, in leach ate is cellulose. 11 percent is hemicellulose and lignin is 15.2 percent. All these 3 are very long complex polymers.

So, nearly 96 percent of the dry weight of leach is complex polymer. So, in the first phase of microbial degradation of leach ate waste, what we have is the high molecular substances the polymers, really complex polymers which are nearly 96 percent of the dry weight are degraded into low molecular compound. For example, the cellulose consists of monomers of cellobiose.

So, these cellobiose units are degraded into cellobiose units, which can be very quickly degraded. Now, in second phase we have acidification. So now, the daughter products are low molecular compounds that undergo acidification and in acidification, organic acids are formed like :- a prop ionic acid, butyric acid, lactic acid, acetic acid along with some ethanol hydrogen and carbon dioxide. So, in this phase we generate acid from these new molecular compounds.

First, we break the long chains and then we acidified them. Microbes convert them into oxidized acids. Some carbon dioxide and hydrogen will also be produced. In the third phase, we have acid to genesis. This is where acetic acid is being produced. We will give you a brief example very soon. So, we have hydrogen carbon dioxide and acetic acid. In the fourth phase, we have as methanogenesis. So, all these are oxidized forms of carbon. Now, they serve as electron acceptor. Eventually, we are left with methane and carbon dioxide.

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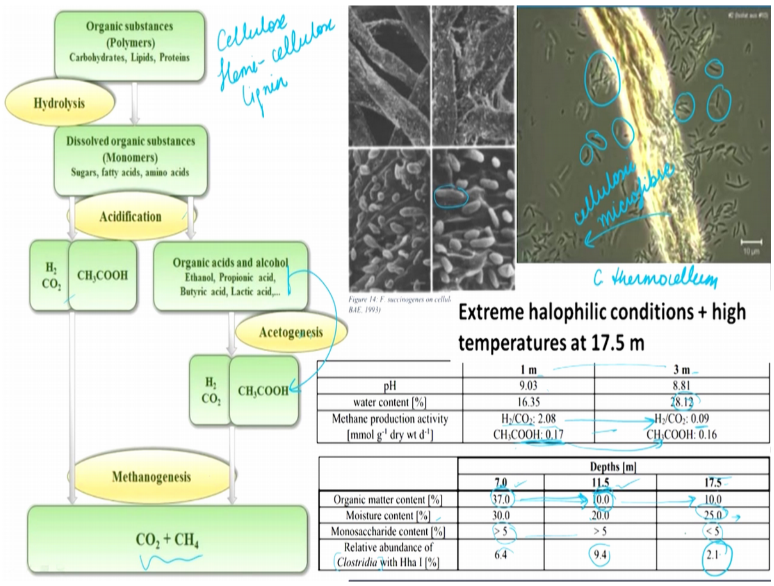

So, this is your typical flowchart for degradation of leach ate. Your organic substances such as carbohydrates, lipids and protein and in case of leach ate you will have cellulose. We have hemi - cellulose and lignin which together form nearly 90 percent of leach ate driveway. They undergo hydrolysis; they make smaller compounds, sugars, fatty acids, amino acids.

Then the acidification, there they form acetate, hydrogen and carbon dioxide or they will form organic acids. The organic acids undergo aceto genesis. So, all these organic acids are converted into acetic acid. Now, this acetic acid undergoes methanogenesis and forms carbon dioxide and methane. This is degradation of the dry weight of your leach ate.

Now, let us look at how microbes do the cellulose degradation. Here, is a very nice picture of clostridium thermocellun. So here, what you are seeing is actually a cellulosic microfiber and these tiny things here, are your bacteria. So, the bacteria are eating. These bacteria have clustered around the cellulosic microfiber and they are degrading it. Now, this is another picture of another microbe of succino genes, which are these small fibers and microbes. They are sticking to cellulosic fibers and they are degrading it.

So, we have a very good evidence showing how microbiologically cellulose is degraded and remember, 51 percent of leach ate to dry weight is cellulose. Now, this is very important to know that cellulose in aerobic under aerobic condition is very recalcitrant. So, unless it is oxidized through fire, cellulose will not be oxidized, it is very recalcitrant, but then over under anaerobic conditions, it undergoes hydrolysis followed by acidification, followed by cytogenesis - methanogenesis and is degraded.

Now, this is from a very cool study that was done recently in Europe, and what they did was they looked at pH water contained methane production at 1 meter depth, 3 meter depth, 7 meter depth, 11.5 meter depth and 17.5 meter depth of a landfill. So, they noticed that as they went below from 1 meter to 3 meter the pH drop, the water content increased because now, the water is going from top to bottom and the methane production activity sort of decreases. Well, in methane production activity, we can say that the hydrogen to carbon dioxide ratio decreases from 2 to less than 1, much less than 1 and the number of the concentration of acetate decreases slightly.

Now, this is same study, different table. We have at deeper depths 7 meter, 11.5 five meter, 17.5 meter; we notice that the organic matter content drops from 7 to 11.5 meter. So here, we have a lot of organic matter that can degrade, but the one at 11.5 meter is more aged. So with age, the waste has been degraded and the BOD the COD have dropped and then after a time they remain constant.

So, if you go back to your diagram here, after a while the BOD and the COD become constant. So now, organic matter has become constant, the moisture content dropped. So, initial would have increased and dropped and then it is slightly increased because now, it is collected at the bottom moisture content at any time depends upon the weather and the flow of water around the flow.

Monosaccharide content initial at the nearly top, 7 meter is not top, but these are newer ways than this one and this one. So, monosaccharide content in the first two is higher and then here, by the time it is really old, it has degraded. The clostridium now is one of the microbes that was first identified to degrade cellulose, of numbers of clostridium were identified to degrade cellulose in a single culture. So, this is your clostridium thermocellum. So, they notice that clostridia increases with age from 7 to 11.5 meter and then, when all the organic come substances have been hydrolyzed and much what is left is just methanogenesis of the acetate that has been formed, then they drop.

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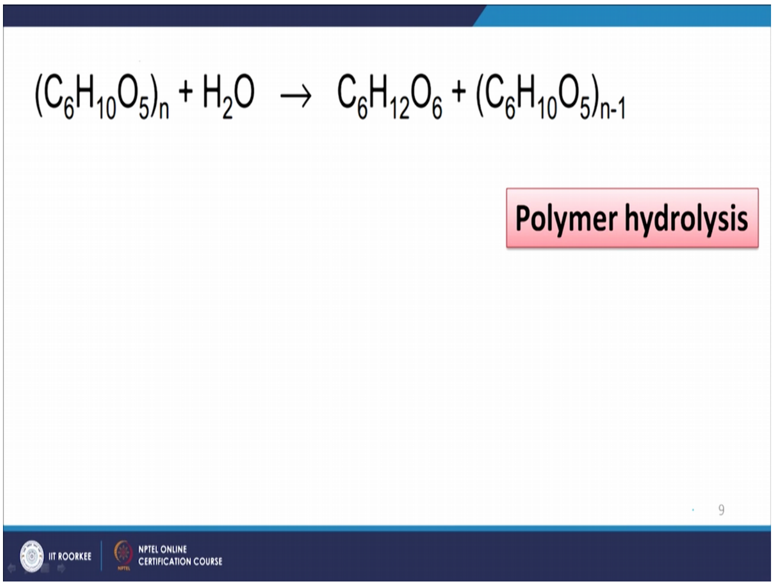

So, we can briefly summarize our degradation of leach ate and waste in the landfill, as the complex polymers who undergo hydrolysis and they are broken into smaller sugars. The sugars are then fermented into as acids.

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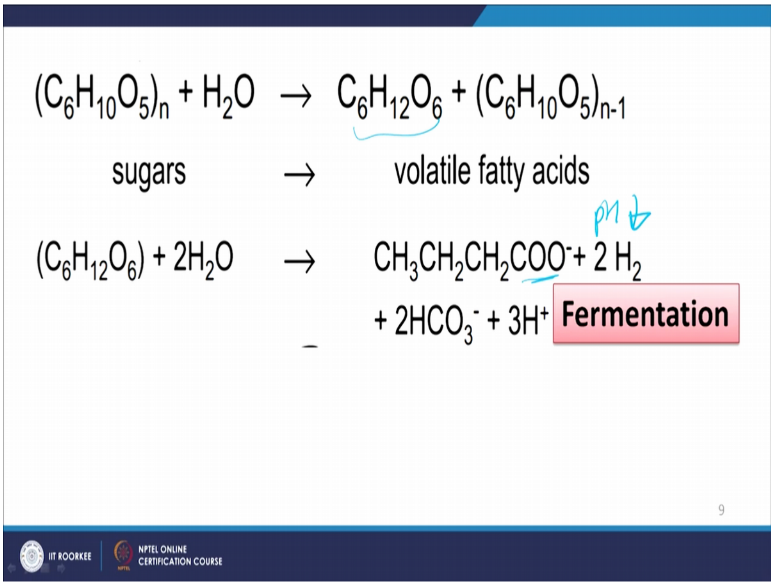

So, these are the acidic the pH drops here.

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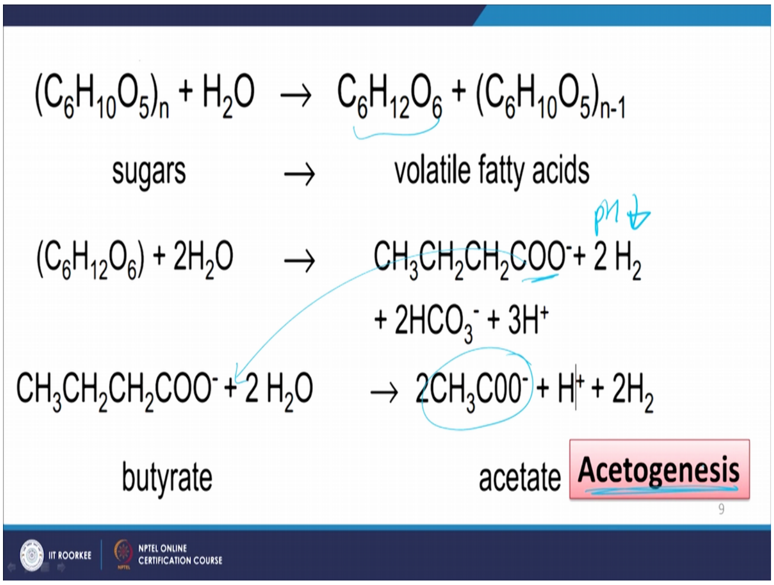

And the next step is when; these acids are converted into acetate. So, this is cytogenesis phase which is a very important phase because then after this, acetate is converted into methane gas.

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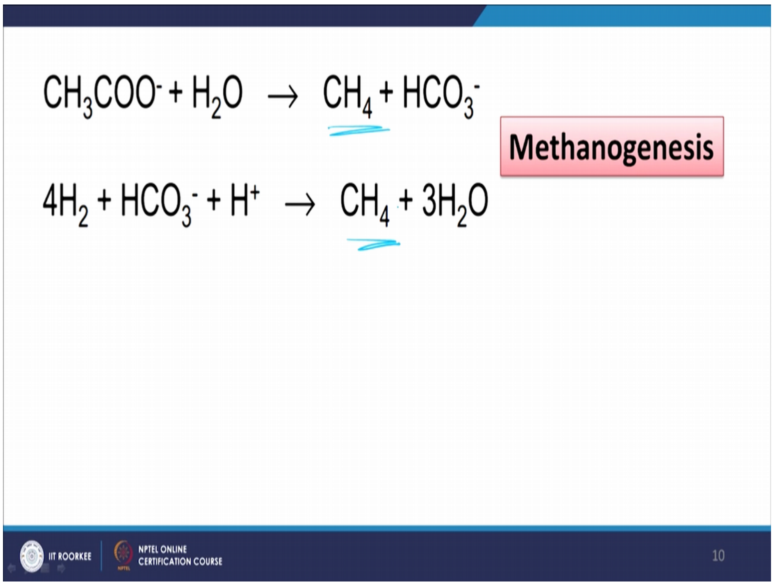

So, remember, in the Bangalore landfill, there was a lot of BOD and thus, there was a very good concentration of methane that was produced; and this is sad because methane is very potent greenhouse gas, and we definitely do not want our people inhaling lot of it. Also, it is highly inflammable. So, there are high chances of accidents and fires. Now, let us look at microbes that were responsible for degradation. With different temperatures, we notice that different kinds of communities are formed. So, as the waste age, the temperatures change and as the temperature in the region changes, the temperature is changed.

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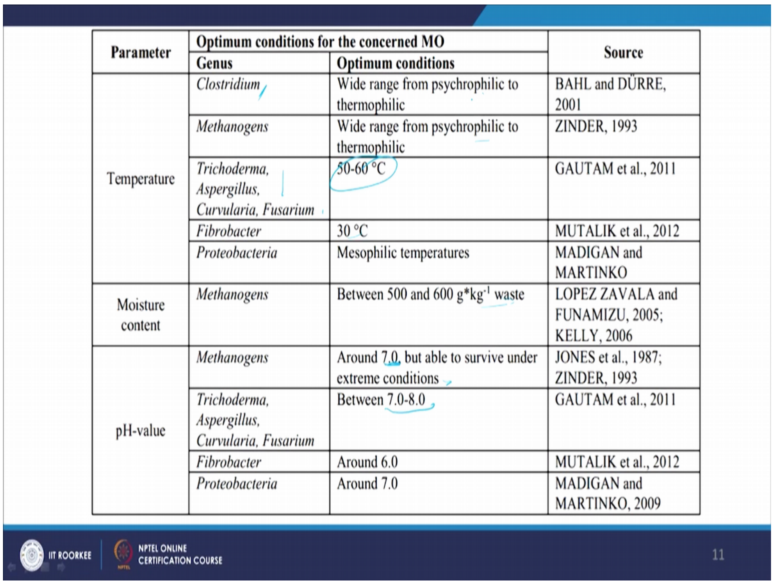

Parameter	Optimum conditions for the concerned MO		Source
	Genus	Optimum conditions	
Temperature	*Clostridium*	Wide range from psychrophilic to thermophilic	BAHL and DÜRRE, 2001
	Methanogens	Wide range from psychrophilic to thermophilic	ZINDER, 1993
	Trichoderma, Aspergillus, Curvularia, Fusarium	50-60 °C	GAUTAM et al., 2011
	Fibrobacter	30 °C	MUTALIK et al., 2012
	Proteobacteria	Mesophilic temperatures	MADIGAN and MARTINKO
Moisture content	*Methanogens*	Between 500 and 600 $g*kg^{-1}$ waste	LOPEZ ZAVALA and FUNAMIZU, 2005; KELLY, 2006
pH-value	*Methanogens*	Around 7.0, but able to survive under extreme conditions	JONES et al., 1987; ZINDER, 1993
	Trichoderma, Aspergillus, Curvularia, Fusarium	Between 7.0-8.0	GAUTAM et al., 2011
	Fibrobacter	Around 6.0	MUTALIK et al., 2012
	Proteobacteria	Around 7.0	MADIGAN and MARTINKO, 2009

So, clostridium, the cellulose degrading microbes are not all cellulose degrading, but the clostridium genus can be found in psychrophilic to thermophilic. So, from really cold temperatures to really hot temperatures, the methanogenesis and methanogenesis are also very diverse, and from very cool to very hot temperatures they exist. Then there are trichoderma, aspergillus, curvularia and fusarium that like thermophilic conditions - really hot fibro bactor, like 30 degree centigrade, Proteobacteria like mesophilic temperatures. Now, the moisture content methanogenesis, when there is 500 to 600 gram of moisture per kilogram of waste, depending on pH value, methanogenesis prefer that the pH should be around 7.

So, when there is an aerobic acidic degradation, methanogenesis will not flourish. They will actually wait for pH to stabilize around 7, before they flourish, they might survive under extreme conditions. Now, again trichoderma, aspergillus and curvularia and fusarium; they like slightly alkaline conditions. So, you can imagine that they will come over after methanogenesis has been started and then we have fiber bactor Proteobacteria, which like around 600 around 7.

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Feature		Note	Characteristic	Source
SAMPLE AGE	Old refuse samples	Lower concentration of acetate Lower microbial activity Lower number of cellulolytic bacteria	Domination of the family *Bacillaceae*	POURCHER et al., 2001 KRISHNAMURTHI and CHAKRABARTI, 2013 HUANG et al., 2003
			Domination of the genus *Bacillus*	HE et al., 2014
			Identification of extremophiles (alkaliphiles and halophiles)	
			High abundance of *Bacillus*-like DNA sequences	HUANG et al., 2005
			Proteobacteria are favoured	HUANG et al., 2005
			Favourable conditions for *Methanosaeta*	KRISHNAMURTHI and CHAKRABARTI, 2013
			Favourable conditions for hydrogenotrophic and formate-using methanogens	UZ et al., 2003
	Young refuse samples	Higher concentration of acetate Higher microbial activity Higher number of cellulolytic bacteria	Unfavourable conditions for gram- bacteria like *Proteobacteria*	KRISHNAMURTHI and CHAKRABARTI, 2013 POURCHER et al., 2001 HUANG et al., 2003
			Favourable conditions for Methanosarcina	CHEN et al., 2003b
			Favourable conditions for acetoclastic, hydrogenotrophic and formate-using methanogens	UZ et al., 2003
	--	--	Significant structure changes of the methanogenic community happen shortly after waste burial	CHEN et al., 2003b
DEPTH	Deeper waste layers	Lower concentration of acetate	Favourable conditions for *Methanoculleus*	KRISHNAMURTHI and CHAKRABARTI, 2013
	Upper waste layers	Higher concentration of acetate	Favourable conditions for *Methanosarcina*	KRISHNAMURTHI and CHAKRABARTI, 2013
			Higher methane production activity	CHEN et al., 2003a
HIGH pH	Incineration ash layer in landfill	--	Dominated by *Bacillus*	MIZUNO et al., 2008

Now, with sample age, we have old refuse samples and young refuse samples. So, this is your old high water age, higher depth, this is lower water age, lower depth or old this is the old landfill site entrance cell, this is young landfill site landfill cell. In the old you had lower concentration of acetate. Because most of it, has been converted into methane. Your lower microbial activities have already done their job and now, they are not very happy because this way, it did a food raft. You have a low level of cellulitic bacteria because, whatever hydrolysis was required to happen, already happen a long time ago.

In this kind of case, you will notice that you will have lot of bacillaceae, out of bacillus, bacillaceae and you will notice extremophiles. 1 thing I did not mention in the previous slides is that, at 17.5 meter depth the researchers of the study notice extreme hemophilic conditions and very high temperatures. So, this is a very high salinity and a very high temperature.

So, as waste ages or in other words if you are doing vertical filling of trenches, then with depth, we notice that the temperature increases and we have extreme conditions. We have extremely salty, extremely alkaline conditions and then again, lot of bacillus like DNA sequences; these are bacillus like organism. We do not know what they are because they have not been cultured. We notice lot of pretty bacteria; we notice methanosaeta, hydrogen atrophic, hydrogen forming and format using methanogenesis.

So, in the younger few samples on the other hand, because they are younger samples, they will have lot of organics to degrade and a lot of celluloid lytic bacteria to carry on the hydrolysis; and once the hydrolysis has happened, the sugars have been converted into acids and acid into acetate, then that is when we will have a lot of acetate and then overall will have a very high amount of microbial activity. Such conditions are not favorable for Proteobacteria, which like the lower microbial activity conditions of old refuse samples. These conditions are very favorable for methanosarcina.

Because of higher concentration of acetate, they are also favorable for acetociastic, hydrogen atrophic format using methanogenesis. Now, a note that they have placed here, it is pretty nice note, let us look into it. Significant structure changes or the methanogenic community, happen shortly after waste per year. Now, why would this happen? Because, right after burning the waste and we have cut off the supply of oxygen and now the degradation has started and methanogenic community will start shifting.

Now, let us look at depth. Now, here depth is representative of the age of the waste material. So, we can notice that in upper waste layers we will have a condition similar to young refuse sample. In deeper waste, they will have conditions similar to older few samples. So, the deeper layers are the older ones, they have higher waste age. The upper ones are the newer ones, they have lower wastage. So, in the deeper ones where the acetate has been consumed, the microbial activity is low, cellularity bacteria is low methanoculleus would survive.

In upper ways, there where the samples are young, microbial activity is higher, is sort of acetate, methanosarcina would survive. Now, high pH; why would a landfill have high pH? Remember one of the most important phase or one of the very important phases of degradation in landfill is the second phase, where we have a lot of acid producing.

So, pH should decrease, but why does pH increase? pH increase would happen when; we take our solid waste means in the same rate then. So, we have ash from there. Now, when that ash is put into a landfill, that ash might increase the pH, in this case the microbial community will be dominated by bacillus.

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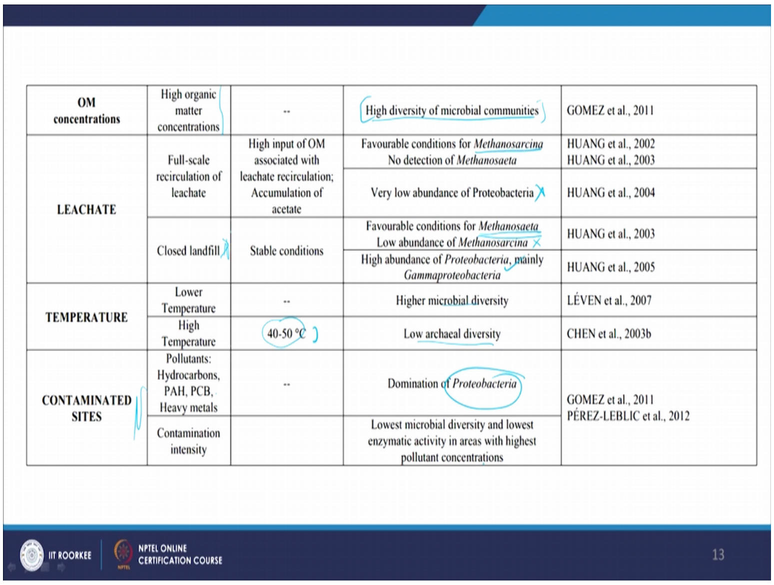

OM concentrations	High organic matter concentrations	--	High diversity of microbial communities	GOMEZ et al., 2011
LEACHATE	Full-scale recirculation of leachate	High input of OM associated with leachate recirculation; Accumulation of acetate	Favourable conditions for *Methanosarcina* No detection of *Methanosaeta*	HUANG et al., 2002 HUANG et al., 2003
			Very low abundance of Proteobacteria	HUANG et al., 2004
	Closed landfill	Stable conditions	Favourable conditions for *Methanosaeta* Low abundance of *Methanosarcina*	HUANG et al., 2003
			High abundance of *Proteobacteria*, mainly *Gammaproteobacteria*	HUANG et al., 2005
TEMPERATURE	Lower Temperature	--	Higher microbial diversity	LÉVEN et al., 2007
	High Temperature	40-50 °C	Low archaeal diversity	CHEN et al., 2003b
CONTAMINATED SITES	Pollutants: Hydrocarbons, PAH, PCB, Heavy metals	--	Domination of *Proteobacteria*	GOMEZ et al., 2011 PÉREZ-LEBLIC et al., 2012
	Contamination intensity		Lowest microbial diversity and lowest enzymatic activity in areas with highest pollutant concentrations	

Now, let us look at the organic matter concentration. When we have high organic matter concentration; obviously, we will have higher diversity of microbial communities. Now, let us look at leach ate. If you are doing for a full scale recirculation of leach ate versus if you have a closed landfill. If you have a closed landfill, the conditions would be relatively stable. These conditions are very nice for methanosaeta, but not for methanosarcina which loves lot of organic material, a lot of acetate. Again, because the condition is stable the Proteobacteria would survive, thrive in full scale recirculation of leach ate, what happens is that with the leach ate recirculation, the organic matter increases.

So, basically we have leach ate that is being collected down, and then it is being re circulated. Every time it comes down, it collects sort of organic matter, and it accumulates sort of acetate, these conditions are very favorable for methanosarcina, which loves high acetate conditions and these are not favorable for Proteobacteria, which likes stable, low microbial activity conditions.

Now, let us look at temperature. At high temperature from 40 to 50 degree Celsius, we will have low alkyl diversity, at relatively lower temperatures compared to this; we will have high microbial diversity. Now, contaminated sites; some of the sites, some of the wastes are very bad and we call them as contaminate sites. In a landfill, if we have for example, an industrial waste landfill or hazardous waste landfill.

And these would be a contaminated landfill. So, if there are hydrocarbons pH's, PCB's or heavy metals or other hazardous waste, proteobacteria will thrive, because over all microbial activity would be low. If depending on the contamination intensity, we will range from lowest microbial diversity and lowest enzymatic activities in areas with highest pollutant concentration and vice versa.

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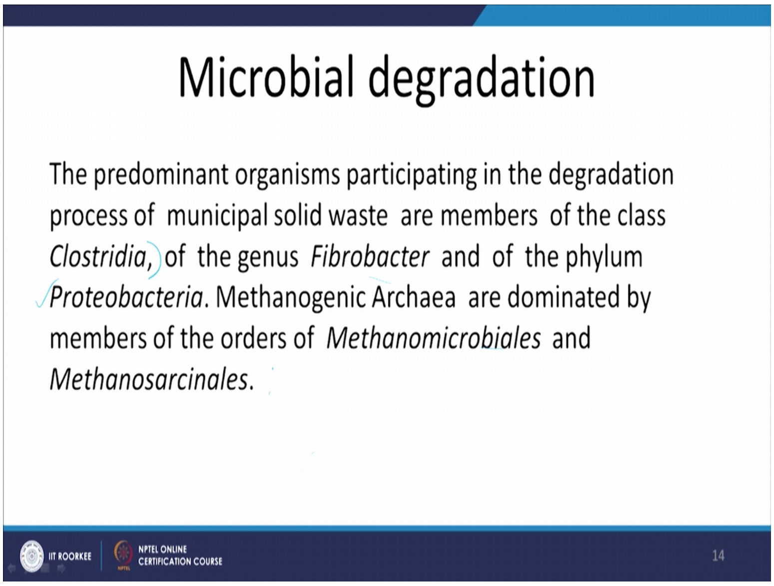

Now, let us look at microbial degradation, which is doing the degradation. Typically, we will notice a lot of clostridia. So, a lot of hydrolysis, we will notice fibrobacter, Proteobacteria, when the microbial activity is low. Methanogenic Achaea would be dominated by methanomicrobiales and methanosarcinales so, which is a lot of methanogenesis happening. In the next lecture, I will take over another and will talk to you about three studies on the microbiology of landfill sites and what are the insights that people have recently generated from the microbiology of landfill degradation. That is all for today,

Thank you very much.