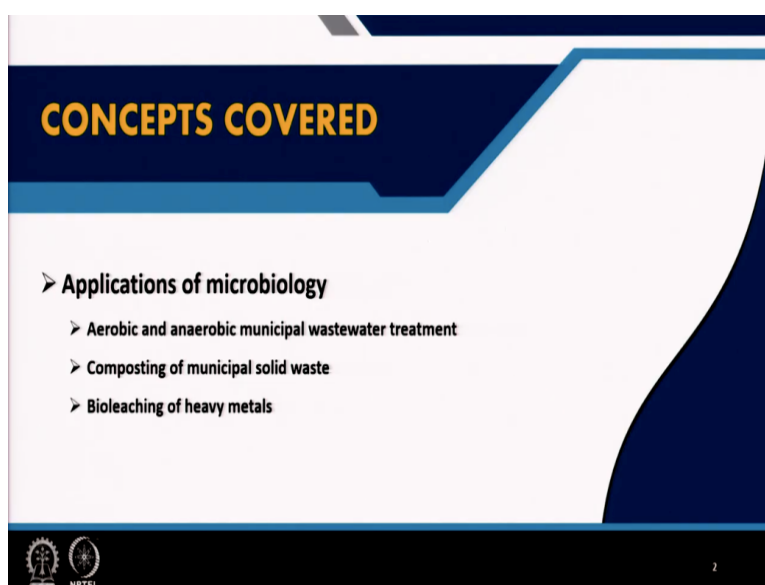


Environmental Chemistry and Microbiology
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Module - 12
Lecture - 61
Metabolic Diversity - VI

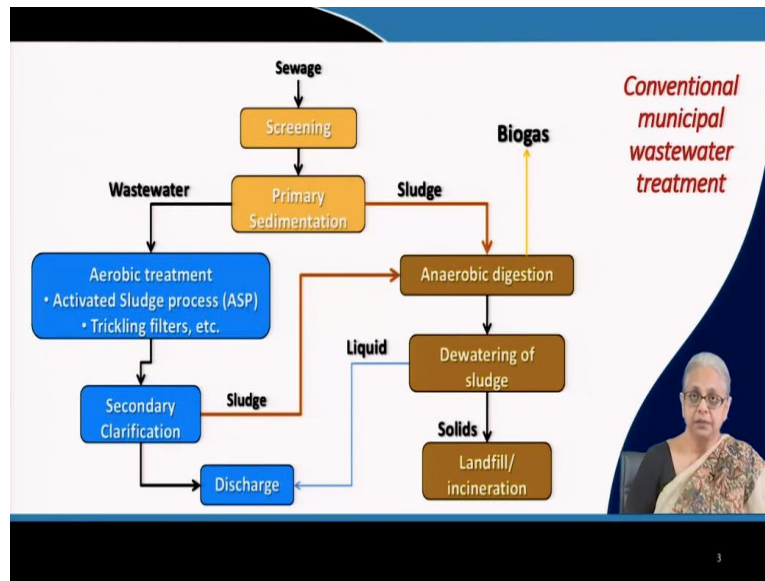
Welcome everyone. This is lecture number 61 of module 12. We are going to look at the final part of Metabolic Diversity.

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So, in this lecture, we are going to go through some more applications of microbiology in Civil and Environmental Engineering. We will start with aerobic and anaerobic municipal wastewater treatment, this is one of the biggest areas, and composting of municipal solid waste or composting of the organic fractions of municipal solid waste is also becoming more and more popular. Finally, we will end with the bioleaching of heavy metals from ores as well as electronic waste.

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So, in case you are not aware of conventional municipal wastewater treatment, this is a very simple schematic or a flowchart that explains the different processes that the wastewater is put through, prior to being discharged. So, here we have sewage. When it comes in, the first thing that we do is screen out all the large materials. So, you have natural materials like twigs and branches, and you have a lot of manmade materials. So, you have plastic bags, you have containers, you have anything that is basically thrown into the sewage will come at this point. Now, these screens are basically of 2 kinds. You can have parallel bars or you can have wire mesh. Depending on the nature of the treatment plant, you will have different types of screens.

After the large useless material has been removed, it will then be taken to a primary sedimentation tank. So, here in the primary sedimentation tank, which may be with or without a grit chamber; it depends on the size of the (waste)water treatment plant and the nature of the wastewater to be treated; all of that will determine whether you have a grit chamber or not. In either case, it is based on the principle of primary sedimentation. Now, this primary sedimentation is based on the principle of discrete settling of particles by gravity, and you get a fair amount of materials that are organic in nature, that will settle by gravity alone. So, this is what is achieved in primary sedimentation. So, this sludge will then be taken to the sludge management part of the wastewater treatment plant.

The clear water; it is not completely clear, it is still wastewater; this wastewater will then be taken into the secondary part of the wastewater treatment plant. At this point, we are giving it what is called aerobic treatment. The most common form of aerobic treatment is activated

sludge process and many treatment plants used to use trickling filters, rotating biological contactors. These are attached growth processes. And even though they may be better in terms of their efficiency, they are not preferred because there are many practical problems associated with running these processes. So, the most popular treatment process to this day is the activated sludge process. And I will show you more details about that in the subsequent slides.

Now, this activated sludge process itself has 2 parts. So, the first part is aeration. And after the aeration tank, you have what is called secondary clarification. So, biomass is generated in the activated sludge process and this biomass has to be removed, and that is done by settling under gravity. So, this biomass has a tendency to form aggregates. These aggregates, when they are heavy enough, will settle by gravity.

So, you have secondary clarification, which will result in an enormous amount of sludge. And this sludge will go for anaerobic digestion. So, both streams of sludge from the primary sedimentation tank as well as the secondary clarifier, will both be taken for anaerobic digestion. This wastewater has been; basically, the BOD level in the wastewater; under ideal conditions, it has been brought down to the discharge standards. So, this water after treatment is now suitable for discharge either on land or in receiving water bodies.

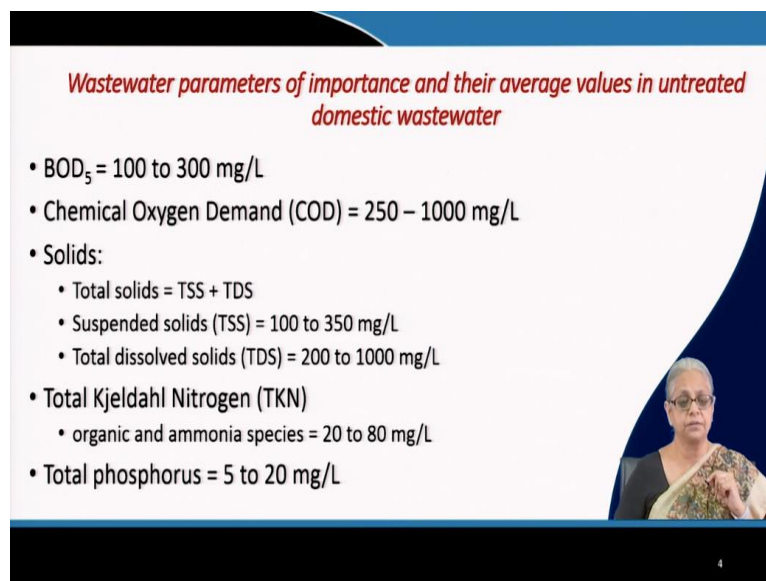
Let us see what happens to the sludge. Now, this sludge, when it goes for anaerobic digestion, one of the major objectives of anaerobic digestion is to reduce the amount of sludge. So, the weight and volume of sludge has to be reduced as much as possible and that is the main objective. In the last few decades, people have been working on making the anaerobic digestion process more and more efficient, so that good amount of biogas is generated; and it is then utilised for either heating of the premises or generating electricity and so many other applications. So, this has become a very big part of the objective of anaerobic digestion. It was not always the case.

The main objective, the primary objective of anaerobic digestion was to reduce the amount of sludge. This sludge, after it has been reduced as far as possible, after biogas has been removed, it can then be dewatered. So, you want to remove the solids and separate them from the liquid material. Now, this liquid material should be good enough to be discharged or reused, either way.

If it is high in nutrients, then it can be reused. Many people prefer to use it for irrigation; and that applies to this stream as well. This liquid; so, like I said, it can be either discharged or reused. And the solids will have to go to a landfill or for incineration. So, many people are incinerating these solids; some of them are dumping them in a landfill and so on. So, these are some of the basic concepts that are behind the entire wastewater treatment plant.

And this is only at the conventional level. Now, there are wastewater treatment plants that have advanced treatment processes. So, we are not going to go there, we are just going to stop at the conventional municipal wastewater treatment level.

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Wastewater parameters of importance and their average values in untreated domestic wastewater

- BOD₅ = 100 to 300 mg/L
- Chemical Oxygen Demand (COD) = 250 – 1000 mg/L
- Solids:
 - Total solids = TSS + TDS
 - Suspended solids (TSS) = 100 to 350 mg/L
 - Total dissolved solids (TDS) = 200 to 1000 mg/L
- Total Kjeldahl Nitrogen (TKN)
 - organic and ammonia species = 20 to 80 mg/L
- Total phosphorus = 5 to 20 mg/L

So, why are we using microbial processes or biological processes for treating wastewater? So, these wastewater parameters that are of importance in untreated domestic sewage or wastewater are basically shown over here in this slide. So, we have BOD₅. Now, remember that is our standard method. So, the BOD values tend to range from 100 to 300 mg/L; COD ranges from 250 to 1000 mg/L.

Within solids we have 2 categories of solids, suspended solids and dissolved solids. So, total solids are equal to TSS and TDS, total suspended solids (TSS) and total dissolved solids (TDS). And this separation of suspended from dissolved is basically based on membrane filtration. Then we have total suspended solids with a range of 100 to 350 mg/L and total dissolved solids which range from 200 to 1000 mg/L.

So, then we come to another parameter and that is called Total Kjeldahl Nitrogen (TKN) and that is a measure of the organic and ammonia species which contain nitrogen. So, organic nitrogen and ammonia nitrogen can be measured using the TKN method and this nitrogen is around 20 to 80 mg/L in sewage. Then, total phosphorus ranges from 5 to 20 mg/L.

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S. No.	Parameter	Inland surface water	Public sewers	Land for irrigation	Marine/coastal areas
2		(a)	(b)	(c)	(d)
1	Colour and odour	See 6 of Annexure-11	See 6 of Annexure-11	See 6 of Annexure-11	See 6 of Annexure-11
2	Suspended solids mg/l. max.	100	600	200	(a) For process waste water (b) For cooling water effluent 10 per cent above total suspended matter of effluent.
3	Particle size of suspended solids	shall pass 850 micron IS Sieve	-	-	(a) Floatable solids, solids max. 3 mm (b) Settleable solids, max. 850 microns
4	pH value	5.5 to 9.0	5.5 to 9.0	5.5 to 9.0	5.5 to 9.0
5	Temperature	Shall not exceed 5°C above the receiving water temperature			Shall not exceed 5°C above the receiving water temperature
6	Oil and grease, mg/l. max.	10	20	10	20
7	Total residual chlorine, mg/l. max.	1	-	-	1
8	Ammonical nitrogen (as N) mg/l. max.	50	50	-	50
9	Total Kjeldahl nitrogen (as N) mg/l. max.	100	-	-	100
10	Free ammonia (as NH ₃) mg/l. max.	5	-	-	5
11	Biochemical oxygen demand (3 days at 27°C), mg/l. max.	30	350	100	100
12	Chemical oxygen demand, mg/l. max.	250	-	-	250

So, what I want to show you here in this slide are the wastewater discharge standards. So, there are 2 main points that I want to make over here, and that is that there are 4 categories of wastewater discharge standards. So, in the first case, we have inland surface water. So, any surface water body that is receiving the treated wastewater; those wastewaters have to abide by these standards. And as I have already mentioned, these are just 12 parameters that are shown over here. The actual CPCB list of parameters is far longer than that and if you ever have to work in this area, you must refer to the original document. There can be situations in which the wastewater is not treated and it is discharged directly into a public sewer.

Then we come to land for irrigation. Now, supposing the treated water is suitable for irrigation, then it can be discharged on land for purposes of irrigation. And finally, we have marine and coastal areas. So, this treated wastewater can be discharged directly into marine and coastal areas. And there are several issues that are related with the discharge of wastewater, even after treatment into marine and coastal areas, because the discharge points should be several metres or even kilometres away from the beach and so on, so that people are not impacted by the discharge of wastewater.

And like I said, there are several issues which I am not going to go into here but that was one major issue that I wanted to highlight, is that these are 4 different categories of standards depending on where the wastewater is to be discharged. That is one point. And then we have ammonical nitrogen; total Kjeldahl nitrogen; free ammonia; biochemical oxygen demand, for 3 days at 27°C; and the chemical oxygen demand. So, all the parameters that I showed you in the previous slide, the discharge standards for each one of them are shown over here.

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Conventional municipal wastewater treatment

- **Primary treatment: removal of objectionable solids (physical processes)**
 - **Screening:** parallel bars with spacing of 2 to 7 cm
 - Wire mesh screens after that with smaller openings
 - Comminuters for grinding of coarse materials are also used
 - Ground material contributes to BOD and COD of WW
 - **Grit chamber**
 - Detention time of a few minutes
 - Long channel to allow heavy particles like sand, and grit to settle
 - **Primary settling**
 - Detention times of 2 to 3 hours
 - Removal of 50 to 65% suspended solids; 25 to 40% of BOD
 - Tanks are either round or rectangular
 - Sludge has to be removed for further processing (see flowchart)
 - Detention time, and overflow rate – key design parameters

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Then we come to primary treatment. Now, primary treatment of wastewater is based on physical processes only. So, I am just giving you a very quick overview of these processes, even though there is no microbiology, no application over here. So, primary treatment is the removal of objectionable solids, it is a physical process. I have already mentioned that parallel bars and wire mesh screens are used.

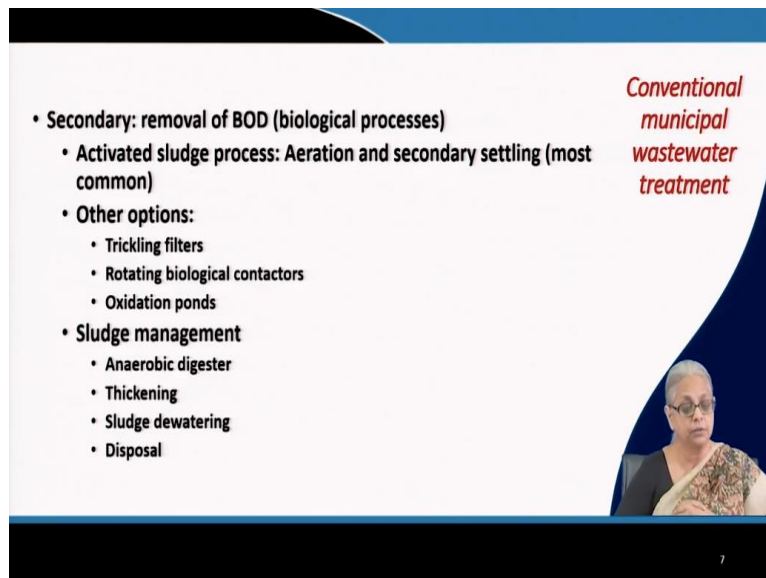
And the wastewater material contains a certain amount of BOD and COD. Now, remember that the biological oxygen demand (organic matter that creates demand for oxygen) is food for the bacteria or the larger group of microbes that may be there. So, comminution, which is the grinding of the coarse materials is used. And if you have a fair amount of vegetation, in the form of twigs and branches and leaves and all of this, that will contribute to both COD and BOD of the wastewater.

So, this material, because it is biodegradable to some extent, can encourage the growth of microorganisms. We also have a grit chamber. This grit chamber has a detention time of a few minutes. It is a long channel that will allow discrete (large and heavier than water), which means heavy particles like sand and grit to settle by gravity. And then we come to primary

settling. Here, the retention time is 2 to 3 hours. You get 50 to 65% removal of suspended solids and 25 to 40% removal of the BOD. These tanks can be either round or rectangular. The sludge has to be processed. I have already shown you the flowchart. So, you know that the sludge coming out of this tank will be taken for anaerobic digestion. So, it may have less BOD compared to the biomass coming out of the activated sludge process, but nevertheless, it is still taken to the anaerobic digester.

And the key design parameters are detention time, overflow rate and so on. We are not going to go through any of the design principles because of lack of time. This is just to illustrate to you how different microorganisms, groups of microorganisms can be utilized and are being utilized in wastewater treatment.

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Conventional municipal wastewater treatment

- **Secondary: removal of BOD (biological processes)**
 - **Activated sludge process: Aeration and secondary settling (most common)**
 - **Other options:**
 - Tricking filters
 - Rotating biological contactors
 - Oxidation ponds
 - **Sludge management**
 - Anaerobic digester
 - Thickening
 - Sludge dewatering
 - Disposal

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Then we come to secondary wastewater treatment. Secondary wastewater treatment has a single objective and that is the removal of BOD. So, whatever BOD is present in the initial untreated water has to be removed as much as possible in these biological processes. So, this is the biggest application of microbiology for civil and environmental engineers. And these days, we have chemical engineers and we have biotechnologists and so many other disciplines that are involved in these works. So, it is truly a multidisciplinary area but I would say that it all begins with civil engineering. So, it used to be called sanitary engineering, and that was because of all these issues.

Then we come to the activated sludge process which is a 2-part process. So, it is aeration plus secondary settling. And like I said, this is the most popular treatment process that is used in wastewater treatment. Within a few minutes, I will come to more details about the activated sludge process. There are several other treatment options for secondary treatment of wastewater. The other more popular option is trickling filters. They are kind of being phased out, I do not see them anymore, but 30 years ago to even more than that, they were around. And they are, like I said, being phased out.

Then we come to rotating biological contactors. They have been built in many places in Europe, in the U.S. and so on, but they have also not been very successful. There are several problems, logistical problems and practical problems in running these kinds of reactors. So, even though they are technically feasible methods, there are too many practical issues that make them unpopular.


And finally, we come to oxidation ponds.

I have already shown you the entire flow chart for a conventional wastewater treatment plant and I have shown you that sludge is one of the biggest streams within the wastewater treatment plant. So, I have already shown you that the sludge from the ASP goes to the anaerobic digester. In fact, the sludge from both the clarifiers, the primary settling tank as well as the secondary clarifier, both of them end up in the anaerobic digester. There are 2 outputs from the anaerobic digester, one is biogas and the other is a reduced volume of sludge. This sludge has to be further thickened and dewatered. And then, the solids are disposed of, either by incineration or by landfilling.

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- **Tertiary: removal of nutrients like N and P**
 - Objective is to prevent eutrophication
 - Biological or chemical processes can be used

Conventional municipal wastewater treatment



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You may have to go for tertiary treatment for the removal of nutrients like nitrogen and phosphorus. The objective here in this case is to prevent eutrophication. And you know from your environmental science, you probably know that eutrophication happens because of excessive algal growth when nutrients like nitrogen and phosphorus are in excess. So, we have biological or chemical processes that can be used at the tertiary treatment level. As it is beyond the scope of what we are doing over here, so I will not be talking about that but that is something for you to keep in mind.

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Suspended growth, mixed flow reactor for BOD removal

- **Activated sludge process (ASP)**
 - Aeration tank to maximize DO levels in water and for biological growth
 - Detention time = 6 to 8 hours of mixing
 - Settling tank or clarifier for solids (biological particles) to settle
 - Recycling of solids from clarifier to aeration tank to ensure a constant bacterial population
 - Remaining solids are taken for sludge handling and disposal
- **Most popular for domestic wastewater treatment**
- **Advantages**
 - Dilution of wastewater; can withstand toxic shock, or shock loads and flow fluctuations
- **Disadvantages**
 - Requires large land area
 - High energy requirements for aeration

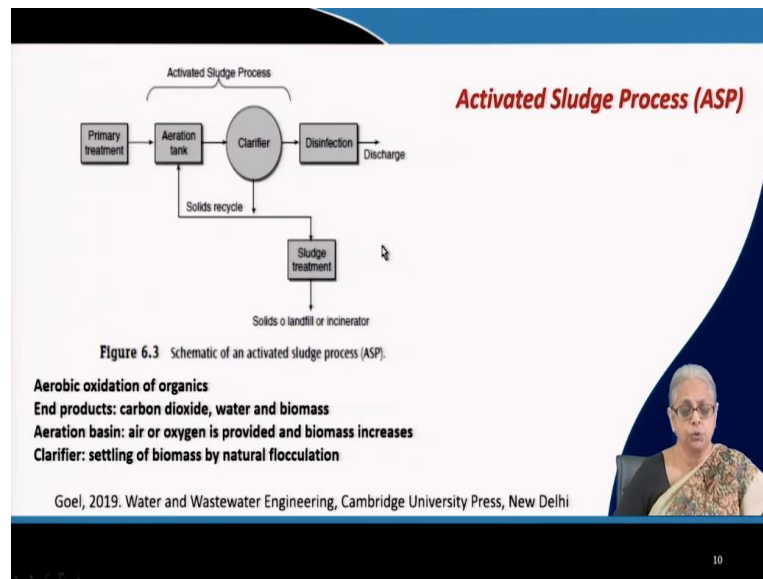
Conventional municipal wastewater treatment: ASP



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As I said, I will go through a little more detail about ASP or activated sludge process. So, we have a suspended growth process with a reactor that is a completely mixed flow reactor.

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So, let me just show you a schematic. So, after the wastewater has gone through primary treatment, it is then taken for activated sludge process. This is a 2-step process, where you have an aeration tank and a clarifier. So, in the aeration tank, you have the wastewater coming in and air is provided. The only objective in this part of the process is to ensure that the microorganisms, especially the bacteria and we believe that they are mostly aerobic heterotrophic bacteria at this stage, and there is a lot of organic matter that is already present in the wastewater. So, when air is added to the aeration tank, these microorganisms have literally everything they need to grow. So, you get an enormous increase in the amount of biomass as the organics in wastewater are now being converted to bacterial biomass, and the oxygen is consumed in this process.

Now, the entire wastewater along with this biomass will go to a clarifier. These clarifiers are circular tanks that are sloped at the bottom with the lowest point at the center of the tank – called the sludge hopper. The biomass, because of the tendency of these bacteria to stick together even though they are capable of living independently, they tend to stick together, partly because of their sticky nature. These bacteria form aggregates and that is the floc and this floc, when it is heavy enough, will settle to the bottom of the clarifier.

Now, in the aeration tank, a certain amount of biomass has to be maintained to make sure that the entire process is run at steady-state conditions. So, some part of the biomass (coming out of the clarifier) will be taken back into the aeration tank, and whatever is in excess will go for sludge treatment.

So, part of the process, the sludge will go to anaerobic digestion and the remaining part will be recycled. So, those are the basics of this process. So, it is aerobic oxidation of the organics in wastewater. The end products along with biomass are carbon dioxide and water. Air and oxygen are provided, biomass increases, and you get natural flocculation of the biomass which helps it to settle.

So, I have already mentioned that this aeration tank maximizes the DO levels. It can have a detention time of anywhere from 3 hours to 8 to 10 hours of mixing. And remember, the more time you provide, the greater the efficiency, but that also increases the cost of treatment. So, it is a fine balance between the economy of the process and the efficiency of the process. So, these 2 factors will determine what the practical detention time is.

Then we have a settling tank or clarifier for removing the particles which are basically nothing but biomass. Recycling of the solids is done to ensure a constant concentration of bacterial cells and the excess material is taken for sludge handling and disposal, which means they actually contribute to the anaerobic digestion and the production of biomass. I have already mentioned a few times that this is the most popular method for domestic wastewater treatment.

Some of the major advantages. Why is it the most popular? The first thing is, it allows dilution of the wastewater. And that is very important, because this dilution provides a shock absorbing capacity which other processes do not have. So, it can withstand toxic shock. So, supposing someone in the city dumps a load of toxic material, that will not kill the biological process, despite the toxicity of the material. So, that is because of the dilution factor.

Similarly, you may have shock loads in terms of volume of the water to be treated. For example, there are daily variations, there are seasonal variations, there may be huge amounts of water released from certain activities within the city; all these events can contribute to shock loading; and flow fluctuations which are either daily or seasonal flow fluctuation. So, all these kinds of changes which are either instantaneous or even over a slightly longer period, all this will contribute to 'upsetting' any process but because there is dilution of wastewater in this process, so the process itself is slightly less vulnerable to all these variations in comparison to processes like trickling filters and rotating biological contactors which have lower dilution factors.

Then we come to what are the problems. The first problem is the large land area. Because you have CSTR type of reactors, the area requirement is very high compared to all the other processes. But like I said, it is a trade-off between these 2 issues. So, most people choose to use a process that has the ability to withstand shock loading. And finally, in terms of energy. We live in a time when energy usage and efficiency are examined more and more carefully, because of the need to conserve energy, the need to reduce CO_2 emissions. So, all these things have also been accounted for in many of these cases. And one of the major issues with ASP is that it requires a high amount of energy for aeration.

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Conventional municipal wastewater treatment: Trickling Filters

- Attached growth, plug flow reactor for BOD removal
- Bed media: ordinary rocks or plastic; bed diameter: as large as 60 m
- Bed depths: with rocks it is restricted to 3 m, with plastic it can be much higher (biological towers)
- Distributor arms sprinkle wastewater evenly at the top
- Void spaces are large and allow air to circulate freely through the media
- Biological growth includes bacteria, algae, fungi, protozoa, worms, insect larvae
- Recycling of treated wastewater (effluent) enables higher efficiency, prevents slime from drying out
- Advantages
 - Removal efficiency is higher than in ASP (suspended growth processes)
 - Less land area requirement than ASP
- Problems
 - Washing out in monsoons and drying out in summer
 - Extreme sensitivity to fluctuations in quantity and quality of influent
 - Cannot withstand toxic or shock loads

Just to give you an idea, there is a little bit information about trickling filters as well over here. So, we have attached growth process. Trickling filters are an attached growth process. They are generally run in plug-flow mode. But if you have a significant amount of recycling of the biomass; the greater the amount of recycling, the closer it begins to resemble a CSTR.

Now, the same objective applies here as well. It is mainly designed for BOD removal. The media, when I said attached growth, it means you have to provide a media on which the bacteria will grow. So, this media can be ordinary rocks, stones or plastic. And these are some of the most common materials that are used. The size of these reactors can be huge in both cases, ASP as well as trickling filters. The bed diameters can be anywhere from 30 m to 60 m or anywhere in that range or even smaller.

So, bed depths are restricted. If you are using rocks and stones, the depth cannot be more than 3 m. But with plastic towers; so, they are called biological towers; these plastic biological

towers can be very high. Then, what we have is distributor arms that sprinkle wastewater evenly at the top of the media. And you can refer to images on the Internet; there are lots of images.

So, there are distributor arms that are going to sprinkle the wastewater evenly at the top. Uniform distribution is highly essential. This is one of the most important parts of the process. And then, we have void spaces. So, if you can just imagine that you have an entire reactor which is packed with stones and rocks and plastic media, whatever type of media you are using, it will have huge empty spaces which are filled with air. That is again crucial for providing air in a passive manner. So, unlike the ASP process where air is provided by either diffusing it through the wastewater or by using aerators. Here, it is passive aeration because you are allowing air to circulate freely through the media. So, it brings the cost down. In terms of the biological organisms that are present, you can have bacteria, algae, fungi, protozoa, worms, insects and even higher organisms.

Now, there are 2 types of trickling filters, you can have it under a cover or you can have it open to the atmosphere. So, if it is under a cover, algal growth can be prevented and is better for process efficiency. However, if it is open to the atmosphere, then obviously light is available and algae will grow. Recycling of treated wastewater will enable higher efficiency. It prevents the slime from drying out. So, this is crucial again for the proper processing of wastewater.

Advantages of this process: The first thing is that the efficiency of all attached growth processes in general is higher than suspended growth processes. So, that is very clear between the 2 types of processes. And because the efficiency is higher, so the land area requirement is also less. These are the major advantages.

What are the problems? The first major issue is the washing out in monsoon. So, imagine you have a trickling filter and it is open to the atmosphere. So, when you have a thunderstorm and a huge amount of water in the form of rainfall comes in, it will wash out all the biomass that has been growing on the surface of the rocks and other media. And in summer, when there is very little humidity, when there is very little water or precipitation, you can have complete drying out and that also will lead to complete death of the biomass, and you may have a reactor that does not operate. So, this is one of the biggest problems with trickling filters.

Any fluctuations in the quantity and quality of the influent are also going to have a huge impact on the quality of the effluent. So, any fluctuations in the input will be reflected directly in the output and this is not the case with ASP. So, this process has no ability to withstand toxic or shock loads.

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*Conventional municipal wastewater treatment:
Anaerobic digestion*

- Processing: anaerobic digestion
 - Objective is reduction in sludge (solids) volume
 - Slower than aerobic digestion, less sludge generation
 - Detention time of 10 to 15 days
 - Production of carbon dioxide and methane gases
 - Complex 3-step process
 - Hydrolysis: conversion of complex polymers to monomers; both extracellular and intracellular processes
 - Acidogenesis: conversion of monomers to organic acids like acetate, propionate, butyrate
 - Faster rate than methane formers (or methanogens)
 - Methanogenesis: fermentation of organic acids to H₂, CO₂, and CH₄

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Then we come to the second microbial process or biological process and that is anaerobic digestion. Now, this process has been getting a lot of attention for 2 reasons. One, it has been used initially for wastewater treatment, but now it is also getting attention for its ability to be used for municipal solid waste treatment. So, the same thing applies to both types of wastes, and it is becoming more and more popular.

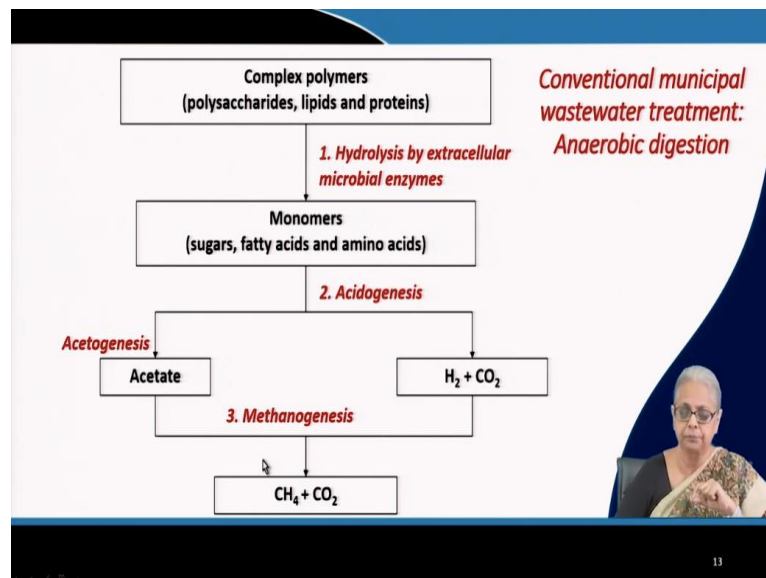
The objective here, like I said, is reduction in sludge volume. So, the initial anaerobic digestion process had only 1 objective, and that was reduction in the solids volume. Because, when the water comes out from the activated sludge process or even the trickling filter, the solids content is very low; it is about 1 to 2% or maybe even lower than that. 1 to 2% solids seem like what comes out of the ASP.

Now, if you can imagine a large city and a wastewater treatment plant for such a city, that is a huge amount of both water as well as material that needs to be taken care of. So, by reducing the volume or by increasing the solids content; so, if you can go from 1 to 5%, 10%, 20%; 22% seems to be the highest; you can reduce the volume in terms of the water content enormously. So, any reduction in volume will have enormous benefit in terms of money. So, that is the main

reason for reducing the volume of the sludge. It is a much slower process than aerobic digestion. We have already seen that aerobic processes are faster compared to anaerobic processes. This is basically a fermentation process, by and large, and it is much slower. The biomass generated is very small in amount.

So, you get very little sludge generation from this process. And you are actually consuming the organics that are coming in from the aerobic processes. A detention time of 10 to 15 days is required for the sludge, and the production is carbon dioxide and methane gases, which we call biogas. It is a complex 3-step process. Some textbooks call it a 4-step process, some call it 3-step; either way, it includes all the basic steps.

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So, let us just go through that. The sludge that is coming out of the primary and secondary settling tanks, it includes mostly complex polymers, mostly of biological origin. So, you have polysaccharides or carbohydrates, lipids, proteins. These are hydrolyzed into monomeric units. Remember that the bacteria cannot take up any of the large polymers. So, the first thing that has to happen is hydrolysis by extracellular microbial enzymes and the end product in that will be monomeric units of.....; basically, you will get sugars, fatty acids, amino acids and so on. These monomeric units can then be taken up by the bacteria.

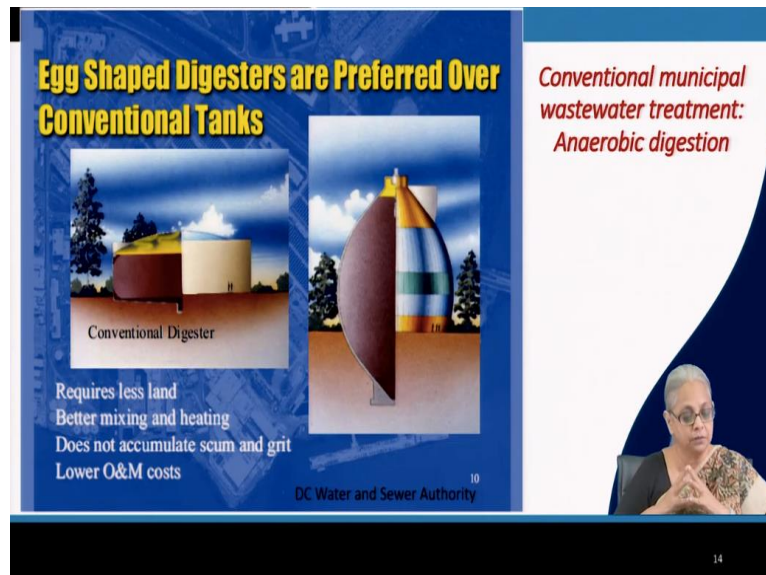
And the next step is what is called acidogenesis. So, acidogenesis, no details are shown in this graphic, you actually get the production of volatile fatty acids. And these volatile fatty acids are further broken down. So, you will get propionic acid, butyric acid. Now, acidogenesis is called acidogenesis for one simple reason. The main step that happens here in the first case is

the generation of volatile fatty acids. These volatile fatty acids are C3, C4, C5, carbon containing acids. So, you have propionic acid, butyric acid and so on. These fatty acids are then converted to acetate in a process which is called acetogenesis. You also get the production of hydrogen and carbon dioxide. And these together are going to be utilised by methanogenic bacteria to get the final end product, which is methane and carbon dioxide.

That in a nutshell is what the 3 or 4-step process of methanogenesis is. So, in all cases, it is fermentation of the initial organic matter, which is mostly in the form of biomass, from the aerobic processes. Now, under strictly anaerobic conditions, it is being converted to monomeric units in the first case; to organic acids which are called volatile fatty acids in the second case. So, you have acetic acid, propionic acid, butyric acid and so on.

And this is a very fast step, compared to the next one which is methanogenesis. So, methanogenesis is our final step, where you get conversion of the organic acids to hydrogen, carbon dioxide and methane. And this biogas, as you know, is a useful end product. If you get sufficient quantity of biogas, it can be separated; the 2 parts, CO_2 and CH_4 can be separated. Methane is a fuel; CO_2 has to be separated because it is a flame retardant. So, methane can be separated, used as a fuel and CO_2 has to be removed.

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These are examples of different types of anaerobic digesters. Way back, 50, 60 years ago, people used to use digesters like these. These are what are called conventional digesters (image on the left). So, they were very large cylindrical tanks. They either had fixed covers or floating

covers. And as the biogas was being generated, it would either be taken off the top, if it was a fixed cover reactor; or if it was a floating cover reactor, then the pressure would be maintained at a constant level, and the biogas would be tapped at the top, because it would accumulate at the top. So, that was very common. That was a common sight in many cities across the world including India, 30 to 50 years ago.

And in the last few decades, people have started shifting to what are called egg-shaped digesters. And this is the size and shape. You can see the scale, the human beings are very tiny compared to these digesters. They are huge, they are literally enormous and you can see the footprint. The footprint of the egg-shaped digester is much smaller than the conventional digester. So, that is the first thing. The second thing is, we are told that you get automatic mixing, and collection of the biogas is more efficient in the egg shape, because of the nature of the shape. So, because of that, the gas collects very easily at the top and it can be drawn off easily. And because it is narrow at the bottom, you do not need to mix it because, as the flow comes in, if the flow is low, it is the narrow part of the digester that is being used. As the flow increases, you get more volume that can be utilized. And this shape helps in providing high efficiency, no matter what the height of the wastewater, or rather the height to which the wastewater is present in the reactor. So, these are some of the advantages of egg-shaped digesters. The third point is, it does not accumulate scum and grit. And the other thing is that because of the shape of the digester, again, the operation and maintenance costs are extremely low compared to the conventional digester.

So, in conventional digesters, you have to periodically empty out the scum and grit that accumulates at the bottom. So, you have downtime for the reactor. You have to have people who are capable of dealing with this kind of material. They have to be trained to be able to go in and clean up the reactor. So, that results in high operation and maintenance costs. Now, egg-shaped digesters, I have been told that because of the egg shape, you do not need to do any cleaning, absolutely no cleaning has been done in this particular case and you get good results in terms of biogas generation.

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Conventional municipal wastewater treatment: Anaerobic digestion

Wikipedia

- The plant's four egg-shaped anaerobic digesters; the first of their kind in the country (UK), they are of reinforced concrete construction, with external cladding.

These are examples of egg-shaped digesters in the UK. This is from the Internet, from Wikipedia. So, you can see that the cladding; this is provided with cladding, and that is simply to provide insulation and keep the heat within the digester. Remember, a lot of heat is being generated in the anaerobic digestion process and it is useful heat. So, that heat has to be kept trapped within the digester, to help the efficiency and to improve the efficiency of the digestion process. So, that is it.

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Composting

- **Compost: the end material after microbial degradation of organic matter in MSW**
- Generally applied to food and garden waste,
- wood waste has to be chipped to small sizes for composting
- **Advantages:**
 - Natural process where the end material is biologically stable
 - Destroys pathogens, insects, etc.
 - Retains maximum nutrients (N, P, K, and other trace nutrients); has low C:N ratio, i.e., high N content
 - Not a substitute for fertilizers but a soil conditioner
 - Generates a product that supports plant growth and improves soil quality
 - Improves cation exchange capacity of soil
 - Improves water retention capacity of soil due to increase in organic content of soil

Here we come to another process and that is composting. Composting in India, we believe has been around for ages, literally. And what is composting? Composting is when you utilize waste organic material and convert it utilizing natural microbial degradation processes, and you get

an end product which we call compost. Now, this compost is considered to be; many people consider it a substitute for fertilizer, but I would say that the best description of compost is that it is a soil conditioner.

So, it helps to improve the quality of the soil and it really does not provide sufficient nutrients for it to serve as a substitute for fertilizers. So, it definitely improves the quality of the soil. You can use it for food and garden waste. And if you want to use it for degrading wood waste, then that wood waste has to be chopped down and then chipped to very small sizes. In fact, if you are using food and garden waste as well, it helps to have smaller sizes.

The smaller the size, the better able the bacteria and fungi are in breaking down this organic material. Then, what are the major advantages of composting? The first thing is, it is a natural process. It is a natural process and the only thing you need to do is, occasionally turn it, mix the content and provide sufficient moisture so that the degradation process continues without any hindrance.

So, the end material is biologically stable. You are providing recycling of the important nutrients. So, nitrogen, potassium, phosphorus, all of these which are very important from an agricultural productivity point of view can be recycled easily using this process. It also helps to destroy pathogenic organisms, insects, larvae; all these materials can be destroyed quite easily in the composting process.

No significant effort is required in many cases, if you do it right. You can convert your organic material from a high carbon to nitrogen ratio, to a low carbon to nitrogen ratio, which means you can increase the nitrogen content of your soil. So, that is why many people call it a fertilizer. You get a product that will help to support plant growth. It will improve the soil quality for 2 reasons.

One is that the organic matter that is part of compost will help to retain moisture. So, the moisture retention capacity of the soil improves because of the organic content. Second, the organic content helps to sequester all these trace nutrients as well as the major nutrients. So, the major and micronutrients will be sequestered by the organic content, and that will help to make the soil more productive, because you do not get a loss of these nutrients. So, it helps to improve the cation exchange capacity as well as the nutrient retention capacity of the soil.

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Aerobic composting of the organic fraction of municipal solid waste

- Preprocessing of MSW
- Aerobic decomposition
- Product preparation and marketing
- Types of composting
 - Pit composting or trench composting
 - Windrow
 - Aerated static pile
 - In-vessel

(A small inset video shows a woman with glasses speaking.)

What is required in aerobic composting? So, composting is an aerobic biodegradation process. You need to segregate; the first most important thing is, you need to separate the organic fraction of municipal solid waste from the remaining part of the waste. Then, that organic material can be decomposed under aerobic conditions. So, the only thing you need to do for maintaining aerobic conditions is to provide some turning.

And if you want to pass air through the pile, that is also possible; people have tried it. And finally, you need to prepare the end product in a way that will make it attractive to the consumer. And in most cases, the consumer is the farmer. So, you have to prepare it and market it in a way that will be beneficial as well as attractive to farmers.

So, we have different methods of composting. The oldest one that is documented in the literature is pit composting or trench composting. And the current popularity in terms of methods is for windrow composting. So, most urban local bodies are going for windrow composting, especially if they have the land area for it. There are other methods that are being examined by different municipalities and as part of research and development. So, we have aerated static pile and we have in-vessel composting.

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**Indore
composting
process**

- **First documented use of composting: Indore process in 1930s in India; published in 1933; several modifications since then**
 - Simplest and oldest process in India
 - Excavation of trench 2 ft deep to ensure that the process is aerobic
- **Successive layers of putrescible material**
 - Solid waste, night soil, animal manure, earth, straw
 - Turn material 3 times in 3 month period; add water 6 times in the same period; see timetable in next slide
 - Liquid released is recirculated or added to drier wastes
 - Degradation by bacteria and fungi; nitrogen fixation was likely
- **Improvements to process later by**
 - More frequent turning to ensure aerobic or facultative processes
 - Vermicomposting by earthworms

Howard, A (1933) The waste products of agriculture: their utilization as humus, *Jour. of Royal Society of Arts*, 82(4229): 84-121.

I am going to start with the pit composting method. That is the oldest one that has been documented. It is also called the Indore composting process. So, it was the first publication, came out in 1933. It was an Indo-British collaborative venture in Pusa, in Bihar. And this is, like I said, the first documented use of composting. There have been any number of modifications to this process since this publication, but it seems like this formula has been more successful than most of the other modifications.


So, let us just take a very quick look at it, for at least understanding the principles that are part of the composting process. So, it is the simplest and oldest process in India. All it requires is that you dig a large trench up to 2 ft deep. You do not want to go more than 2 ft deep, because it is only up to 2 ft that the process is somewhat aerobic; beyond that, it becomes anaerobic.

So, if you want to keep it aerobic, you have to ensure that the height or rather the depth is not more than 2 ft. In the Indore composting process, what was done was that solid waste, night soil, animal manure, earth and straw were piled up in layers, one layer after the other. I will show you the timetable of the entire process.

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No.	Event	Days
1	Charging of compost pit	1 to 6 days
2	Fungal growth established	10 days
3	First watering	12 days
4	First turning, inoculation of pit with compost from 31 day old pit	16-17 days
5	2 nd watering	24
6	2 nd turning	30
7	3 rd watering	38
8	4 th watering	45
9	3 rd turning	60
10	5 th watering	67
11	6 th watering	75
12	Removal to field	90

Indore composting method (1933)



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So, it takes about a week to put in all the layers into the pit. So, 1 to 6 days for what is called charging of the compost pit. During that period and for another 2 to 3 days, you allow fungal growth, which is natural. You will find that happening any time you try to do this, even in a vessel or any kind of container. So, fungal growth will be established within 10 days. And you add some amount of moisture in terms of sprinkling it with water. And that was done after 12 days. The first turning; and in this case, no aeration was done; simply mixing of the contents was done and compost from a 31-day old pit was brought into the new pit, to get sufficient microbial inoculum. This was done at 16 to 17 days. So, at 24 days, a little more moisture was added. At 30 days, the whole thing was turned over again. At 38 days, moisture was added again. So, it is not done on a daily basis, it is not done on a weekly basis, only 3 times; the material was turned only 3 times in a 3-month period and 6 times, water was applied to it, over the same period of 90 days. At the end of 90 days, you get good quality compost that can be applied directly to the field. So, that in a nutshell is how simple the initial process was.

A significant amount of liquid is generated; we call it leachate. So, this leachate, if it is fairly large in terms of quantity, can be recirculated and added back into the heap; and that helps to keep the waste from drying out. So, it is organic material, it has a high microbial population, so it works in decomposing the waste even further. Now, what they found is that degradation was done by a combination of bacteria and fungi. And they also think that the nitrogen content of the final compost was higher than what was put into the pit. So, it is quite likely that nitrogen-fixing bacteria were active during the composting process (mineralization of organic carbon to carbon dioxide is another reason for the higher N content of the compost). There are several improvements to this process and people have tried to improve the process by making it more

aerobic, by making the turning frequency higher, by providing aeration, and so many other modifications have been tried.

And there are ways of doing this process as a facultative process. So, you make the pit or the container deeper and you allow aerobic as well as anaerobic composting to be done; all that has been tried.

Another new modification is vermicomposting. So, vermicomposting is done by earthworms, not by microbial organisms, except those that inhabit the gut of the earthworms. So, vermicomposting is a totally different kind of method, and it is outside the scope of our course. So, I would not go into it, but that is also proving to be more and more popular and it gives good quality compost.

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Different types of composting processes

- Windrow:**
 - Dimensions are 3 to 4 m wide, 2 m high and tens of m in length
 - Turn 1 or 2 times/wk in open field for 4 to 5 weeks, turning provides oxygen and maintains high biodegradation rates
 - 2 to 8 weeks in open fields is sufficient for stabilization
 - High temp inside pile as biodegradation results in generation of heat
 - Organic matter is converted to biomass and CO_2
 - Off-gases of NH_3 and H_2S can be objectionable
- Static pile (Aerated)**
 - No turning, air blown into pile
- In-vessel**
 - Plug-flow or dynamic (agitated or CSTR type or mixed)
 - Popular now because of greater control over process and odor
 - Faster throughput, less detention time (1 to 2 wks)
 - Lower labor costs, smaller area requirements

Curing time in all systems remains 4 to 12 weeks

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Let us take a look at the other processes. So, we have windrows. Windrows are also quite popular these days. They require a large land area. The biggest problem with windrows is, they are very efficient but the land area required is very high. So, the dimensions are 3 to 4 m wide, 2 m in height and tens of metres in length. Turning is done 1 or 2 times per week in an open field for 4 to 5 weeks.

The turning provides oxygen and allows high biodegradation rates. 2 to 8 weeks is generally considered sufficient for stabilization of the organic material. So, the biodegradation stops and there is no off-gassing of malodorous gases like ammonia and hydrogen sulphide. The organic matter is reduced and it is converted to biomass and CO_2 .

The biggest advantage of composting, all kinds of composting, the biggest advantage comes from the fact that in aerobic composting, a large amount of heat is generated. And this heat is responsible for destroying pathogenic species. So, once the temperature inside the compost pile is around 50°C or higher, anywhere from 50 to 70 °C, you are going to get destruction of any pathogenic organisms that we are concerned about. So, this heat generation is crucial to the success of the composting process. And this high temperature is easily achieved. If it is done right, the high temperature of 65 to 70 °C or even higher in some cases has been achieved and the higher the temperature, the better it is in terms of microbial quality.

Static pile is when there is no turning, only aeration, and air is blown into the pile, through pipes into the pile. So, you have a pile of waste. You have pipes, perforated pipes at the bottom. You can either blow air using pumps or you can allow passive aeration by allowing air to flow in and out of these perforated pipes. No turning is required, no mechanical energy is used.

Then we come to in-vessel composting. Now, the biggest advantage of in-vessel composting is that the footprint will be very small in comparison to the other two, or in fact, in comparison to all the other 3 processes which have a large footprint, i.e., land area requirement.

In-vessel, it can be run in either plug-flow mode or CSTR or mixed-mode; it depends on how much energy you want to put into the process. This process has become popular for the simple reason that you have more process control, and malodorous gases like ammonia and hydrogen sulphide which can cause problems; the residents in the areas where the other processes are used generally object to these odours; that can be controlled in in-vessel composting processes. You should be able to do this in 15 days instead of 3 months. However, that has not been as easy as it sounds.


I think there are claims that you get a faster throughput, less detention time, less labour requirement and smaller area requirement. Curing or maturing of the compost: So, after it has stabilized, you have a curing period in which the biodegradation activity is almost zero.

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Bioleaching of heavy metals from ores and e-waste

Applications of microbiology

- Biometallurgy or biohydrometallurgy is the use of microbes in an aqueous environment for recovering metals like gold, copper and uranium
- Quarter of the world's copper production is by bioleaching of copper from ore (to be covered in Lecture 63)
- The same principles can be used to recover minute amounts of heavy metals from e-waste
- One of the most common microbial species involved in bioleaching is *Acidithiobacillus ferrooxidans*
- These bacteria are acidophilic, obligate autotrophs and are common in acid mine drainage and mine tailings
- Can use ferrous iron and elemental sulphur as electron donors



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Then we come to the last application that I want to cover in this course, and that is bioleaching of heavy metals from ores and electronic waste. These processes are collectively called biometallurgy or biohydrometallurgy, for the simple reason that you cannot have biological processes without water. So, biometallurgy implies the presence of water, and therefore it is also called biohydrometallurgy.

In this case, microbes can be used in an aqueous environment for recovering metals like gold, copper, uranium from either ore or from other waste materials. One-quarter of the world's copper production is considered to come from the bioleaching of copper from the ore. So, this has proved to be the most popular way of extracting copper from ore and the same principles that are used in bioleaching of copper from ore, can be used to recover these heavy metals from electronic waste.

One of the most common species that has been used in bioleaching is *Acidithiobacillus ferrooxidans*. These are acidophilic bacteria, they are obligate autotrophs and they are very commonly found in acid mine drainage and mine tailings.


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Biometallurgical methods

Table 12.9 Biometallurgical methods for metal recovery from electronic waste

Materials	Metals recovered %		Microorganism used	References
	Nickel	Cadmium		
Ni-Cd battery	96.50	100	<i>A. ferrooxidans</i>	Cerruti et al. (1998)
	66.10	100	Indigenous acidophilic thiobacilli in sewage sludge	Zhu et al. (2003)
	–	84	<i>A. ferrooxidans</i> and <i>A. thiooxidans</i>	Velgosova et al. (2010)
Electronic scrap	Cu, Sn, Ni, Pb, Zn, Al		<i>A. ferrooxidans</i> , <i>A. thiooxidans</i> , <i>A. niger</i> and <i>P. simplicissimum</i>	Brandl et al. (2001)
Electronic waste	Cu, Zn, Al, Ni		<i>Thermosulfidooxidans sulfobacillus</i> + <i>Thermoplasma acidophilum</i>	Ilyas et al. (2010)
Electronic waste	Cu, Pb, Zn		<i>A. ferrooxidans</i> , <i>A. thiooxidans</i> and mixture	Wang et al. (2009)
Electronic waste	Cu		<i>A. ferrooxidans</i> and <i>A. thiooxidans</i>	Mražiková et al. (2013)

Source: Willner et al. (2015) and Natarajan et al. (2015) Dutta and Goel, 2017



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So, I will just show you a table from a book chapter and these are examples of different types of metals that were recovered from electronic waste. The percent recovered is based on the total amount of metal. So, nickel-cadmium batteries; nickel and cadmium both of them were recovered using this particular bacterium - *Acidithiobacillus ferrooxidans*, which is also present in sewage. So, it is not difficult to find, and it can be used to recover these metals. From electronic waste, copper, tin, nickel, lead, zinc, aluminium, all of them have been recovered. So, this is a very promising method for recovering different types of metals. The basic process, I think I have already explained it in a previous topic. What can be done is that you can combine it with ferrous iron and elemental sulphur which can serve as the electron donors. I will be talking about this process at the end of the last lecture. So, the biogeochemical cycles; we will go into details about this particular process.

Thank you.