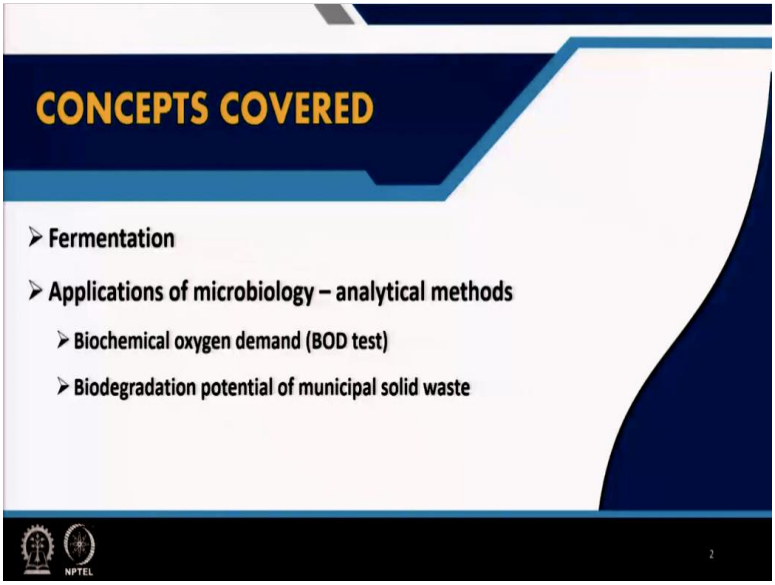
Environmental Chemistry and Microbiology

Dr. Anjali Pal
Dr. Sudha Goel
Department of Civil Engineering
Indian Institute of Technology – Kharagpur

Module - 12
Lecture - 60
Metabolic Diversity – V

Welcome everyone. This is part 5 of Metabolic Diversity. This is lecture 60 of module 12.

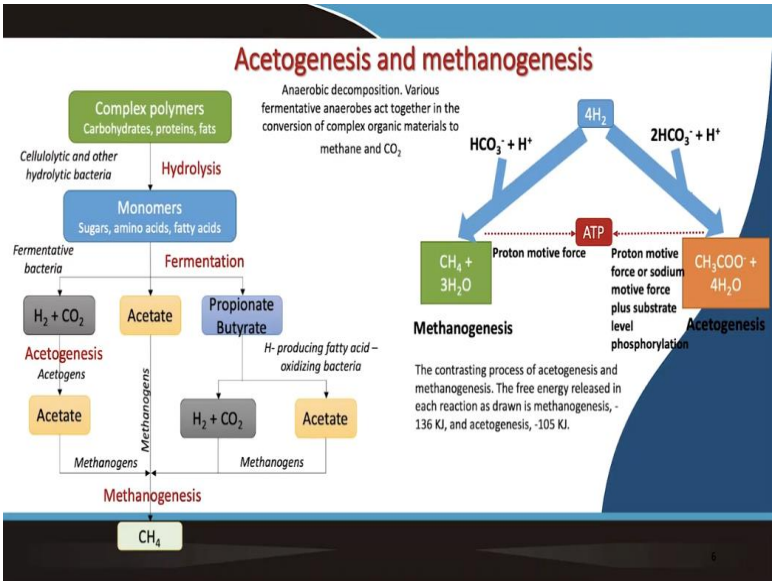
(Refer Slide Time: 00:36)



So, in this lecture, we are going to complete the last part of Metabolic Diversity in terms of the metabolic pathways that bacteria can utilize for again utilizing different types of substrates under different environmental conditions. So, the last pathway that we are going to focus on is fermentation. After that, I will come to some practical applications of microbiology.

So, how are microbes used in the lab or even in the field to achieve certain purposes? We will start with analytical methods. So, the 2 analytical methods that we are, most of us are familiar with are, biochemical oxygen demand (BOD) test. This is used for determining the biodegradation potential of organics in any wastewater. And then, we will look at the biodegradation potential of organic material that is present in municipal solid waste. That is a topic that my research group has been working on. So, we are doing quite a lot of work in that area. So, I can spend some time talking about that.

(Refer Slide Time: 01:54)



Now, in a previous lecture, I have mentioned that anaerobic digestion or fermentation is very common in wastewater treatment. Remember that the sludge from activated sludge processes is taken to an anaerobic digester. There are many different types of anaerobic digesters. But the first thing that you see over here in this slide is that the complex polymers that are part of the biomass of this sludge, which is contained in this sludge, have to be broken down into monomers before the cells can even begin to absorb these nutrients and utilize them for energy and growth.

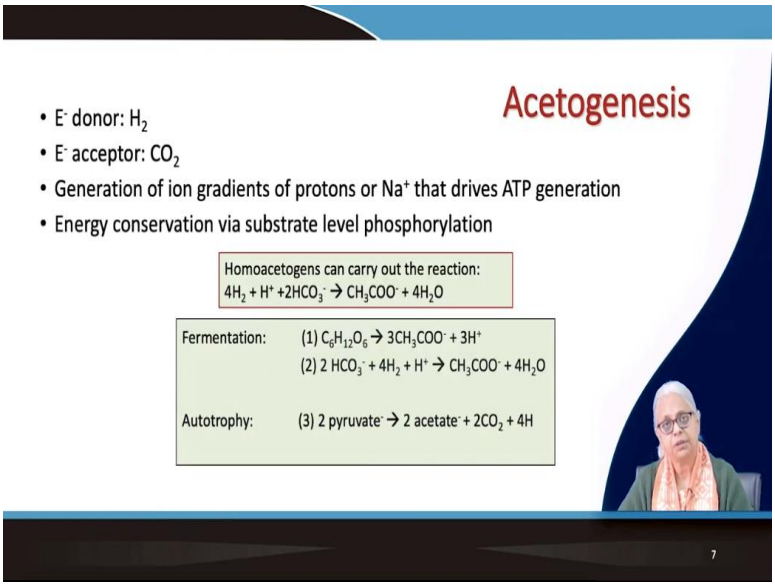
So, we have these complex polymers like cellulose, polysaccharides, proteins and so on. So then, these bacteria are extruding or excreting cellulolytic as well as hydrolytic enzymes; and these bacteria are also called (cellulolytic or hydrolytic) bacteria for the same reason and they are breaking down these polymers into their monomeric units by hydrolysis reactions. So, these monomeric units are going to be sugars, amino acids, nucleotides and so on, the fatty acids, all of that.

They will then be fermented by various groups of fermentative bacteria. So, we have fermentative bacteria that will utilize these monomeric forms and convert them to 3 key intermediates. So, we have $H_2 + CO_2$, we have acetate and we have acetate, which is C2, propionate is C3, butyrate is C4 and higher numbers of organic acids. So, these are what we also call volatile fatty acids (C2 to C6 compounds).

There are acetogenic bacteria that will convert H_2 and $CO_2$to acetate. This acetate will be taken up by methanogenic bacteria and converted to methane. The organic acids will be taken up by hydrogen producing fatty acid-oxidising bacteria. They will again form these 2 intermediates of hydrogen, carbon dioxide and acetate, which will again be taken up by methanogenic bacteria and converted to methane.

So, we have 2 major processes. We have methanogenesis and acetogenesis. So, the formation of these, or rather the formation of methane gas depends on conversion of hydrogen and carbon dioxide to acetate; acetate directly to methane and the conversion of fatty acids to these intermediates as well. So, you can see that the starting point, the key to this process is hydrogen gas. This hydrogen gas is going to be key to the success of the anaerobic digestion process. Because, as it is being produced, it has to be utilized; and I will come back to this in the processes of methanogenesis and acetogenesis as well. So, the electron donors are hydrogen, nitrate, thiosulphate; and electron acceptors are considered to be carbon dioxide.

(Refer Slide Time: 05:24)

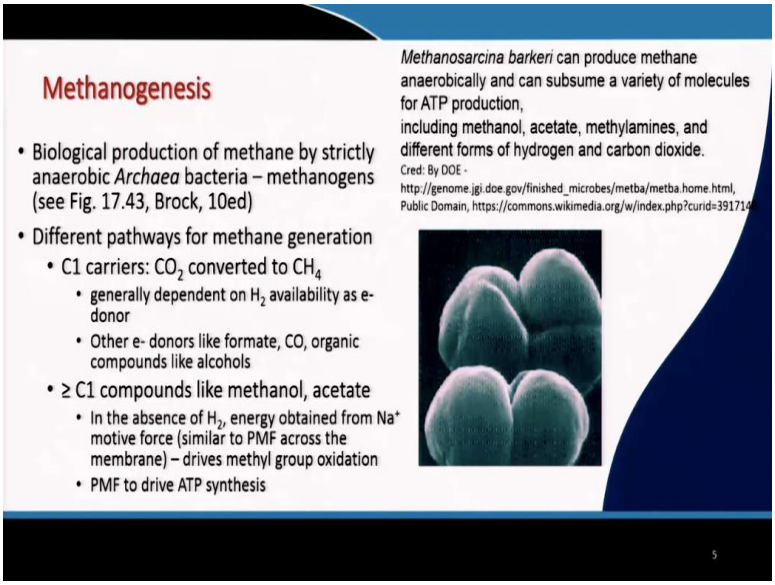


So, the electron donor, like I said, is hydrogen; electron acceptor is CO_2; the generation of ion gradients of protons or sodium that drive ATP generation is the principle behind this process. Energy is conserved in the substrate level phosphorylation reaction. So, these homoacetogenic bacteria will utilize hydrogen along with protons and bicarbonates, convert it to acetate and water.

Fermentation is the conversion of larger organic compounds like glucose (C6) to acetate (C2), and from bicarbonates to acetate. You can have fermentative bacteria and you can have

autotrophic bacteria. So, if you have autotrophic bacteria, then you get disproportionment and you have pyruvate being converted to acetate and CO_2.

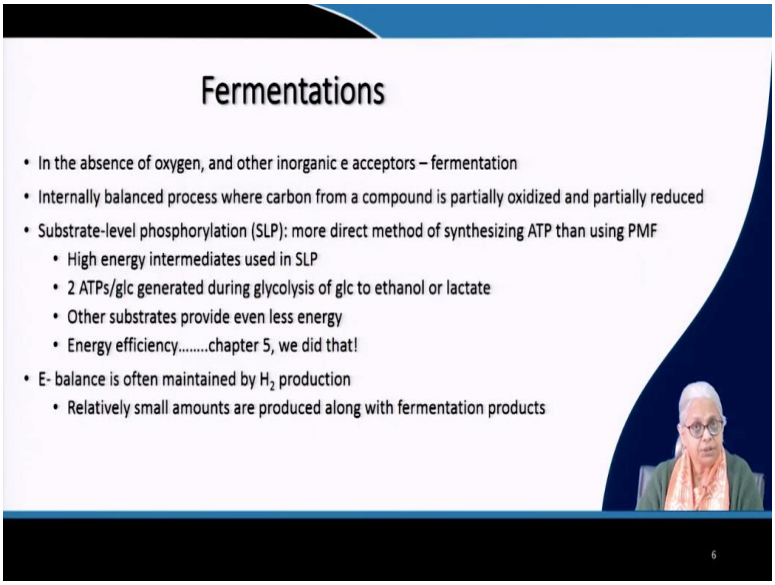
(Refer Slide Time: 06:22)



What happens in methanogenesis? Methanogenesis is the production of methane by anaerobic archaebacteria, which are also methanogens. So, you can see some methanogenic bacteria in this particular photo. The cells are about 1.7 microns and they are fluorescing under blue light. So, they are visible only with fluorescence. So, we have different pathways for methane generation. If you have a C1 compound, CO_2 is converted to methane (CH_4).

This depends on the availability of hydrogen as an electron donor. You can have other electron donors like formate, carbon monoxide; organic compounds like alcohols and others. And then, you can have other C1 compounds like methanol and higher carbon containing compounds; so, methanol, acetate and so many other compounds. If hydrogen is not present, energy can be obtained using sodium. And instead of proton motive force, you have sodium motive force and this can drive methyl group oxidation. So, PMF or NaMF can drive ATP synthesis.

(Refer Slide Time: 07:46)



In the absence of oxygen and other inorganic electron acceptors, that is what we call fermentation. It is an internally balanced process where carbon from the compound is partially oxidized and partially reduced. You can also have substrate level phosphorylation (SLP). It is a more direct method of synthesizing ATP, rather than using proton motive force.

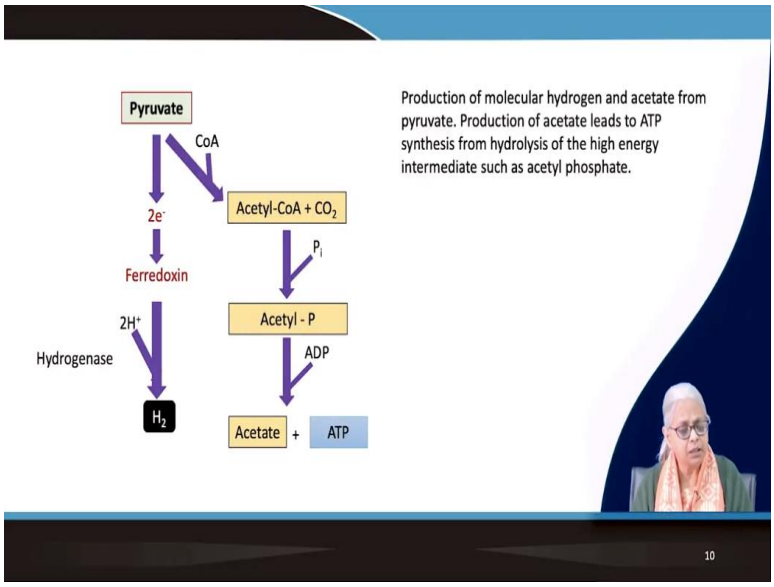
Remember, we have gone through the 3 biochemical pathways. If you have oxidative phosphorylation, then you can utilise PMF or proton motive force to generate ATP. If you do not have that, then you have a fermentation set of reactions where oxidative phosphorylation does not happen, proton motive force is not generated and you have just substrate level phosphorylation. So, you have high energy intermediates that are used in SLP.

You have 2 ATPs per glucose molecule that are generated during the glycolysis of glucose to ethanol or lactate. Other substrates can provide less energy, and we have looked at the energy efficiency of these reactions under a previous topic. One key to our understanding of all these processes is that the electron balance has to be maintained. How is the electron balance maintained?

So, whatever (electrons are) being donated by one substrate, they have to be picked up by the electron acceptor. And that is why, when I said, the simplest way of looking at fermentation is, think about glucose. Split it equally into the most reduced form which is methane and the most oxidized form which is CO_2. That is a way of maintaining electron balance. But here we have a key intermediate, and that is H_2 production. So, relatively small amounts of H_2 gas are

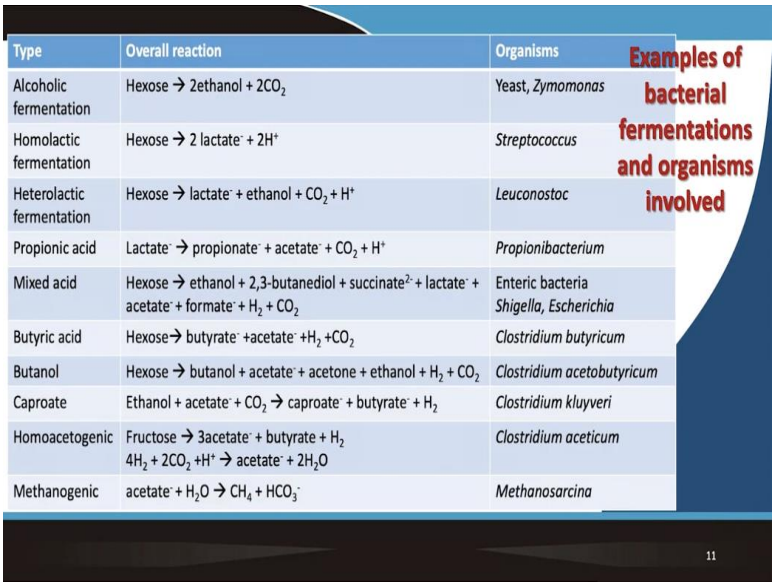
produced along with the fermentation products, and whatever is produced has to be taken up very quickly.

(Refer Slide Time: 09:54)



We then come to the production of molecular hydrogen and acetate from pyruvate. Again, if I remind you, in the 3 biochemical pathways, one thing that is common is glycolysis, and the end point in glycolysis is pyruvic acid or pyruvate. This pyruvate will pick up electrons and result in the production of hydrogen or in the production of acetate and ATP.

(Refer Slide Time: 10:18)



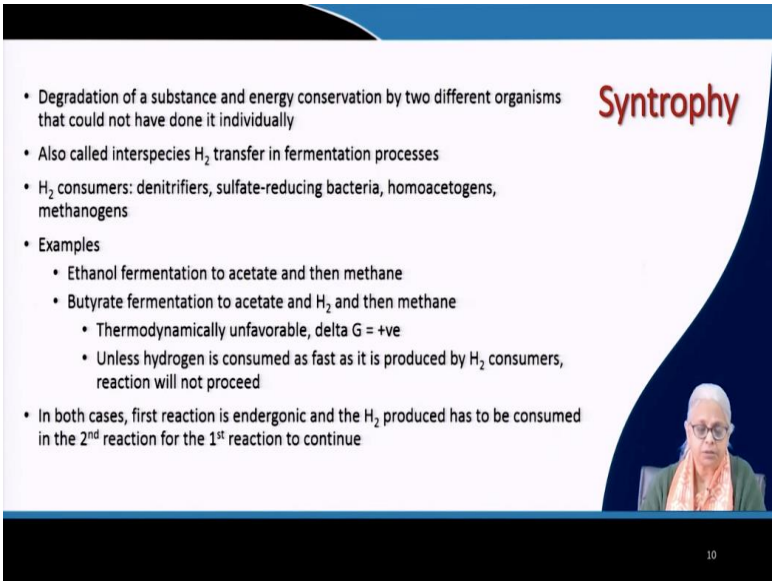
Type	Overall reaction	Organisms
Alcoholic fermentation	Hexose → 2ethanol + $2CO_2$	Yeast, *Zymomonas*
Homolactic fermentation	Hexose → 2 $lactate^-$ + $2H^+$	*Streptococcus*
Heterolactic fermentation	Hexose → $lactate^-$ + ethanol + CO_2 + H^+	*Leuconostoc*
Propionic acid	$Lactate^-$ → $propionate^-$ + $acetate^-$ + CO_2 + H^+	*Propionibacterium*
Mixed acid	Hexose → ethanol + 2,3-butanediol + $succinate^{2-}$ + $lactate^-$ + $acetate^-$ + $formate^-$ + H_2 + CO_2	Enteric bacteria *Shigella, Escherichia*
Butyric acid	Hexose→ $butyrate^-$ +$acetate^-$ +H_2 +CO_2	*Clostridium butyricum*
Butanol	Hexose → butanol + $acetate^-$ + acetone + ethanol + H_2 + CO_2	*Clostridium acetobutyricum*
Caproate	Ethanol + $acetate^-$ + CO_2 → $caproate^-$ + $butyrate^-$ + H_2	*Clostridium kluyveri*
Homoacetogenic	Fructose → 3$acetate^-$ + butyrate + H_2 $4H_2$ + $2CO_2$ + H^+ → $acetate^-$ + $2H_2O$	*Clostridium aceticum*
Methanogenic	$acetate^-$ + H_2O → CH_4 + HCO_3^-	*Methanosarcina*

These are examples of common fermentation reactions which have some commercial applications as well. So, formation of alcohols is based on sugars. The sugar can be derived from any source; it can be rice; it can be wheat; it can be any other grain; it can be nuts (and

fruits); the number of alcohols that come from different sugars is endless. So, we have hexose being converted to 2 ethanol and 2 CO_2, by either yeast or *Zymomonas*.

Then you have homolactic fermentation. So, the best example, like I said, is milk being converted to yoghurt which is lactic acid, and then further conversion to other products. So, you have the sugar lactose being converted to lactate and ethanol and carbon dioxide and protons. Then you have propionic acid; lactate to propionate, acetate and carbon dioxide. Butyric acid; hexose goes to butyrate, acetate, hydrogen and so on. And you can see the number of organisms or bacterial species that are capable of fermenting these substrates.

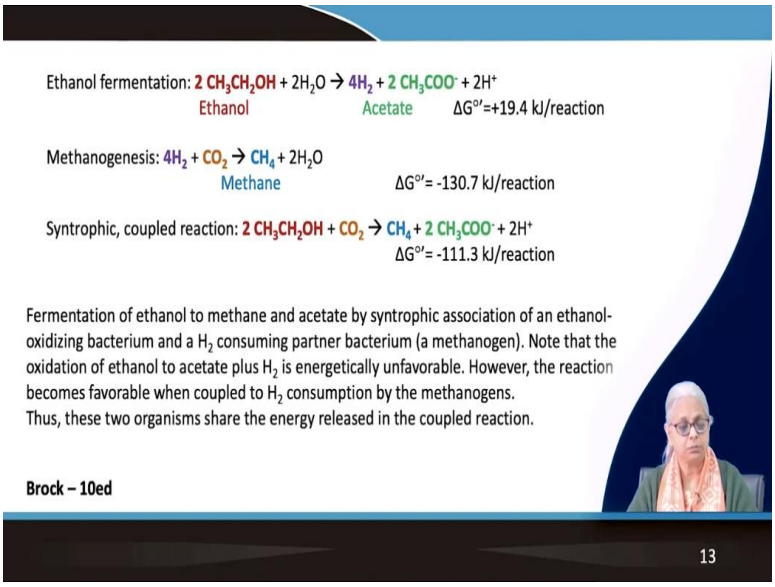
(Refer Slide Time: 11:45)



We then go to syntrophy. What is syntrophy? Syntrophy is the degradation of a substance and energy conservation by 2 different organisms or 2 different species which by themselves are not capable of doing it. In the fermentation process, this is also called interspecies hydrogen transfer. So, I have already mentioned to you that you have acetogens which are generating hydrogen and acetate, and you have methanogens.

So, these 2 groups of bacteria have to transfer hydrogen from one point to another and this is the syntrophy between them. So, what are the hydrogen consuming organisms? We have denitrifiers, we have sulphate reducing bacteria, we have homoacetogens, and we have methanogens; all of them are (capable of) consuming hydrogen. Examples are, ethanol fermentation to acetate and then methane; butyrate fermentation to acetate and hydrogen and then methane. In the first case, in the case of butyrate fermentation;

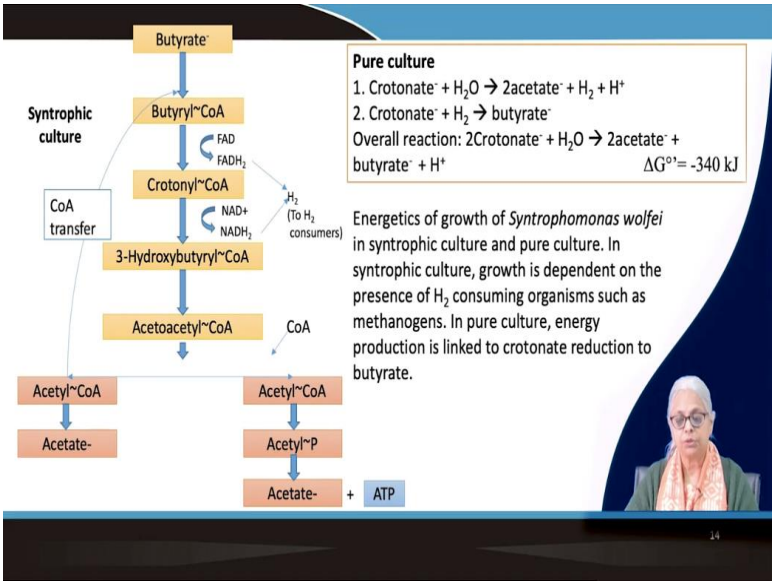
(Refer Slide Time: 12:54)



So, if we take ethanol fermentation to acetate. So, here we have ethanol being converted to hydrogen and acetate. The ΔG for this particular reaction is positive. So, by itself, it is not going to give any energy to the bacteria. So, the bacteria will not be able to utilize it. However, when it is coupled; so, when you have an ethanol oxidizing bacterial species in syntrophic relation with a methanogen, what will happen?

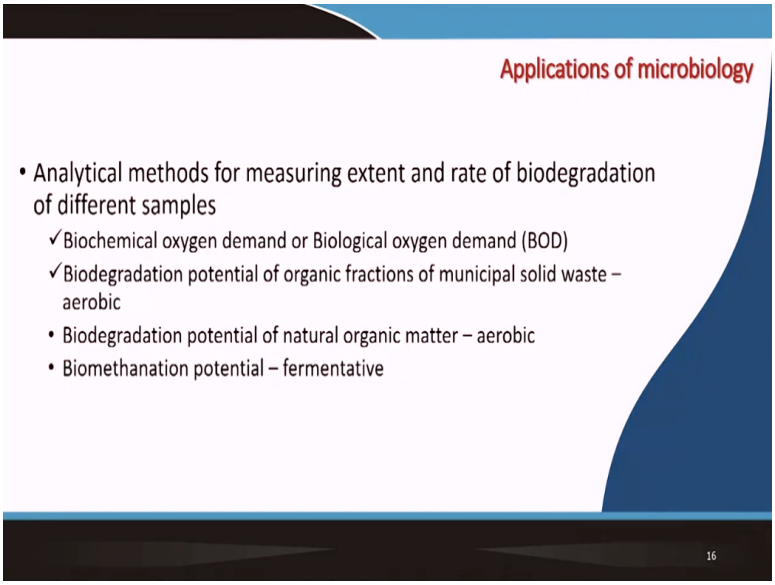
The methanogenic reaction, $H_2 + CO_2 \rightarrow CH_4$ is favourable. So, when these 2 reactions are combined by these 2 bacterial species which have a syntrophic relationship, then the net ΔG for this reaction is negative and therefore possible (favorable); which is not the case otherwise. In both cases, the first reaction is endergonic and H_2 is produced. H_2 that is produced has to be consumed in the second reaction, otherwise you have a problem, because the first reaction will not proceed. So, if I were to say it in another way, this hydrogen has to be consumed as fast as it is formed for this (reaction) to be complete.

(Refer Slide Time: 14:26)



So, here is an example of one of these bacteria. So, we have *Syntrophomonas wolfei* and it has been tested in a syntrophic culture as well as in a pure culture. This particular species is capable of converting crotonate to acetate or to butyrate using proton motive force. But if it is in a syntrophic culture, it will convert butyrate to acetate, and in the process produce hydrogen, and this hydrogen will be taken up by methanogens. Therefore, you have complete conversion to acetate. In the other case, you do not get complete conversion, you still have butyrate.

(Refer Slide Time: 15:11)



So, from this point onwards, we are going to look at some of the applications of microbiology, which have special importance in both Civil and Environmental Engineering and Science and so forth. Because of lack of time, I am going to focus only on 2 analytical methods. 4 of them

are mentioned over here, but we will be looking at 2 of the major analytical methods that are noted over here, the first 2.

So, by now, you know that in the environment, aerobic heterotrophic bacteria are the most common group of bacteria. And from an environmental perspective, we want to be in a position to measure the extent and rate of biodegradation of different types of samples. Now, the organic matter for heterotrophic bacteria can be present in water, it can be present in soil or it can be present in solid waste material.

So, the 4 methods that I have mentioned over here, the first one is biochemical oxygen demand or some people call it biological oxygen demand (BOD). This is associated with organic material that is present in any type of wastewater. So, if you are dealing with municipal wastewater or with industrial wastewater and you want to know how much of the organic material in that wastewater is biodegradable, then that is measured using the BOD test.

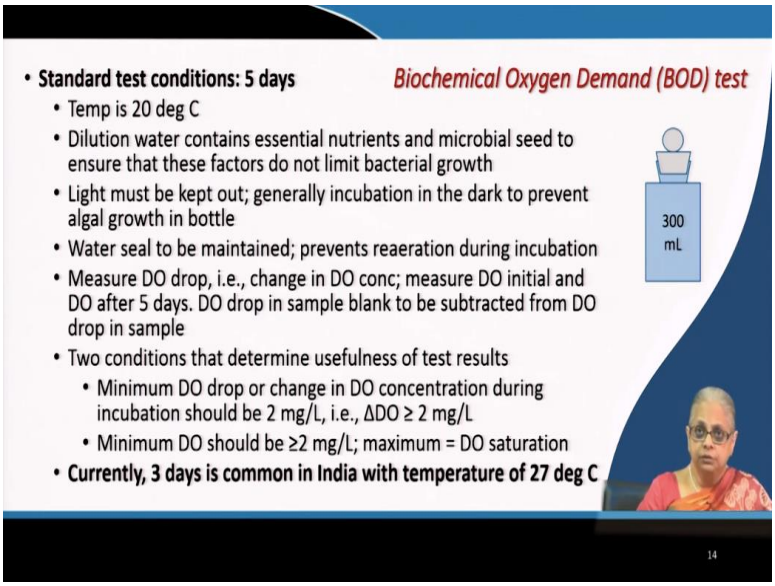
So, we are going to go through some details about the BOD test. Then we come to the degradation potential or you can also call it the biodegradable fraction of the organic fractions of municipal solid waste. Now, this seems like a mouthful. Let me explain. So, municipal solid waste is a mixture of different types of materials. It is got recyclable materials like paper, plastic, glass, metals and so on; and it also has food waste, garden waste, street sweepings; so, you have soil, you have dirt, all kinds of materials are present in municipal solid waste. We are interested in knowing how much of that mixed solid waste has organic material. So, we know that food waste is organic in nature. We know that grass and twigs and branches and leaves, all this, leaf litter or you might say vegetation; all of that is organic. You also have paper and plastic, which are also organic in nature, but we know that they are very different in their biodegradability compared to let us say food waste.

So, we have a large number of organic fractions in municipal solid waste. From a management perspective, if I want to design treatment processes, I need to know what the biodegradation potential of each fraction of municipal solid waste is and we can do this under aerobic conditions as well as under anaerobic conditions. So, when we measure the biodegradation potential of organic fractions of municipal solid waste, we can do them under, like I said, aerobic or anaerobic conditions.

I will go through some of the work that my research group has been involved with, and they have been measuring the aerobic biodegradation potential of different organic fractions. The same thing can also be done for natural organic matter. This natural organic matter is a problematic group of contaminants that is present in water supplies. So, whether your drinking water or any other; basically, municipal water supplies are the ones that are most affected by the presence of natural organic matter. It creates many problems in the treatment as well as distribution, especially of drinking water. So, in the past we have measured the biodegradation potential of natural organic matter under aerobic conditions and that has, like I said, several applications in terms of water supply systems. There is another set of research that has been gaining a lot of attention, and that is measuring the biomethanation potential of different organic fractions, after treatment, with or without treatment. And various types of treatment methods have been used. And this is under fermentative condition. So, you can see that we are looking at how different organic fractions will behave in the presence of different types of microbes under different conditions of oxygen availability.

So, these are some major areas of research. And obviously, I am not going to cover all 4 of them, I will just cover the first 2.

(Refer Slide Time: 20:20)



So, let us start with the biochemical oxygen demand test or BOD test. Now, this is a standard test that is used to determine the biodegradation potential of any wastewater. So, this has been in existence for probably 100 years or so (it was selected as a standard method in 1908). The standard test conditions are for 5 days. The temperature is standardized to 20℃. I think it started in the UK, and it has been more or less unchanged since then.

We have made changes in India, and I will talk about that at the end of this (topic). So, this standardized test has several parts to it. Now, you know that wastewater, whether it is municipal wastewater or sewage or any industrial wastewater will have varying degrees of biodegradable material. If you think about municipal wastewater, we often take a ratio of 2, it may be 3 or even 4 (COD:BOD ratio).

And industrial wastewater can be completely non-biodegradable or completely degradable. So, for example, wastewater coming out of, let us say, a food processing plant, will have completely biodegradable material. Something coming out of a paper and pulp industry may be a mix of biodegradable and non-biodegradable material. Something coming out of a pesticide manufacturing plant may have no biodegradable material, it may have only toxic chemicals and so on. So, depending on the nature of the wastewater, you will have varying degrees of biodegradation potential of the material that is in the wastewater. Now, you have to start with some kind of guestimate of how much of the material is going to be biodegradable. So, from this point onwards, I will take the example of municipal wastewater, because that is what we deal with for the most part.

Let me also add a few more things here. BOD test bottles come in a standard size of 300 mL. So, if your municipal wastewater, we know, broadly speaking, it may have about 300 to 500 mg/L of COD, and maybe half of it is biodegradable. So, it has to be diluted. You cannot add the entire thing, because, you may be knowing that the DO concentration in water cannot exceed 14 to 15 mg/L. Dissolved oxygen has very low (saturation) concentration in water. Oxygen is considered a sparingly soluble gas, and the maximum concentration is, let us say, at 0℃, no salinity; you will get around 14 to 15 mg/L of oxygen. And under our test conditions of 20 ℃, your maximum DO level is going to be around 10 to 11 mg/L.

Now, when we are monitoring the DO levels; this entire test is based on monitoring the DO levels during the test period of 5 days. So, we have to do 2 things. The first thing we need to do is to dilute our wastewater, because if we take undiluted wastewater, we will get a DO level of 0 at the end of the test period, because it has highly biodegradable organic matter. So, the first thing we do is dilute it.

So, in a 300 mL bottle, you will probably add about 10 to 30 mL of wastewater to stay within this range of DO drop. And I will come to more details about the DO drop in a little bit. What is the remaining content of this 300 mL bottle? After adding your original sample in a very small amount, maybe 10 to 30 mL, maybe 50 mL, you will fill the rest of the bottle with dilution water. This dilution water contains all essential inorganic materials. We have already gone through the law of the minimum. In this case, we design our analytical methods to ensure that these tests are carbon-limiting only. All the other inorganic nutrients: nitrogen, phosphorus, sulphur, iron, calcium, magnesium, manganese, you name it, any of the nutrients have to be in surplus amount, and it should be limited by carbon only to ensure that none of these micronutrients will limit bacterial growth.

So, you are in a sense optimising the growth of the bacteria and ensuring that only one nutrient, in this case, the carbon containing substrate is going to be the limiting nutrient. So, that is the first thing. The second thing is, if you are dealing with industrial wastewater, let us say, a pesticide. So, let us say we have wastewater from a pesticide manufacturing plant. Now, this toxic material is not going to contain any microbes to begin with. So, you may have to add microbial seed to ensure that there is at least a good initial bacterial population. On the other hand, in municipal sewage, we do not need to add microbial seed, because sewage has millions to billions of cells/mL. So, no need for adding other seed. There is a sufficient native population, sufficient amount of native bacteria that will be capable of degrading the organics in the wastewater.

So, like I said, this is the first part of the test. So, one more thing to notice that the BOD bottle is, basically it is manufactured in a particular way. The main difference between other bottles and a BOD bottle is at the top end of the bottle. So, it is a cylindrical glass borosilicate bottle, and the top part of the bottle has an inverted frustum of a cone. So, it is got an inverted top and the stopper is also made of glass, but it is frosted glass. And when you fill the bottle, the bottle is filled to the brim. This glass topper is literally dropped into the bottle to create what is called a water seal. Now, this water seal will prevent any reaeration during the incubation period. In fact, there is one more point that I need to mention that once the dilution water is added, it is also aerated. So, the aeration is done prior to filling the bottle, to ensure that this dilution water is completely saturated with dissolved oxygen.

So, when it is added and the water seal is created, no further reaeration is allowed during the incubation period of 5 days. Now, these bottles are then incubated in the dark. So, we normally put them in an incubator shaker. These bottles are kept in shaking systems within the incubator, and there is no light inside the incubator. And this is also a very essential part of the test. Light has to be kept out, and this is to prevent algal growth in the bottles. Because, if you are taking, let us say, a natural river water sample, it will contain algal cells. Now, these algal cells will grow if light is present; and if they grow, they will contribute to the oxygen levels in water. So, we do not want that to happen, because that will create interference in the results of the tests. So, that is why incubation is always done in the dark, to keep the algal growth out of the system.

Then we come to the end of the 5-day test. At the beginning of the test, we take the DO concentration in the mixed bottle. So, you sacrifice the first bottle; determine the DO in the mixed contents of the bottle. At the end of 5 days, you measure the DO concentration again. So, you have a measure of the initial DO and the final DO. Now, DO these days can be measured in many different ways.

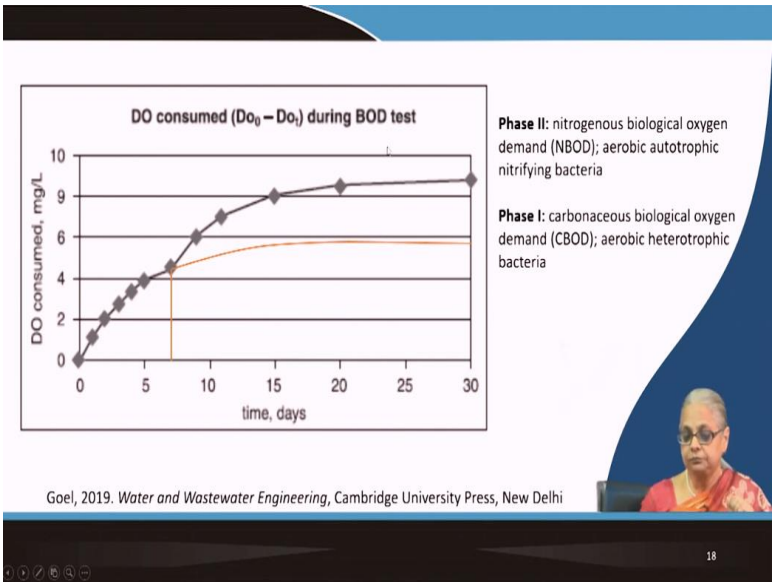
There are titrimetric methods, there are electrode sensor-based methods and we also have spectrophotometric methods. So, there are several methods that can be used for measuring DO concentration. Now, this $\Delta DO\ or$ drop from the initial DO to the DO after 5 days has to be measured. And if you suspect that your dilution water has some amount of organics in it, significant enough to contribute to the DO drop, then you need to run what is called a sample blank; and the Δ DO or the DO drop in the sample blank has to be subtracted from the DO drop in the sample.

So, these are the test conditions. Then we come to the next point and that is, how to determine whether the results of this test are useful or not? So, there are 2 thumb rules that are used over here. One is that the $\Delta DO\ or$ drop within the 5-day period should exceed or be equal to 2 mg/L. So, the minimum DO drop or change in DO concentration should be greater than 2 mg/L.

The minimum Δ DO should be greater than 2 mg/L. The maximum DO by definition will be DO saturation. So, these are the 2 conditions that will determine whether the results of the test can be useful or not.

Now, in India, we have 2 issues. The temperature in the ambient environment is much higher than what you have in comparison to a country like the UK. So, the temperature that the CPCB has chosen is 27 ℃ because reaction kinetics are much higher at a higher temperature. So the 3-day result; we are using 3 days rather than 5 days to get a result. And these 5-day results correspond to about 60 to 70% of the entire biodegradable fraction of the wastewater content. So, the 3-day result at 27 degree centigrade is expected to give similar results.

(Refer Slide Time: 31:59)



So, here we have the results of a hypothetical BOD test. Now, in a BOD test, we normally take only initial DO concentration and final DO concentration. And you can, once you know what your DO saturation is, you determine the concentration in the sample and you will get your DO consumed by subtracting it. So, $DO_0 - DO_t$. Like I said, that is a simple 2-point test.

But supposing you were a little more ambitious and you were to set up your test for 1 month and take samples at more frequent intervals. So, supposing you were to take a sample at time t = 0. For 7 days, you were to take samples almost every day, and then with lesser frequency over a 30-day period. If you were to do that, you would get a curve that has this shape. Now, what we are doing in a BOD test is just 5 days; that is a standard BOD test.

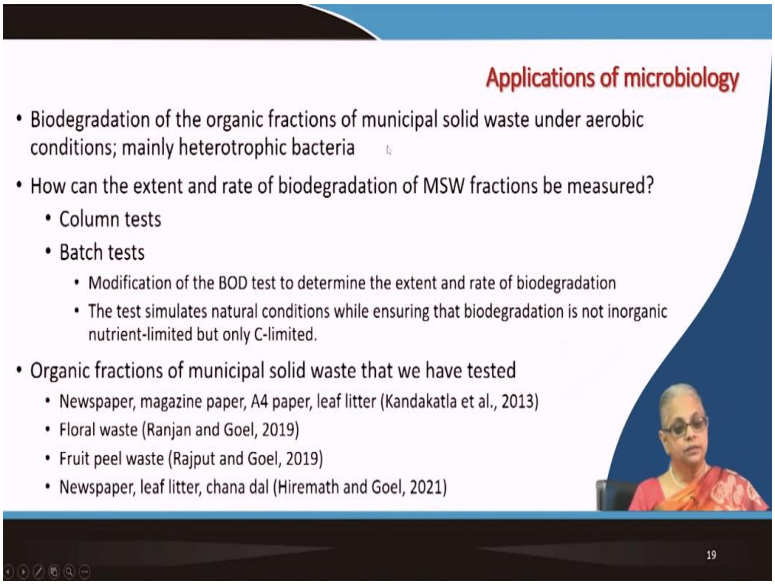
You can see, there is a slight inflection in the curve at about 7 days. I am not teaching you all of these, but you can always refer to any textbook about water pollution. But there are 2 parts to the BOD test, which is not captured in the 5-day BOD test. Now, in the 5-day BOD test, what happens is that you have a very high concentration of organic matter, but you also have a

significant concentration of nitrogen (organic nitrogen). I have already mentioned that nitrifying bacteria are slow growers and they are autotrophs. Aerobic heterotrophic bacteria are fast growing bacteria, and in the presence of oxygen, they will multiply very fast, as long as the carbon containing substrate is present. So, in this first week of the BOD test, aerobic heterotrophic bacteria are going to dominate. Their food supply is higher, oxygen levels are high and in comparison, to the nitrifying bacteria, they are going to grow faster. So, when they grow faster, their population will dominate, and that is what we are calling phase 1 of the BOD test. So, this is what we call the carbonaceous biological oxygen demand or biochemical oxygen demand (CBOD) and this is entirely being consumed by aerobic heterotrophic bacteria.

Then we come to the second part of this curve, where there is an inflection. This second phase is when your carbon-containing substrate is beginning to dwindle, oxygen levels are still reasonably high, enough to keep this group of bacteria happy. Aerobic autotrophic nitrifying bacteria will still continue to grow under these conditions, because now they are no longer outcompeted by the heterotrophic bacteria. So, these slow growers will start making their impact felt in the second phase, which is not captured in the standard BOD test. So, this inflection reflects what we call nitrogenous biological or biochemical oxygen demand (NBOD). So, if you remember what I said, ammonia plus oxygen is being converted to nitrite and nitrate. So, this second phase is exactly that reaction where ammonia is being converted to nitrite and nitrate, and oxygen is consumed in that process.

So, this is the BOD test if it were to be carried out over a long period of time. And this orange line which is an extension of the first part of this curve, is telling you what the ultimate carbonaceous BOD (CBOD) is. So, this is the ultimate carbonaceous BOD, and the remaining part is the nitrogenous BOD (NBOD).

(Refer Slide Time: 36:38)



Let us now come to the second part. So, now we can take a look at another test. So, using the same principle as the BOD test, we have now come up with an aerobic biodegradation test to determine, or rather, let me put it another way; using the same principles that are part of the BOD test, we have taken the same ideas and transferred them to measuring the biodegradable fractions or the biodegradable potential for different organic fractions of municipal solid waste under aerobic conditions.

So, here again, aerobic heterotrophic bacteria are being utilized, even though our results tell us a slightly different story, but I will come to that later. So, the question that we are trying to answer in these tests is, how can we measure the extent and rate of biodegradation of the organic fractions of municipal solid waste? Now, what I did not mention in the previous talk about BOD test; with the kind of data that I showed you over there, you can come up with the reaction rate constants, which are very important when you are designing treatment processes, because the design is based on our understanding of the reaction kinetics.

So, the same thing is done over here. So, you can do it in column studies or you can do it in batch studies. It is much simpler to do it in the batch studies, because you have more control over all the parameters, and it is much easier to measure the various parameters that you need to. So, like I said, it is a modification of the BOD test, and we are doing 2 things; we are measuring the biodegradable fraction, and we are measuring the rate at which biodegradation is happening.

The test simulates natural conditions just like the BOD test with one major difference, and that is, we are ensuring that the system is carbon limited only, that the microbes are growing under carbon limiting conditions and all other inorganic nutrients are there in excess of the microbial requirement. So, then we come to the organic fractions of the municipal solid wastes that we have already tested.

So, we have done (tested) newspaper, magazine paper, different types of paper and remember that newspaper has very high lignin content. It is more or less pulp that has; it is basically the process of creating newspaper is called mechanical pulping. There is very little chemical addition, bare minimum of chemical addition in comparison to magazine paper and A4 paper which have high degree of chemical processing along with mechanical pulping.

So, the weight, gram per gram for newspaper versus magazine and A4 paper; you will find that there are very different quantities of organic matter in each of these paper fractions. So, those were measured, and so was leaf litter. We have also done studies with floral waste, fruit peel waste and we have also repeated studies with newspaper, leaf litter and chana dal. Chana dal is a leguminous grain, a legume or a pulse that comes from plants.

(Refer Slide Time: 40:15)

Sample		Floral waste		Leaf litter (Kandakatla, et al., 2010)	
Days of Incubation		6th Aug. to 3rd Nov. 2015 (89 days)		89 days	
		Biotic	Control	Biotic	Control
pH	Initial	6.92 ± 0.11	6.905 ± 0.015	6.94 ± 0.06	6.95
	Final	7.11 ± 0.12	7.025 ± 0.095	6.98 ± 0.13	7.02
Conductivity (µS/cm)	Initial	1367.5 ± 31.5	1329.5 ± 12.5	1460 ± 300	1300
	Final	1516.25 ± 6.75	1499 ± 10	4540 ± 150	3900
TSS (g/L)	Initial	0.57 ± 0.02	0.44 ± 0.05	0.82 ± 0.05	0.71
	Final	0.37 ± 0.02	0.38 ± 0.01	0.2 ± 0.02	0.61
	Degradation	35.08%	13.63%	75.6%	14.08%
VSS (g/L)	Initial	0.55 ± 0.02	0.46 ± 0.02	0.75 ± 0.03	0.83 ± 0.03
	Final	0.31 ± 0.03	0.39	0.18 ± 0.02	0.68
	Degradation	43.64%	15.21%	76%	18.07%

Ranjan, Ved Prakash and S Goel [2019] Biodegradation of floral waste under aerobic conditions with different microbial inocula and aeration methods, *Treatment and disposal of solid and hazardous waste*, Capital Books, Delhi and Springer, Germany.

18

So, these are some of the results that we have seen and I will not go into any of the details because of lack of time, but you can refer to the papers which are referenced at the end of this slide, at the end of this PPT. So, I will just point out some of the major results. We were measuring TSS and VSS. TSS means total suspended solids; VSS means volatile suspended solids.

Volatile suspended solids can be associated with the microbial biomass or with the organic content of the material itself. So, the loss of VSS is the main result in our case, because that is the biodegradable fraction that is lost, that is mineralised. So, if you remember our key equation of

$C_6H_{12}O_6 + O_2 \rightarrow CO_2 + H_2O$

so, here we have

$Organic\ Matter + O_2 \rightarrow CO_2 + H_2O.$

So, any loss in VSS is basically attributed to mineralisation of the carbon. So, we got 43.6% loss of organic matter in floral waste as compared to 76% in leaf litter. So, just comparing these results tells us that floral waste, (this is) very difficult to understand even; is more resistant to biodegradation compared to leaf litter and the tannin lignin content was more biodegradable.

Now, this is kind of contrary to what you might expect, for the simple reason that the tannin lignin in floral waste is likely to be low molecular weight compared to leaf litter which has high molecular weight tannin and lignin. Now, this high molecular weight material is likely to be resistant compared to the floral waste. Now, there are other studies in the literature, which have also pointed out that floral waste is less biodegradable compared to leaf litter and other materials like that.

(Refer Slide Time: 42:31)

Sample		Floral waste (with passive aeration)		Floral waste (with no aeration)	
Types of bacterial seed added		Soil inoculum		Cow dung supernatant	
Days of Incubation		6th Aug. to 3rd Nov. 2015 (89 days)		23rd Jan. to 20th April (89 days)	
		Biotic	Control	Biotic	Control
pH	Initial	6.92 ± 0.11	6.905 ± 0.015	6.58 ± 0.05	6.57
	Final	7.11 ± 0.12	7.025 ± 0.095	6.31 ± 0.19	6.40
Conductivity (µS/cm)	Initial	1367.5 ± 31.5	1329.5 ± 12.5	1377.67 ± 8.33	1386
	Final	1516.25 ± 6.75	1499 ± 10	1991.67 ± 18.33	1595
	Increment	**13.99%**	**11.30%**	**44.57**	**15.08**
TSS (g/L)	Initial	0.57 ± 0.02	0.44 ± 0.05	0.58 ± 0.02	0.55
	Final	0.37 ± 0.02	0.38 ± 0.01	0.34 ± 0.01	0.50
	Degradation	**35.08%**	**13.63%**	**41.38%**	**9.1%**
VSS (g/L)	Initial	0.55 ± 0.02	0.46 ± 0.02	0.56 ± 0.02	0.52
	Final	0.31 ± 0.03	0.39	0.31± 0.02	0.48
	Degradation	**43.64%**	**15.21%**	**44.64%**	**7.7%**

19

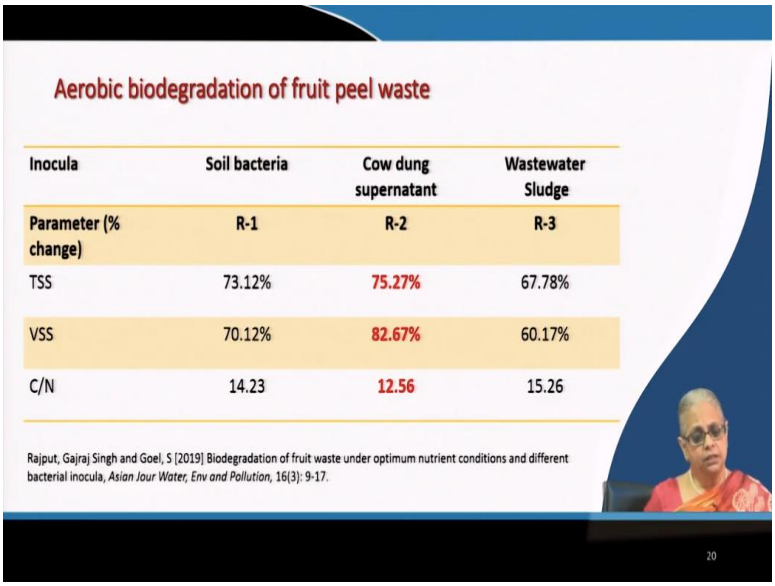
So, here we have a few more results. And in this case, 2 different sets of conditions were examined with floral waste. So, in the first case, we had floral waste with passive aeration.

Passive aeration was provided by using vented caps on the test tubes. So, air could pass in and out, without any bacterial contamination; because the tortuous path of air would prevent the bacteria from entering, but allow air to enter and the inoculum in this case was soil.

In the second case, the BOD bottles were used along with floral waste. So, no aeration during the incubation period was provided; and cow dung was used as the source of bacterial seed and you can see the difference. This condition without aeration and with cow dung as the inoculum, gave better results, not a huge difference, but significantly better results compared to floral waste with aeration and soil inoculum, in terms of TSS as well as VSS.

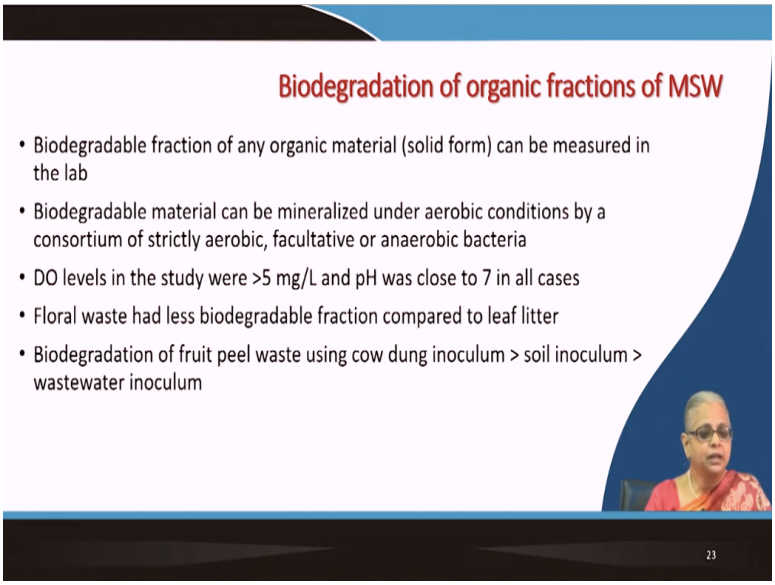
VSS, the results are insignificantly different; they are almost equal. And in terms of tannin lignin removal also, it is no significant difference. So, whether you provide aeration in terms of vented caps or whether you use cow dung or soil inoculum, at least in this set of experiments, there was no significant difference.

(Refer Slide Time: 44:06)



In another set of experiments with fruit peel waste, there was a significant difference with different inocula. So, soil bacteria gave us 70% removal in terms of VSS; cow dung supernatant gave us 83% removal of VSS; and wastewater sludge which is teeming with bacteria and which we thought might give good results gave us only 60% removal of VSS. It made a small difference to the C/N ratio as well. But what is most important in terms of the measurement was the biodegradable fraction which is measured in terms of change in VSS.

(Refer Slide Time: 44:52)



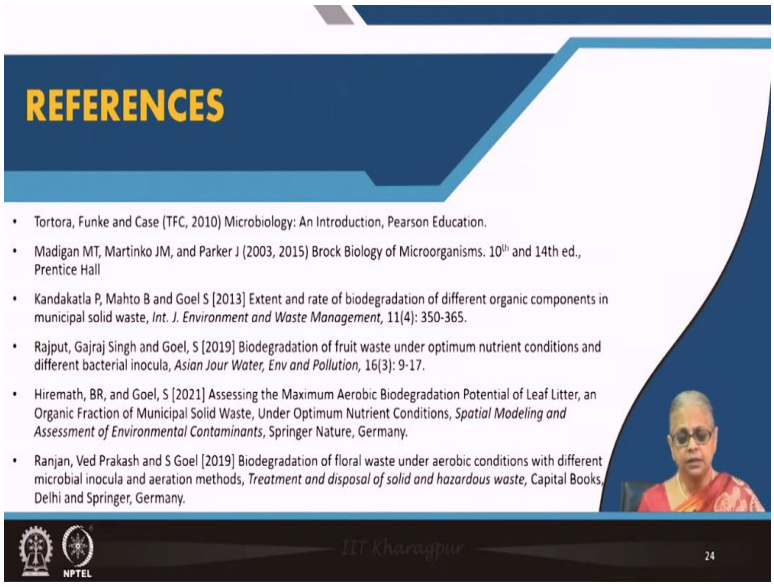
So, to summarize some of our major findings, we have a method for determining the biodegradable fraction of any organic material in the lab. This biodegradable organic material can be mineralized under aerobic conditions by a consortium of bacteria. Now, these bacteria, like I said, can be from soil, they can be from cow dung, they can be from wastewater sludge or any other group of organisms from any other part of the environment.

These consortia of bacteria will have aerobic, facultative, as well as anaerobic bacteria. We have done some stoichiometric calculations of the level to which oxygen is likely to be used, if it was a strictly aerobic pathway that was being utilized by all the bacteria. So, we have clear indications from our results that it was not just the aerobic bacteria that were responsible for the biodegradation of this material, but a combination of all of these bacteria, which are often present in any of these consortia.

Then we have, in terms of DO levels in all of our tests, we had adequate DO in the water, in the media. So, it was always greater than 5 mg/L. And the pH was maintained using a buffer that provided neutral pH as well as phosphate or phosphorus for growth. So, in all cases, there was minimal change in pH, and it was always just around 7. We found that floral waste had less biodegradable material compared to leaf litter.

And the biodegradation of fruit peel waste was affected by inoculum type, but not floral waste. So, these are some of the major findings. And this is just a very small snapshot of everything that has been done, but for obvious reasons of time limitations, I am not going too far.

(Refer Slide Time: 47:13)



And these are some of the references. Anyone who is interested in this material can refer to the papers that are listed over here along with the textbooks. Thank you.