

**Biological Process Design for Wastewater Treatment**  
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**Lecture: 08**  
**Wastewater Characterization-V**

Welcome everyone in this NPTEL Online Certification Course on Biological Process Design for Wastewater Treatment. So, in the previous lectures we have studied regarding various physico chemical parameters and then biological or biochemical water quality parameters. In particular, in the last few lectures we studied regarding the chemical oxygen demand, then biochemical oxygen demand, then total Organic Carbon, theoretical oxygen demand etc.

Now, in today's lecture, we will try to summarize whatever we have learned with this by taking few examples and solving some problems related to BOD, COD, ThOD and TOC.

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**Problem**

- If 250 mg/L Glucose is completely oxidized to  $\text{CO}_2$ , find:
  - a) Theoretical Oxygen Demand (ThOD)
  - b) Chemical Oxygen Demand (COD)
  - c) Ultimate Biochemical Oxygen Demand ( $\text{BOD}_u$ )
  - d)  $\text{BOD}_5$ , if  $k_{20}=0.023 \text{ day}^{-1}$
  - e) Total Organic Carbon (TOC)

So, the first problem that we are taking today, the question is given here that a wastewater sample contains 250 milligram per liter of glucose and during the test it is oxidized to  $\text{CO}_2$  suppose, so what we have to do is that? We have to find that theoretical oxygen demand that chemical oxygen demand the ultimate biochemical oxygen demand that is  $\text{BOD}_u$ ,  $\text{BOD}_5$  if  $k_{20}$  is known, we could have asked the  $\text{BOD}_3$  also.

So, this is possible and then find out the total Organic Carbon also. So, this actually tests represents a standard condition in which a many times dissolved glucose and we try to check whether our instruments are calibrated well, we are analyzing the all these things correctly or

not? So, this is the test through which we can cross check out the testing systems with respect to all these parameters.

So, coming back to the problem, this is 250 milligram per liter of glucose.

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**Solution**

a. We begin by writing a balanced equation for the reaction.

$$\checkmark \text{C}_6\text{H}_{12}\text{O}_6 + \text{6O}_2 = \text{6CO}_2 + \text{6H}_2\text{O}$$

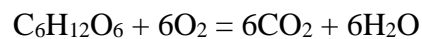
$\checkmark$   $\frac{180}{\text{C}_6\text{H}_{12}\text{O}_6}$        $\frac{192}{\text{6O}_2}$        $\frac{360}{\text{6CO}_2}$        $\frac{72}{\text{6H}_2\text{O}}$

Glucose	Oxygen
$6\text{C} = 6 \times 12 = 72 \checkmark$	$(6)(32) = 192$
$12\text{H} = 12 \times 1 = 12 \checkmark$	
$6\text{O} = 6 \times 16 = 96 \checkmark$	
<u>Total = 180</u>	

Thus, it takes 192 g of oxygen to oxidize 180 g of glucose to CO<sub>2</sub> and H<sub>2</sub>O.

The ThOD of 250 mg/L of glucose is  $(250 \text{ mg/L of glucose}) \times \left( \frac{192 \text{ g of O}_2}{180 \text{ g glucose}} \right) = \underline{266.7 \text{ mg O}_2/\text{L}}$

We begin by writing a balanced equation for the reaction.



So, what we do is that first we write a balance equation for the reaction. So, glucose formula is C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> and it is converted into CO<sub>2</sub> and H<sub>2</sub>O and we balance will there be 6 6 these will be the coefficient that will be coming. Now, for glucose if we try to find out what its molecular weight, so it has 6 carbon atoms 12 Hydrogen atoms 6 oxygen atom, so we can see here this is 6 into 12 this is 12 into 1 and then here again 6 into 16, so this is there.

So, if we calculate this is the respect to molecular weight and then the total is 180. So, that means, the glucose is having 180 molecular weight. Similarly, the oxygen which is actually getting consumed, so for this it will come out to be 192 value. Now, since we learn to concentrate on the oxygen initially and then carbon also later on, so does it takes 192 gram of oxygen to oxidize 180 gram of glucose to CO<sub>2</sub> and H<sub>2</sub>O.

So, that theoretical oxygen demand is that 250 milligram of glucose will require 250 milligram per liter of glucose into 192 gram of oxygen divided by 180 gram of glucose. So,

this and if we solve this, we will be getting 266.7 milligram of oxygen per liter. So, the ThOD value is 266.7 milligram of oxygen per liter.

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**Cont....**

b. Since no substance other than Glucose is present, therefore COD is equal to ThOD.  
 $COD = 266.7 \text{ mg/L O}_2$ .

c. Glucose is itself biodegradable. So, Ultimate BOD = COD = 266.7 mg/L O<sub>2</sub>.

d.  $BOD_5 = BOD_{ult} [1 - \exp(-kt)]$  if  $k = 0.023 \text{ day}^{-1}$   
 $BOD_5 = 0.6833 \times BOD_{ult} = 0.6833 \times 266.7 = 182.25 \text{ mg/L O}_2$ .

e. TOC present in 1 mg of C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> =  $6 \times 12 / 180 = 0.4 \text{ mg TOC / mg C}_6\text{H}_{12}\text{O}_6$   
 TOC present in 250 mg/L of C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> =  $250 \text{ (mg C}_6\text{H}_{12}\text{O}_6\text{/L)} \times 0.4 = 100 \text{ mg TOC/L}$

$$BOD_5 = BOD_{ult} [1 - \exp(-kt)] \quad \text{if } k = 0.023 \text{ day}^{-1}$$

$$BOD_5 = 0.6833 \times BOD_{ult} = 0.6833 \times 266.7 = 182.25 \text{ mg/L O}_2$$

**Solution**

a. We begin by writing a balanced equation for the reaction.

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$$

$180$	$192$		
Glucose		Oxygen	
$6C = 6 \times 12 = 72$	$12H = 12 \times 1 = 12$	$(6)(32) = 192$	
$6O = 6 \times 16 = 96$	<u>Total = 180</u>		

Thus, it takes 192 g of oxygen to oxidize 180 g of glucose to CO<sub>2</sub> and H<sub>2</sub>O.

The ThOD of 250 mg/L of glucose is  $(250 \text{ mg/L of glucose}) \times \left( \frac{192 \text{ g of O}_2}{180 \text{ g glucose}} \right) = 266.7 \text{ mg O}_2\text{/L}$

Now, the b case is that we had to find out COD, since no substance other than glucose is present. Therefore, the COD value will be equal to ThOD and then the glucose also does not contain any nitrogen. So, glucose for glucose COD is equal to ThOD itself a COD is also 266.7 milligram per liter. Now, again glucose itself is totally biodegradable. So, the ultimate BOD will also be equal to COD itself.

So, that means the  $BOD_u$  is also equal 266.7 milligram oxygen per liter. So, this is also now known. Now we have to find out the  $BOD_5$ , it was asked that find out the  $BOD_5$   $k_{20}$  is known at  $k$  at 20 degrees Centigrade known. Now, here again we can see we can use this formula which was given earlier and  $k$  is also known. So,  $BOD_5$  is equal to 0.6833 into.

So, here if we put this  $k$  value here, and then we can find out this, this term will come out 0.6833 and ultimately 182.25 milligram of oxygen per liter of the sample that will be the  $BOD_5$  value. Similarly, we could have found out  $BOD_3$  also it is possible to find out  $BOD_3$  also. But for that we you we required the  $k$  value to be known at 27 degrees centigrade, because  $BOD_3$  is determined at 27 degrees centigrade.

So, it was possible to calculate from  $k_{20}$  also given the theta value is known. So, if theta value is known, we could have calculated using this formula and this theta value is generally like 1.047 suppose, so  $k_{27}$  was also known, so we could have determined the  $BOD_3$  also using this particular formula  $1 - \exp(-k_{27} \times 3)$ , so this is known, so this is already known so we can find out  $BOD_3$  also though it were not asked in this question.

Now, where to find out ultimately we had to find out the TOC present in the one milligram of this. So, from the equation which was given here, we can see that the 6 into 6 into 12 that means 72 milligram of carbon is present in 180 milligram of glucose. So, using this formula, we find out that TOC present in one milligram of this is 0.4 per this.

So, from 250 we can calculate and it will be come out 100 milligram of TOC per liter. So, through this we can calculate to this, this particular question gives lot of idea that how we can calculate the different values if we knew that before and that what is a present in the water and what is the concentration.

So, this was a simple case where fully biodegradable glucose was only present in the water and with no other compound present in the water. Now, there may be a case where a number of compounds are present in the water and with different concentration. So, let us take that example.

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**Problem**

A wastewater contains all of the following: 150 mg/L ethylene glycol; 100 mg/L phenol; 125 mg/L ethylene diamine hydrate; and 40 mg/L sulfide ( $S^{2-}$ ).

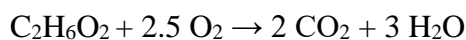
(a) Compute the COD. ✓  
(b) Compute the ThOD. ✓  
(c) Compute the TOC. ✓

**Solution.**

(a) Ethylene glycol

$$C_2H_6O_2 + 2.5 O_2 \rightarrow 2 CO_2 + 3 H_2O$$
$$COD = \frac{2.5 \times 32}{62} \times 150 \frac{mg}{L} = 193.5 \frac{mg}{L} \quad | \quad ThOD = 193.5 \frac{mg}{L}$$
$$TOC = \frac{2 \times 12}{62} \times 150 \frac{mg}{L} = 58.1 \frac{mg}{L}$$

Ethylene glycol



$$COD = \frac{2.5 \times 32}{62} \times 150 \frac{mg}{L} = 193.5 \frac{mg}{L}$$

$$TOC = \frac{2 \times 12}{62} \times 150 \frac{mg}{L} = 58.1 \frac{mg}{L}$$

$$ThOD = 193.5 \frac{mg}{L}$$

So, in this problem is wastewater contains all of the following that means, it contains 150 milligram per liter of ethylene glycol, 100 milligram per liter of phenol, 125 milligram per liter of ethylene diamine hydrate and 40 milligram per liter of sulfide that means, 4 different compounds are present in the water. Now, we have to calculate the COD, ThOD, and TOC.

So, let us what we do is that we try to solve this problem by taking individual compounds first and then add together different values. So, we have to remember we have to find out COD, ThOD, and TOC. So, for each compound COD, ThOD, and TOC values will be formed out and then later on they will be added.

So, for the first compound the ethylene glycol which formula is  $C_2H_6O_2$ , the reaction is written here. So, this is a compound which cannot contain nitrogen it contains only carbon, hydrogen and oxygen. So, we can easily write. So, we can see that 2.5 moles of oxygen is required per mole of ethylene glycol.

Now, the COD can be calculated as the remember the molecular weight of ethylene glycol is 62 milligram. So, from this we can get the 2.5 into 32 per mole which is 62 and the wastewater sample contains 150 milligram of ethylene glycol. So, that means a COD which will be incurred because of ethylene glycol is 193.5 milligram per liter.

Now, we have to find out that ThOD, for ThOD the same formula applies. So, therefore, the ThOD is same as COD 193.5 milligram per liter. Now, the TOC value we can see that 62 the molecular weight contains 2 carbon element. So, that means 2 into 12 divided by 62 which is a molecular weight of ethylene glycol into 150 which is the total concentration.

So, that means, the TOC value for ethylene glycol is 58.1 if it would have been present individually, so this is one compound is done.

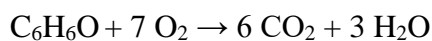
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(b) **Phenol**  
 $C_6H_6O + 7O_2 \rightarrow 6CO_2 + 3H_2O$  ✓  
94  
 $COD = \frac{7 \times 32}{94} \times 100 \frac{mg}{L} = 238.3 \frac{mg}{L}$  | ThOD=238.3 mg/L |  $TOC = \frac{6 \times 12}{94} \times 100 \frac{mg}{L} = 76.6 \frac{mg}{L}$

(c) **Ethylene diamine hydrate**  
 $C_2H_{10}ON_2 + 2.5O_2 \rightarrow 2CO_2 + 2H_2O + 2NH_3$   
78  
 $COD = \frac{2.5 \times 32}{78} \times 125 \frac{mg}{L} = 128.2 \frac{mg}{L}$  |  $TOC = \frac{2 \times 12}{78} \times 125 \frac{mg}{L} = 38.5 \frac{mg}{L}$

### Phenol

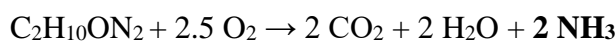


$$COD = \frac{7 \times 32}{94} \times 100 \frac{mg}{L} = 238.3 \frac{mg}{L}$$

$$TOC = \frac{6 \times 12}{94} \times 100 \frac{mg}{L} = 76.6 \frac{mg}{L}$$

$$ThOD = 238.3 \frac{mg}{L}$$

### Ethylene diamine hydrate



$$COD = \frac{2.5 \times 32}{78} \times 125 \frac{mg}{L} = 128.2 \text{ mg/L}$$

$$TOC = \frac{2 \times 12}{78} \times 125 \frac{mg}{L} = 38.5 \text{ mg/L}$$

Now, let us go to phenol. So, let us calculate for phenol to for phenol the reaction is written here. Phenol requires 7 mole of oxygen for its complete oxidation to CO<sub>2</sub> and H<sub>2</sub>O. Now, the amount of phenol in the wastewater sample is given to be 100 milligram per liter. So, here again the COD is calculated COD is equal to 7 into 32 divided by 94 which is the molecular weight of phenol. Now, so this is 238.3.

The ThOD value will also be same as the COD value because phenol does not contain any nitrogen or sulfur and now, the TOC value is calculated and this is equal to 6 into 12 because, 6 moles of carbon are present the 6 into 12 divided by 94 into 100 this is 76.6. So, this is also easier and we have calculated for phenol.

Now, going further for ethylene diamine hydrate. So, this is the where difference will come. Now, remember for COD and ThOD in the previous lecture we had studied that when we write a balance equation for COD, nitrogen is always only converted into ammonia whereas, for ThOD we convert the nitrogen into HNO<sub>3</sub>. So, this is the difference.

So, first what we do is that we write an equation which will be used where the nitrogen is converted into ammonia and we do the balance. So, this is the molecular formula for ethylene diamine hydrate, which is having a molecular weight of 78 if we calculate, and we convert this nitrogen into ammonia, remember ammonia and this will be used for calculation of COD and TOC only not for ThOD.

And for ThOD will have to convert this into HNO<sub>3</sub>, for HNO<sub>3</sub> then only we can use now, we could calculate the COD value and COD is since only 2.5 moles of oxygen are required. So, 2.5 into 32 divided by 78 into 125 milligram per liter which is the actual concentration of ethylene diamine hydrate present in the water sample in the present question.

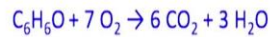
So, the COD value comes out to be 128.2 milligram per liter. From the same equation the TOC value is calculated and TOC value 2 into 12 because ethylene diamine hydrate contains only 2 moles of carbon. So, 2 into 12 divided by 78 into 125 this is 38.5.

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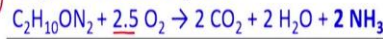
## Cont....

### Phenol



$$\text{COD} = \frac{7 \times 32}{94} \times 100 \frac{\text{mg}}{\text{L}} = 238.3 \frac{\text{mg}}{\text{L}} \quad \text{ThOD} = 238.3 \frac{\text{mg}}{\text{L}} \quad \text{TOC} = \frac{6 \times 12}{94} \times 100 \frac{\text{mg}}{\text{L}} = 76.6 \frac{\text{mg}}{\text{L}}$$

### Ethylene diamine hydrate



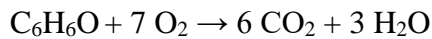
$$\text{COD} = \frac{2.5 \times 32}{78} \times 125 \frac{\text{mg}}{\text{L}} = 128.2 \frac{\text{mg}}{\text{L}} \quad \text{TOC} = \frac{2 \times 12}{78} \times 125 \frac{\text{mg}}{\text{L}} = 38.5 \frac{\text{mg}}{\text{L}}$$

### For ThoD



$$\text{ThOD} = \frac{6.5 \times 32}{78} \times 125 \frac{\text{mg}}{\text{L}} = 333.3 \frac{\text{mg}}{\text{L}} \quad \text{ThOD} = 333.3 \text{ mg/L}$$

## Phenol

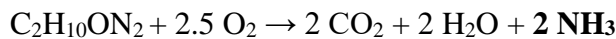


$$\text{COD} = \frac{7 \times 32}{94} \times 100 \frac{\text{mg}}{\text{L}} = 238.3 \text{ mg/L}$$

$$\text{TOC} = \frac{6 \times 12}{94} \times 100 \frac{\text{mg}}{\text{L}} = 76.6 \text{ mg/L}$$

$$\text{ThOD} = 238.3 \text{ mg/L}$$

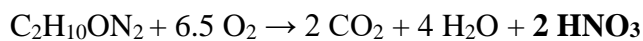
## Ethylene diamine hydrate



$$\text{COD} = \frac{2.5 \times 32}{78} \times 125 \frac{\text{mg}}{\text{L}} = 128.2 \text{ mg/L}$$

$$\text{TOC} = \frac{2 \times 12}{78} \times 125 \frac{\text{mg}}{\text{L}} = 38.5 \text{ mg/L}$$

For ThOD



$$\text{ThOD} = \frac{6.5 \times 32}{78} \times 125 \frac{\text{mg}}{\text{L}} = 333.3 \text{ mg/L}$$

Remember, we have not calculated ThOD for calculating ThOD we use this equation. So, for calculating the ethylene diamine hydrate for ThOD this equation use and here this is the formula for ethylene diamine hydrate. We see here because there are 2 moles of nitrogen and we have studied previously for each mole of nitrogen 2 additional mole of oxygen is required when we convert into HNO<sub>3</sub>.

So, we can see here, for COD and TOC only 2.5 oxygen was required, whereas here it is 6.5 and this is 4 difference and 4 is different because there are 2 moles of nitrogen. So 2 into 2, 4 additional moles of oxygen are required when we calculate for ThOD. So we can calculate what ThOD  $6.5 \times 32$  divided by  $78 \times 125$ , 333.3 this is the ThOD for the c case.

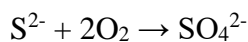
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**Cont....**

② Sulfide  
 $S^{2-} + 2O_2 \rightarrow SO_4^{2-}$

$COD = \frac{2 \times 32}{32} \times 40 \frac{mg}{L} = 80 \frac{mg}{L}$	ThOD = 80 mg/L	$TOC = \frac{0 \times 12}{32} \times 40 \frac{mg}{L} = 0 \frac{mg}{L}$
<b>Total COD = 640 mg/L</b>	<b>Total ThOD = 845.1 mg/L</b>	<b>Total TOC = 173.2 mg/L</b>

### Sulfide



$$COD = \frac{2 \times 32}{32} \times 40 \frac{mg}{L} = 80 \frac{mg}{L}$$

$$ThOD = 80 \text{ mg/L}$$

$$TOC = \frac{0 \times 12}{32} \times 40 \frac{mg}{L} = 0 \text{ mg/L}$$

Now we go back further for D case is where the sulfides is present in the water. The sulfide is converted into  $SO_4$ . And for this we required 2 moles of oxygen. So and sulfide itself contains 32 is the molecular weight 2 into 32 divided by 32 and 240. So 80 is the COD 80 is the ThOD and since there is no carbon, so the TOC value is 0. So, what we do is that? We add all the COD values.

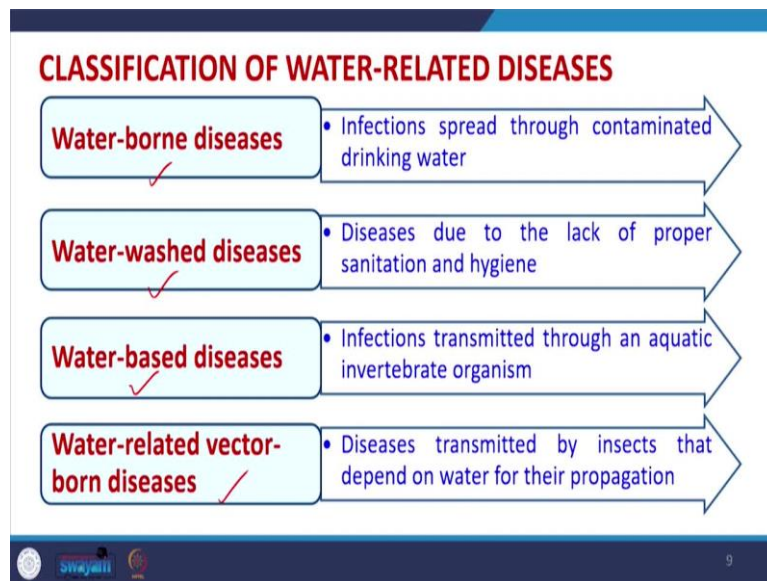
So the COD value was like 238.3, 128.2, then again here 80 so if we add on the COD value for all the 4 compounds, it will come out to 640 milligram per liter. Similarly, for ThOD, there will be some difference will be there. So, because the third compound ethylene, this ethylene diamine hydrate required 4 additional moles of oxygen, so ThOD value is higher than the COD value.

And this is because in the COD we get only converted into, nitrogen is converted into ammonia, whereas for ThOD we get convert that nitrogen into  $HNO_3$ . The TOC value is

173.2 milligram per liter. To this way, we can calculate the COD, ThOD, TOC values for complex wastewater samples also. So, this example give an idea.

Now of going further, we will try to some go to the fourth parameter which is called bacteriological parameter. And here we try to find out the different types of microorganism, but and this is so because there are different types of water related diseases which are occurring. So, there are essentially 4 types of diseases which are related to water.

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So and they are classified as water borne diseases, water washed disease, water based disease and water related vector borne diseases. So, these are the 4 classification of water related diseases. Now, infections spread through contaminated drinking water. So if any drinking water is contaminated, and the infection is spread through that, it is called waterborne disease.

Now, diseases which are caused due to lack of proper sanitation and hygiene, they are called water washed disease diseases, because we are not cleaning sanitation is not proper, hygiene is not proper, and because of which the diseases are occurring then it is called water washed. Then water based infections, which are transmitted through an aquatic invertebrate organism. So they are called water based diseases.

Then water related vector borne diseases are diseases transmitted by insects that depend upon water for their propagation.

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**FEW EXAMPLES OF WATER RELATED DISEASES**

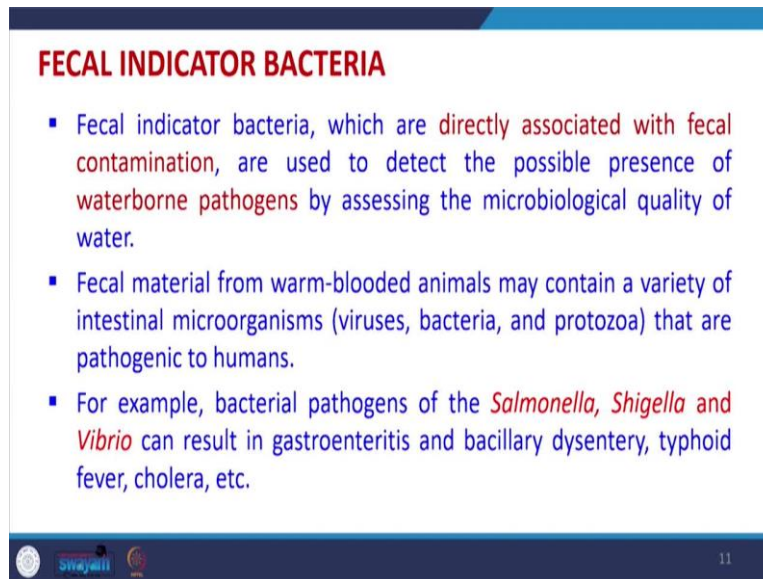
<b>WATER-BORNE DISEASES</b>	<ul style="list-style-type: none"><li>• Diarrhoeal, Parasitological, Skin, Eye diseases</li><li>• Typhoid Fever</li></ul>
<b>WATER-WASHED DISEASES</b>	<ul style="list-style-type: none"><li>• Ascariasis (=roundworm infection)</li><li>• Ancylostomiasis (=hookworm infection)</li></ul>
<b>WATER-BASED DISEASES</b>	<ul style="list-style-type: none"><li>• Schistosomiasis (Bilharzia)</li></ul>
<b>WATER-RELATED VECTOR-BORNE DISEASES</b>	<ul style="list-style-type: none"><li>• Malaria</li><li>• Lymphatic filariasis</li><li>• Onchocerciasis</li><li>• Japanese encephalitis</li></ul>

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The examples are given here are waterborne diseases include like diarrhea, then parasitological skin, there are many other skin eye diseases typhoid fever etc. Then there are water washed diseases also the roundworm, hookworm infections, then we have water based diseases also. The Bilharzia is one of the examples then water related vector borne the various vectors which are we depend upon water like malaria, then lymphatic filariasis, then there are other types of diseases which are water related vector borne diseases.

So all these are borne because of water related issues and for determining these diseases it is not that simple, water quality determination will not help. We require a specialist for determining such diseases and bacteria and set microcosms and thus be required Pathologies for determination of these diseases, and these vectors and other infections. Now, the simple indicator which is used in water or water quality estimation is called fecal indicator bacteria.

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**FECAL INDICATOR BACTERIA**

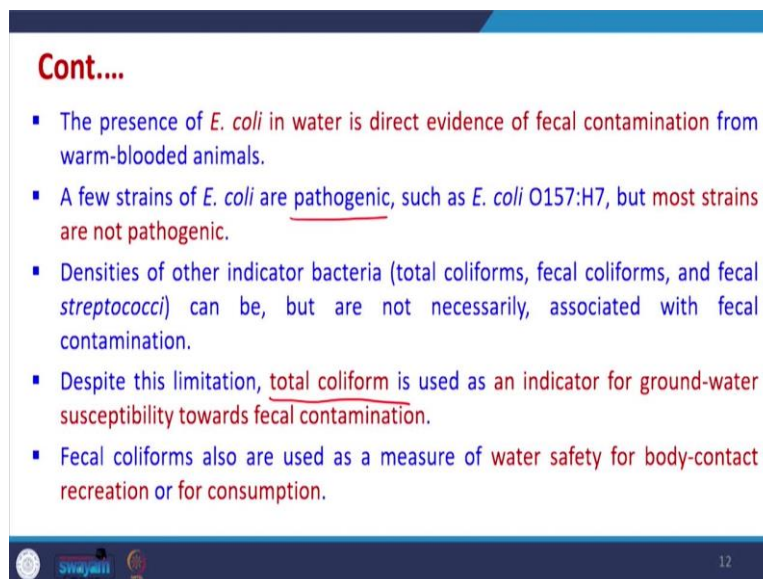
- Fecal indicator bacteria, which are directly associated with fecal contamination, are used to detect the possible presence of waterborne pathogens by assessing the microbiological quality of water.
- Fecal material from warm-blooded animals may contain a variety of intestinal microorganisms (viruses, bacteria, and protozoa) that are pathogenic to humans.
- For example, bacterial pathogens of the *Salmonella*, *Shigella* and *Vibrio* can result in gastroenteritis and bacillary dysentery, typhoid fever, cholera, etc.

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So we determine the fecal indicator bacteria, which is directly associated with fecal contamination, and it is used to detect the possible presence of water and borne pathogens. And this can be determined by simple water chemistry persons or simple persons who which are doing the analysis of water in the lab.

Fecal material from warm blooded animals may contain a variety of intestinal microorganisms, including viruses, bacteria and protozoa and which are pathogenic to humans. And the example include bacterial pathogens of salmonella, Shigella, and Vibrio that can result in various gastroenteritis and bacillary dysentery, typhoid fever, cholera, etc.

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**Cont....**

- The presence of *E. coli* in water is direct evidence of fecal contamination from warm-blooded animals.
- A few strains of *E. coli* are pathogenic, such as *E. coli* O157:H7, but most strains are not pathogenic.
- Densities of other indicator bacteria (total coliforms, fecal coliforms, and fecal streptococci) can be, but are not necessarily, associated with fecal contamination.
- Despite this limitation, total coliform is used as an indicator for ground-water susceptibility towards fecal contamination.
- Fecal coliforms also are used as a measure of water safety for body-contact recreation or for consumption.

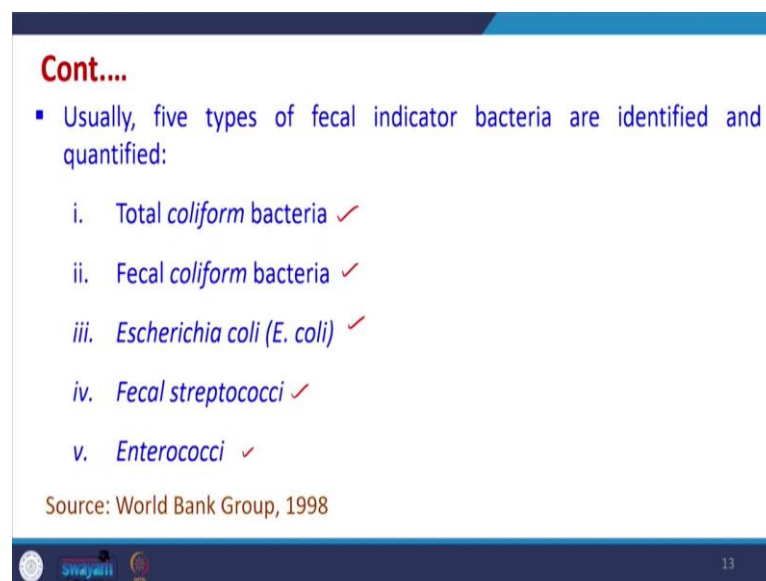
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What we do is that in water quality estimation, we estimate the presence of E. Coli in the water. And if the E. coli is present, it is a direct evidence that fecal contamination is there in the water. A few strains of E. coli are pathogenic, such as these, but most systems are not pathogenic, densities of other indicator bacteria like total coliform, fecal coliform and fecal streptococci, can be, but are not always associated with fecal contamination.

Despite this limitation, total coliform is one of the indicator for groundwater susceptibility towards fecal contamination and this is determined. So, you always try to find out that total coliform, fecal coliform also are used to measure water safety, for body contact recreation and for consumption.

So, in in those places like where the river water has to be used for bathing and also similarly in water parks, so that determination of these parameters is essential, before actually bathing should happen. So, this has to be determined daily and periodically it has to measured that so that we can check that water can be used for bathing, drinking et cetera or not.

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**Cont....**

- Usually, five types of fecal indicator bacteria are identified and quantified:
  - i. Total *coliform* bacteria ✓
  - ii. Fecal *coliform* bacteria ✓
  - iii. *Escherichia coli* (*E. coli*) ✓
  - iv. Fecal *streptococci* ✓
  - v. *Enterococci* ✓

Source: World Bank Group, 1998

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Usually five types of fecal indicator bacteria are identified and quantified. These include total coliform bacteria, fecal coliform bacteria, E. coli, fecal Streptococci, and enterococci. So, this is these are determined to check the vulnerability of water sample with respect to fecal indicator bacteria.



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**Cont....**

- Following methods can be used to test for indicator bacteria:
  - Total count of bacteria:
    - a. In this method, total number of bacteria present in a milliliter of water is counted. The sample of water is diluted; 1 mL of sample water is diluted in 99 mL of sterilized water.
    - b. Then 1 mL of diluted water is mixed with 10 mL of agar or gelatin (culture medium). This mixture is then kept in incubator at 37 °C for 24 h or at 20°C for 48 h.
    - c. After that, the sample is taken out from incubator and colonies of bacteria are counted by means of microscope.
    - d. The product of the number of colonies and the dilution factor gives the total number of bacteria per mL of undiluted water sample.



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

Following methods can be used to test the indicator bacteria total count of bacteria is one of the method in this method the total number of bacteria present in one milliliter of water is counted the sample is diluted 1 mL of sample is diluted in 99 ml of sterilized water and then this diluted water is mixed with 10 ml of agar or gelatin this mixture is then kept in the incubator at 37 degrees centigrade for 24 hours or at 20 degrees centigrade for 48 hours.

After that, the sample is taken out from the incubator and colonies of the bacteria are counted. The product of number of colonies obtained and the dilution factor the suppose we have diluted 100 times we have to multiply it by 100. In this way, we can report the total number of bacteria per ml of undiluted water sample this is the total count of bacteria.

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**Cont....**

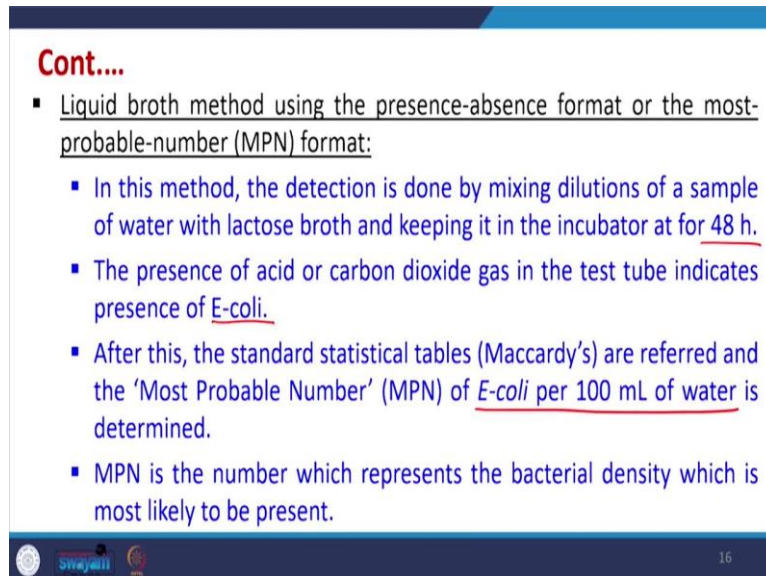
- Membrane-filtration method:
  - In this method, the sample is filtered through a sterilized membrane of special design due to which all bacteria get stained on the membrane.
  - The member is then put in contact of culture medium in the incubator for 24 hours at 37°C.
  - The membrane after incubation is taken out and the colonies of bacteria are counted by means of microscope.



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There are membrane filtration method in this method the sample is filtered through a sterilized membrane of shell design due to which all bacteria get strained on the membrane itself. And the membrane is then put in contact with the culture medium in the incubator for 24 hour at 20 or 37 degrees centigrade. The membrane after incubation is taken out and the colonies of bacteria are counted by means of microscope this is membrane.

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**Cont....**

- Liquid broth method using the presence-absence format or the most-probable-number (MPN) format:
  - In this method, the detection is done by mixing dilutions of a sample of water with lactose broth and keeping it in the incubator at for 48 h.
  - The presence of acid or carbon dioxide gas in the test tube indicates presence of E-coli.
  - After this, the standard statistical tables (Maccardy's) are referred and the 'Most Probable Number' (MPN) of E-coli per 100 mL of water is determined.
  - MPN is the number which represents the bacterial density which is most likely to be present.

Now, there is another method a liquid broth method using the presence or absence format and the most probable number this MPN is reported, in this method the detection is done by mixing dilutions of a sample of water with lactose broth and keeping it in the incubator for 48 hours. The presence of acid or carbon dioxide gas in the test tube indicates presence of E. coli.

After this, the standard tables are referred and most probable number of E coli per 100 ml of water sample is determined. MPN actually represents the bacterial density which is most likely to be present. So we can report the MPN also for complete assessment of the quality of aquatic environment.

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**COMPLETE ASSESSMENT OF THE QUALITY OF THE AQUATIC ENVIRONMENT**

- Chemical analyses of water and aquatic organisms.
- Biological tests such as toxicity tests and measurements of enzyme activities.
- Descriptions of aquatic organisms including their occurrence, density, biomass, physiology and diversity.
- Physical measurements of water: temperature, pH, conductivity, light penetration, particle size of suspended and dissolved material, flow velocity, hydrological balance, etc.

Source: [http://www.inspectapedia.com/septic/BOD5\\_Wastewater\\_Test.php](http://www.inspectapedia.com/septic/BOD5_Wastewater_Test.php)

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So any water if you have to determine that complete quantification, determination and etc, we have to perform the physical water quality parameters, chemical on chemical water quality parameters, so we had to perform a chemical analysis of water and aquatic organisms present in the sample.

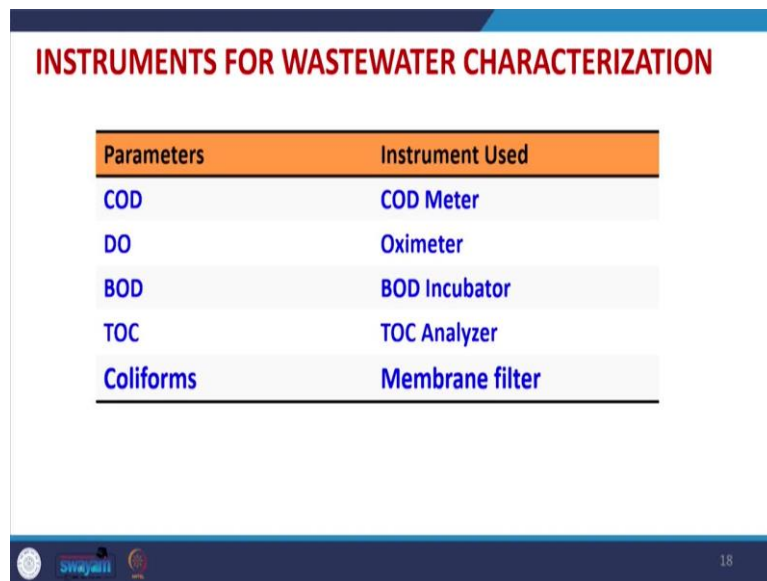
Then the biological tests such as toxicity tests and measurements of enzyme activity, they have to be performed but generally, sometimes these tests are not done for regular water. But now, for some water samples, we have to determine these toxicity tests enzymes activities etc also then description of aquatic organisms which are present in the water including their occurrence, density, biomass, physiologic diversity, all need to be reported.

So, that means, we require a specialist working in this area to completely assess the quality of aquatic environment in any condition. Along with the measurement of all the physical measurement of water, including the temperature, pH conductivity, light penetration, particle size of suspended and dissolved materials, flow velocity, everything.

So, physical water quality parameters, chemical water quality parameters, logical water quality parameters, biochemical water quality, water quality parameters are need to be determined to completely assess the quality of aquatic environment. For these we require persons from different backgrounds which can test the water with respect to these parameters along with the biological test, toxicity tests etc.

And thus we can report the quality of the aquatic environment or the quality of the water or wastewater sample.

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Parameters	Instrument Used
COD	COD Meter
DO	Oximeter
BOD	BOD Incubator
TOC	TOC Analyzer
Coliforms	Membrane filter

So, this is there. Few instruments which are generally required for COD, DO BOD, TOC coliform etc are listed here. There are large number of instruments which are required for completely assessing the chemical water quality parameters. So, I have already given a complete analysis, how to be done using different instruments and other things in my another course on physico chemical waste water quality parameters, you can go back and study all the chemical water quality parameters using those instruments.

There are a large number of instruments which are required for analysis, we can use titration method, Gravimetric method, etc., we can use colorimetric method also but that those are studying and teaching all those is beyond the scope of this course, you can always refer to other courses for determining those water quality parameters.

So if we can determine the physical water quality parameter chemical, then biochemical and the bacteriological we can report all these water quality parameters before going for treatment and after going, after treating the water, so as to find out the efficacy of our water treatment system.

So we will continue further with the other sections of this biological water quality treatment. So we will study other sections of this particular course in the next lecture onward will now complete we have completed the water quality characterization analysis, and we will continue further in the next class. Thank you very much.