

7. Laboratory Analysis

7.1 BOD Analysis

- The Biochemical Oxygen Demand (BOD) test estimates the amount of biodegradable material present by measuring the amount of oxygen used by the bacteria to break down the organic waste in the sample incubated at 20 deg. C over a five-day period . The BOD test provides an indication on the strength of wastewater in terms of how much oxygen could be depleted if that wastewater was introduced into another receiving water. Complete stabilization of a sample may require a period of incubation too long for practical purposes; therefore, 5 days has been accepted as the standard incubation period.
- As the regular BOD test includes estimation of oxygen nitrifying bacteria consumes in the process of converting inorganic forms of ammonia and nitrogen to nitrite and nitrate, its value represents oxygen used for removing both, organic material and nitrogenous matter. As this BOD value does not quite represent the organic strength of the wastewater, the normal BOD test is modified by introducing a chemical inhibitor - 3 mg of 2-chloro-6-(trichloro methyl) pyridine (TCMP), which suppresses the growth of the nitrogenous bacteria so that the resultant BOD measured represents the oxygen depletion associated with the depletion of the organic matter only. This is the Carbonaceous biochemical oxygen demand or cBOD.

Thus $t\text{BOD} = n\text{BOD} + c\text{BOD}$

- Wastewater BOD measurement involves testing a sample set consisting of several sample dilutions along with a "Blank". "Blank" is a sample with only the dilution water with no wastewater added.
- The dilutions are made based upon the expected BOD concentration of the sample. Using the final dilution volume of 300 ml, the initial sample volume can be estimated using the formula:

$$\text{Sample Volume}(ml) = \frac{\left[\text{Oxygen Depletion} \left(\frac{mg}{l} \right) \right]}{\text{Anticipated BOD} \left(\frac{mg}{l} \right)} * 300 \text{ ml}$$

- For example, if testing an influent wastewater BOD with an expected BOD value of 250 mg/l, a range of sample volumes for dilution around sample volume of $\frac{4 \frac{mg}{l}}{250 \frac{mg}{l}} * 300 \text{ ml} = 5 \text{ ml}$.
- The data obtained for each of the dilutions after the 5-day incubation period must meet the following criteria for the sample value to be acceptable for calculating the BOD.
 - A residual DO of at least 1 mg/L,
 - A DO depletion of at least 2 mg/L
- Additionally, the whole sample set is rejected if the Blank shows an oxygen depletion of >0.2mg/l.
- BOD is calculated for each sample dilution value using the following formula:

$$\text{BOD} \left(\frac{mg}{l} \right) = \frac{\text{Initial DO} - \text{DO Day 5}}{\text{Sample Volume (ml)}} * 300 \text{ ml}$$

7.2 Wastewater solids

7.2.1 Total (TSS) and Volatile (VSS)

- A known volume of wastewater sample is filtered through a pre-weighed filter paper
- The suspended solids will be retained by the filter
- The water with the dissolved solids will pass through the filter
- The filter paper with the filter solids is rinsed with distilled water to remove
- The filter paper with the solids is dried in the oven and then weighed
- The difference between the weight of the dried filter paper with the solids and the pre-weighed filter paper, measured in mg, will be the suspended solids in: mg per the original quantity of wastewater sample taken. This value can be converted to give the suspended solids content in mg/l
- A filter paper with the dried solids is incinerated in a muffler furnace
- The difference in the weight of the solids, before and after incineration is the fixed solids
- The difference between the weight of the solids before incineration and the fixed solids is the volatile solids

$$\begin{aligned}\text{Total Suspended Solids - TSS } TSS \frac{mg}{l} &= \frac{\text{weight of solids gms}}{\text{volume of sample ml}} * \frac{1000 ml}{l} * \frac{1000 mg}{gms} \\ &= \frac{\text{weight of filter paper with dried solids} - \text{weight of filter paper}}{\text{volume of sample (ml)}} * 1,000,000\end{aligned}$$

Volatile Suspended Solids - VSS

$$\begin{aligned}VSS \frac{mg}{l} &= \frac{\text{weight of volatile solids gms}}{\text{volume of sample ml}} * \frac{1000 ml}{l} * \frac{1000 mg}{gms} \\ &= \frac{\text{wt. of filter paper with dried solids} - \text{wt. of filter paper incinerated residue}}{\text{volume of sample (ml)}} * \\ &\quad 1,000,000 \\ VSS(\%) &= \frac{\text{weight (gms) of volatile solids}}{100 gms \text{ total solids}} = \frac{gms \text{ volatile solids}}{\cancel{gms \text{ total solids}}} * \frac{100 \cancel{gms \text{ total solids}}}{100 gms \text{ total solids}} \\ &= \frac{\text{wt. of filter paper with dried solids} - \text{wt. of filter paper incinerated residue}}{\text{wt. of filter paper with dried solids} - \text{wt. of filter paper}} * 100\end{aligned}$$

7.2.2 Wastewater and Sludge Total & Volatile Solids

- A certain quantity of wastewater (by volume) or sludge (by weight) is taken in a pre-weighed dish and weighed. Note: the sample is not filtered.
- The dish with the sample is dried in an oven
- The difference in the weight of the pre-weighed dish from that of the dish with the dried sample is the total solids
- The dried solids are incinerated in a muffler furnace
- The difference in the weight of the solids, before and after incineration is the fixed solids
- The difference between the fixed solids and the total solids is the volatile solids
- Total solids of a sludge sample is reported as a % of the sludge weight. A 7% sludge has 7 lbs of solids for every 100 lbs of sludge.

For sludge samples, volatile solids is typically reported as the volatile solids fraction in % of the total solids content of the sludge. For example, if a 8% sludge (i.e sludge which has 8% TS or 80,000mg/l solids), is reported to have 70% volatile, it means that 70% of the total solids - $0.7*8\% = 5.6\%$ or 56,000mg/l is the sludge volatile solids content. 70% volatile does not meet the sludge has 700,000mg/l volatile solids

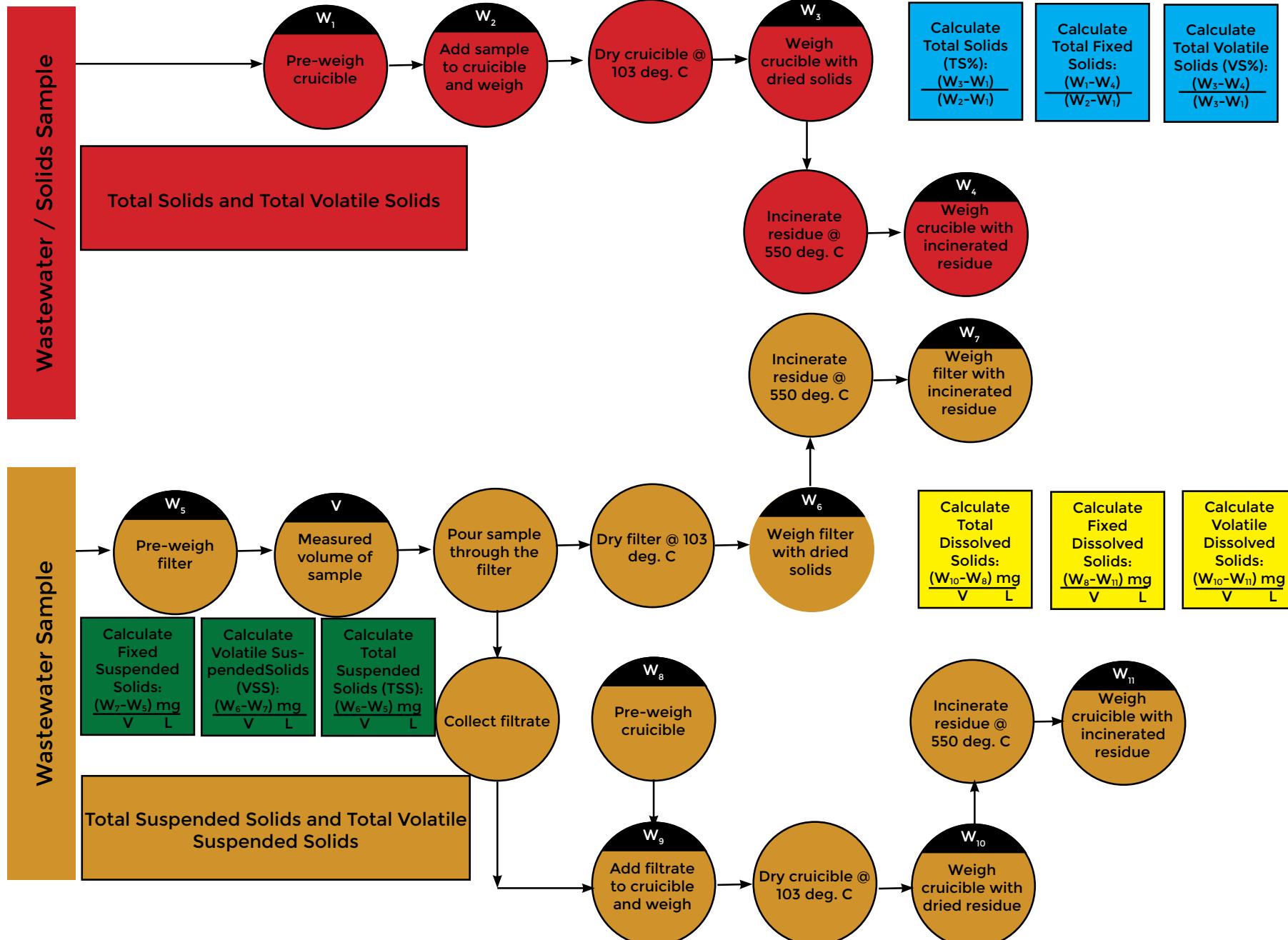
$$\text{Total Solids - TS } TS(\%) = \frac{\text{weight of solids (gms)}}{100 gms \text{ of sample}} = \frac{gms \text{ solids}}{gms \text{ sample}} * 100$$

$$= \frac{\text{weight of crucible with dried solids} - \text{weight of crucible}}{\text{weight of crucible with sample} - \text{weight of crucible}} * 100$$

$$\text{Total Volatile Solids - VS } VS(\%) = \frac{\text{weight of volatile solids (gms)}}{100 gms \text{ of total solids}} = \frac{gms \text{ volatile solids}}{gms \text{ total solids}} * 100$$

$$= \frac{\text{wt. of crucible with dried solids} - \text{wt. of crucible incinerated residue}}{\text{wt. of crucible with dried solids} - \text{wt. of crucible}} * 100$$

Solids Analysis



7.2.3 Sample BOD and solids analysis math problems

1. BOD tests are run on the final effluent from an activated sludge plant with and without the use of a "nitrification inhibitor". Three hundred milliliter bottles (300 ml) are used in these tests. The raw data for these tests are presented below. What **percentage of the average total BOD is the average nBOD?**

Sample Volume, ml	10	20	30	40	Blank
Initial DO, mg/l	9.0	8.9	8.8	9.1	9.1
Final DO, mg/l	6.9	4.8	2.5	1.1	9.0

BOD Test with "inhibitor" added (cBOD)

Sample Volume, ml	10	20	30	40	Blank
Initial DO, mg/l	8.9	8.9	9.0	9.0	9.1
Final DO, mg/l	7.5	6.2	5.0	3.3	9.0

Solution:

Blanks for both tBOD and cBOD are both $\leq 0.2\text{mg/l}$ - thus sample sets are acceptable

Sample Volume, ml	10	20	30	40
tBOD Diff., mg/l	2.1	4.1	6.3	8
tBOD, mg/l	$2.1 \times 300/10$	$4.1 \times 300/20$	$6.3 \times 300/30$	$8.0 \times 300/40$
	=63.0	= 61.5	= 63.0	= 60.0
cBOD Diff., mg/l	1.4	2.7	4.0	5.7
cBOD, mg/l	Reject	$2.7 \times 300/20$	$4.0 \times 300/30$	$5.7 \times 300/40$
		= 40.5	= 40	= 42.75

$$tBOD(\text{avg}) = (63 + 61.5 + 63 + 60)/4 = 61.9 \quad cBOD(\text{avg}) = (40.5 + 40 + 42.75)/3 = 41.1$$

$$\text{nBOD} = \text{tBOD} - \text{cBOD} \implies \text{nBOD} = 61.9 - 41.1 = 20.8 \implies \text{nBOD}(\%) = 20.8/61.9 * 100 = \boxed{33.6\%}$$

2. Calculate percent total solids and percent volatile solids of a sludge sample given the following data:

$$\begin{aligned} \text{Weight of dish} &= 104.55 \text{ gms} \\ \text{Weight of dish and wet sludge} &= 199.95 \text{ gms} \\ \text{Weight of dish and dry sludge} &= 108.34 \text{ gms} \\ \text{Weight of dish and ash} &= 106.37 \text{ gms} \end{aligned}$$

Solution:

$$\text{Weight of dish} = 104.55 \text{ gms}$$

$$\text{Weight of dish and wet sludge} = 199.95 \text{ gms}$$

$$\text{Weight of dish and ash} = 106.37 \text{ gms}$$

$$\implies \text{Weight of sludge} = 199.95 - 104.55 = 95.40 \text{ gms}$$

$$\implies \text{Weight of dry sludge (solids)} = 108.34 - 104.55 = 3.79 \text{ gms}$$

$$\implies \text{Weight of volatile solids} = 108.34 - 106.37 = 1.97 \text{ gms}$$

$$\text{Total solids(TS\%)} = \frac{\text{gms solids}}{100 \text{ gms sludge}} = \frac{3.79}{95.40} \frac{\text{gms solids}}{\text{gms sludge}} * \frac{100 \text{ gms sludge}}{100 \text{ gms sludge}} = \boxed{3.97\%}$$

$$\text{Total volatile solids(VS\%)} = \frac{1.97}{3.79} \frac{\text{gms volatile solids}}{\text{gms total solids}} * \frac{100 \text{ gms total solids}}{100 \text{ gms total solids}} = \boxed{52.0\%}$$

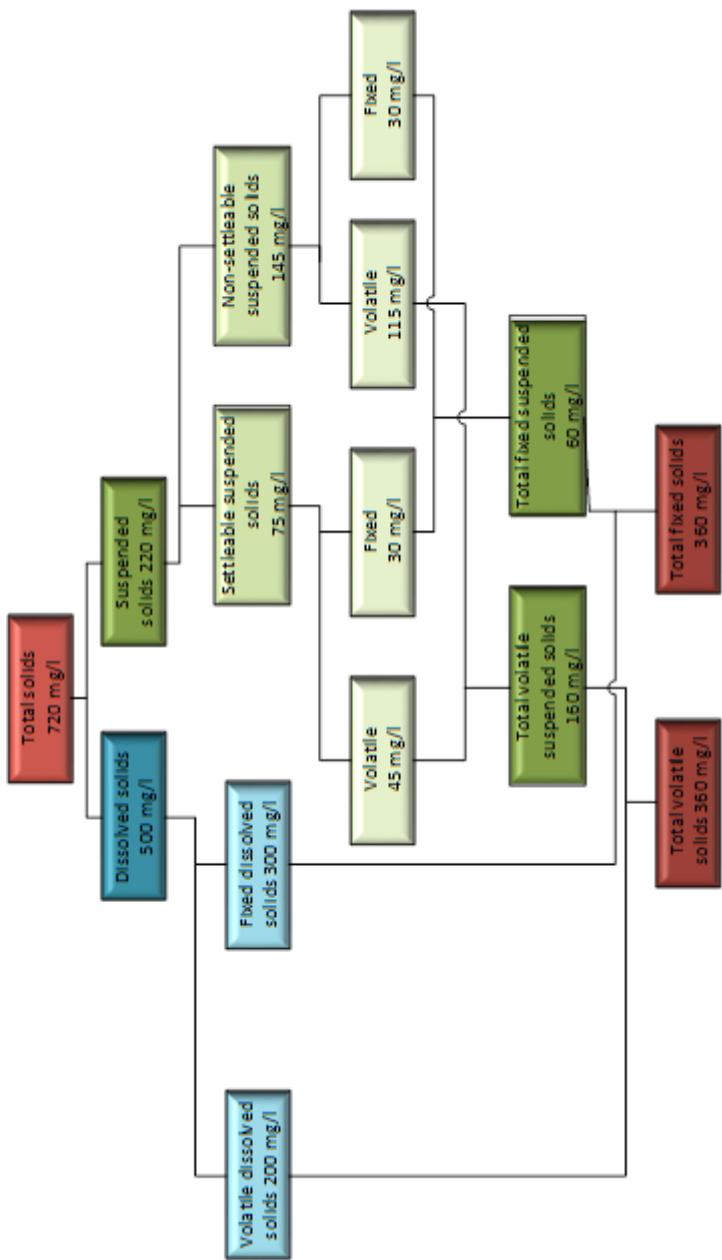
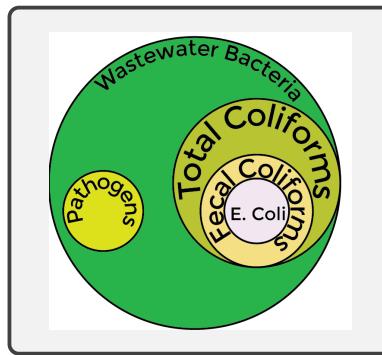


Figure 7.1: Typical Wastewater Solids Concentrations

7.3 Bacteriological Enumeration

- Involves bacteriological testing of the wastewater effluent and the surface water impacted by the wastewater discharge
- Conducted in-order to:
 1. Meet the requirements of a wastewater discharge permit
 2. Monitor the pathogen impact of treated wastewater discharge
 3. Assess the level of contamination of a public body of water
 4. Bacteriological tests involves detection and quantification of one or more of the following bacteria: total coliforms, fecal coliforms, *E. Coli*, and *Enterococci*.



Wastewater Bacteria

5. In wastewater, fecal coliforms originate in the intestines of warm-blooded animals. Aerobic bacteria including coliforms partake in the metabolism of the organic matter as part of the secondary treatment process
6. Fecal coliforms are seldom pathogenic under normal circumstances and are easily cultured, their presence indicates the potential presence of pathogens
The reason why these bacteria such as coliforms and enterococcus are used:
 - (a) It is not practical to detect and quantify all pathogens associated with wastewater
 - (b) These bacteria originate from feces and indicate fecal contamination and thus serve as an indicator organisms for pathogens of wastewater origin
 - (c) They are also:
 - abundant
 - potentially less harmful, and
 - easy to detect
 - (d) *E. coli* has been shown to be a better predictor of the potential for impacts to human health and therefore many newer wastewater discharge permits require *E. Coli* testing in lieu of fecal coliform testing requirements.

7.3.1 Bacteriological Testing Methods

The methods for wastewater bacteriological tests include: multiple-tube fermentation (MTF) technique, membrane filtration (MF) and quanti-tray testing. When using the MTF and MF methods, it is not possible to exactly quantify the number of bacteria present, a statistical based - Most Probable Number (MPN) approach is utilized

7.3.2 The Multiple-Tube Fermentation (MTF) technique

This involves adding three volumes – 10 ml, 1 ml and 0.1 ml of the sample, each to a set of five tubes containing Lauryl Tryptose broth and an inverted tube (Durham tube), followed by incubating the tubes at for a specified time. The Lauryl Tryptose broth produces color and/or turbidity change due to the growth of the target bacteria and the inverted tube collects the gas produced by the bacterial respiration. At the end of the process, the number of tubes showing bacterial growth are counted for each volume of sample and using this information the concentrations of organisms in the original sample are established using Statistical Tables. The test is conducted in three parts – presumptive, confirmative and completed. A schematic of the MTF used for quantifying total coliforms and fecal coliforms is provided below.

PRESUMPTIVE

Wastewater Sample

Make required serial dilutions (See Note 1)

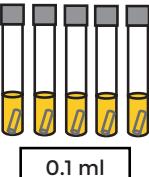
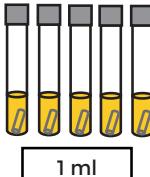
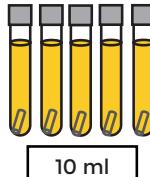
Example: Expected coliform value range 5000 - 50000.

First dilution (1:10): 11 ml sample + 99 ml dilution water

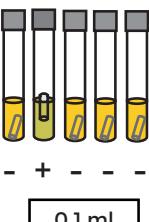
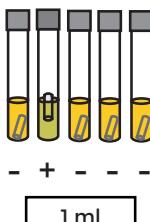
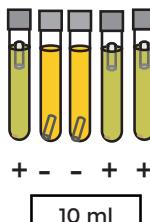
Second dilution (1:100): 11 ml of first dilution + 99 ml dilution water

Third dilution (1:1000): 11 ml of second dilution water + 99 ml dilution water

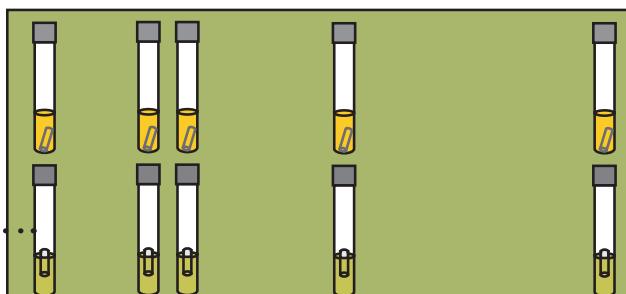
Setup 15 tubes with Lauryl Tryptose Broth and an inverted vial (Durham tube)
5 tubes each for 10 ml, 1 ml and 0.1ml sample



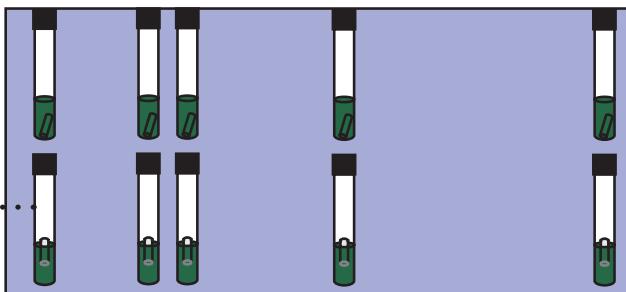
Incubate 24 hrs – check tubes for positive results marked by : 1) broth turning cloudy, and 2) gas collection in the inverted tube. If no gas collection or media clouding is observed, incubate for another 24 hrs and then check again to see if the tubes are positive



Inoculate each positive into bacteria specific broths – EC broth for fecal coliforms and Brilliant Green Bile (BGB) broth for total coliforms with inverted tubes. Incubate for upto 24 hrs for fecal coliforms and 24 hours for total coliforms and observe for positive results



EC Broth (for fecal coliform)
Note: EC Broth with MUG is used for E. Coli



BGB Broth (for total coliform)

Count number of confirmed positives for each set of the three sample volumes
Example above: 10 ml - 3, 1 ml - 1, 0.1 ml - 1

Establish the MPN using the statistical MPN Table

Example 3-1-1 From Table - MPN = 14 MPN/100 ml. Given 1:1000 dilution, MPN = 14 * 1000 = 14,000 MPN/100 ml

COMPLETED



Streak agar plates & incubate

Use colonies to inoculate an agar slant and nutrient broth

If after 24 hours, from the gram staining of the colonies from the agar slant, nonsporing gram negative bacteria can be identified and the lactose broth shows gas formation, the completed test is deemed positive

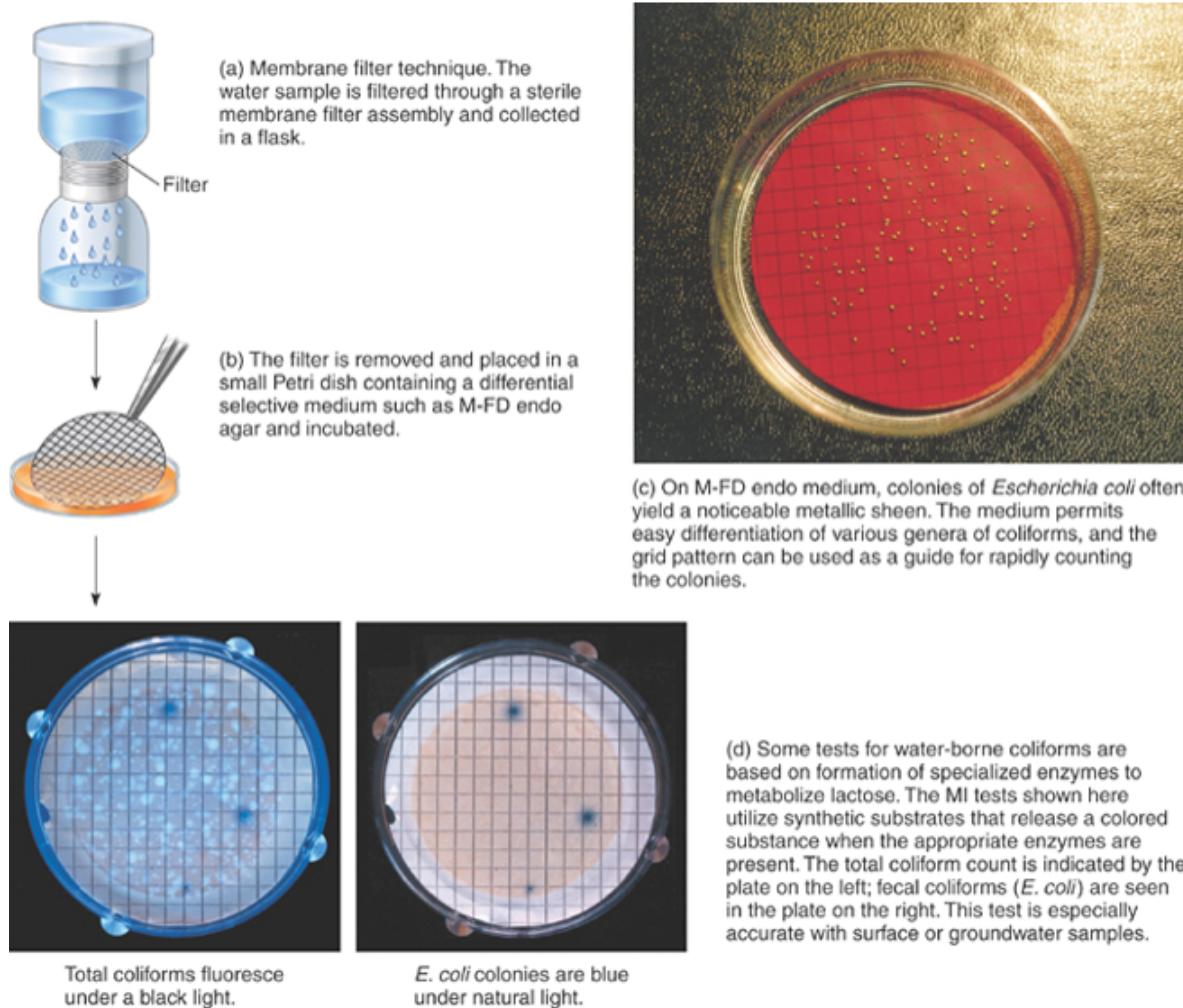
Most Probable Number Table

Number of tubes giving a positive reaction for a 5-tube set			MPN (per 100 ml)	95% Confidence Limits	
10 ml	1 ml	0.1 ml		Low	High
0	0	0	<2	<1	7
0	1	0	2	<1	7
0	2	0	4	<1	11
1	0	0	2	<1	7
1	0	1	4	<1	11
1	1	0	4	<1	11
1	1	1	6	<1	15
2	0	0	5	<1	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	0	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	78
4	3	0	27	9	80
4	3	1	33	11	93
4	4	0	34	12	93
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	110
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	150
5	2	0	49	17	130
5	2	1	70	23	170
5	2	2	94	28	220
5	3	0	79	25	190
5	3	1	110	31	250
5	3	2	140	37	340
5	3	3	180	44	500

7.3.3 The Membrane Filtration (MF) method

This is a faster way to estimate bacterial populations in water. In this method, an appropriate sample volume is passed through a membrane filter with a pore size small enough (0.45 micron) to retain the bacteria present. The filter is placed on an absorbent pad (in a petri dish) saturated with a culture medium that is selective for coliform growth. The petri dish containing the filter and pad is incubated, upside down, for 24 hours at the appropriate temperature. After incubation, the colonies that have grown are identified and counted using a low power microscope. A MUG medium is used for E. Coli. If E. Coli is present, it will make the MUG fluorescent when viewed in UV light.

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7.3.4 Quanti-trays tests

This test used for the detection and quantification of specific microorganisms is being used increasingly mainly because it is a quicker test than the MTF. Colilert and Enterolert are the quanti tray based tests for E. Coli and Enterococcus. This method involve the use of specific enzymes and overcomes the drawbacks of the MTF which include false positives and negatives due to the more generic nature of the media used

STEP-BY-STEP GUIDE TO USING QUANTI-TRAYS

Step 1



Add sample/reagent to tray

Step 2



Seal tray in Quanti-Tray sealer

Step 3



Incubate, count positive wells and refer to appropriate MPN Table