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Experiment No. 15

termination of pathogenic microorganisms (Total Coliform and Fecal Coliform) in water and wastewater

ODUCTION

iety of different microorganisms are found in untreated water. Most of these organisms do se a health hazard to humans. Certain organisms, referred to as pathogens, cause disease to ns which include species of bacteria, viruses and protozoa. These organisms are not native uatic systems and usually require an animal host for growth and reproduction. They can, vér, be transported by natural water systems, thus becoming a temporary member of the ic community. Many species of pathogens are able to survive in water and maintain their tious capabilities for significant periods of time. From the perspective of human use and mption, the most important organisms in water are the pathogens. Table 1 lists the more non waterborne diseases and the organisms that cause the diseases as well as the disease ptoms.

e 1: Waterborne Diseases

terborne iseases	Causative Organism	Source of organism in water	Symptom
roenteritis	*Salmonella* (bacteria)	Animal or human feces	Acute diarrhea and vomiting
yphoid	*Salmonella typhosa* (bacteria)	Human feces	Inflamed intestine, enlarged spleen, high temperature – fatal
ysentery	*Shigeila* (bacteria)	Human feces	Diarrhea- rarely fatal
holera	*Vibrio Comma* (bacteria)	Human feces	Vomiting, severe diarrhea, rapid dehydration, mineral loss - high mortality
fectious epatitis aundice)	Virus	Human feces	Yellowed skin, enlarged liver, abdominal pain - low mortality, lasts up to four months
moebic ysentery	*Entamoeba histolytica* (protozoa)	Human feces	Mild diarrhea, chronic dysentery
iardiasis	Giardia lamblia (protozoa)	Suspect wild animal feces	Diarrhea, cramps nausea and general weakness - lasts 1week to 30 weeks, not fatal

ARACTERSTICS OF PRIMARY WATERBORNE PATHOGENS

major characteristics of bacteria, viruses and protozoa are briefly described below:

teria:

y are unicellular prokaryotic organisms and arc the lowest form of life capable of

synthesizing protoplasm from the surrounding environment. They can grow on artificial laboratory media. Cell multiplication is usually by binary fission.

Viruses:
They are the smallest biological structures known to contain all the generic information necessary for their own reproduction. They require a host in which to live. They do not grow on artificial media, rather require living cells within which they are reproduced. Electron microscope is required see viruses. Multiplication takes place by replication.

Protozoa:
They are unicellular eukaryotic organisms and are more complex in their functional activity than bacteria and viruses. Some can be grown on artificial media and some are parasites. Reproduction is by asexual and sexual processes.

INDICATOR ORGANISMS

One might assume that a water sample is analyzed to search for pathogenic organisms. This is not true, however for the following reasons:

- Pathogens are likely to gain entrance sporadically, and they do not survive for very long period of time; consequently they could be missed in a sample submitted to the laboratory.
- If they are present in very small numbers, pathogens are likely to escape detection by laboratory procedures.
- Although it is possible to detect the presence of various pathogens in water, the isolation and identification of many of these is often extremely complicated and seldom quantitative.
- Analysis of water for all known pathogens would be a very time-consuming and expensive proposition.

Tests for specific pathogens are usually made only when there is a reason to suspect that those particular organisms are present. At other times, the microbiological quality of water is checked using indicator organisms. An indicator organism is one whose presence presumes that contamination has occurred and suggests the nature and extent of the contaminations.

CHOICE OF INDICATOR ORGANISMS

It is known that most pathogens that are likely to be transmitted via the water route are shed in human and/or animal feces. Hence, and indicator organism should be a microorganism whose presence is the evidence of fecal contamination of warm blooded animals. Indicators may be accompanied by pathogens, but typically do not cause disease themselves. The ideal indicator organism should have the following characteristics:

- be applicable to all waters
- always be present when pathogens are present
- always be absent where pathogens are absent
- numbers should correlate the degree of pollution
- be present in greater number than pathogens

- there should be no after-growth or re-growth in water
- there should be greater or equal survival time than pathogens
- be easily and quickly detected by simple laboratory tests
- should have constant biochemical and identifying characteristics
- harmless to humans

organisms or group of organisms meet all of these criteria; but the coliform bacteria fulfill st of them, and this group is most common indicator in use today. Each person discharges ut 100 to 400 billion coliforms each day. Thus, the presence of coliform organisms is taken an indication that pathogenic organisms may also be present, and the absence of coliform anisms is taken as an indication that the water is free from pathogens. Total coliforms are ined as gram negative bacteria which ferment lactose at 35°C or 37°C, with the production of d, gas and aldehyde within 24 or 48 hours. They are cytochrome oxidase negative and non-re forming.

Total coliform (TC) = Fecal coliform (FC) + Non-fecal coliform.

cal coliforms (thermo-tolerant coliform) are a subgroup of total coliforms, which live in the rm blooded animals and have the same properties as the total coliforms but tolerate and grow high temperature.

ETERMINATION METHODS FOR INDICATOR ORGANISMS

) *Multiple Tube (MT) Method:*

he multiple tube method is applicable to all kinds of water samples. It can cope with clean, lored, or turbid waters containing sewage or sewage sludge, or mud and soil panicles, ovided that the bacteria are homogenously distributed in the prepared test samples. neoretically, the technique is sufficiently sensitive to measure low levels of bacteria in water mples, although bigger containers to take larger volumes of sample are needed to be used as lture vessels. However for normal water analysis, 10 ml is usually the largest volume used. his method is often preferred by microbiologists.

) *Membrane Filter (MF) Method:*

contrast to the multiple-tube (MT) method, the membrane filter (MF) method gives a direct unt of total coliforms and fecal coliforms present in a given sample of water. The method is sed on the filtration of a known volume of water through a membrane filter consisting of a llulose compound with a uniform pore diameter of 0.45 μm; the bacteria are retained on the rface of the membrane filter. When the membrane containing the bacteria is incubated in a erile container at an appropriate temperature with a selective differential culture medium, haracteristic colonies of coliforms and fecal coliform develop, which can be counted directly. his technique is popular with environmental engineers.

his method is not suitable for turbid waters, but otherwise it has several advantages. Its articular advantages and limitations are as follows:

dvantages:

- Results are obtained more quickly as the number of coliform can be assessed in less than 24 hours, whereas the multiple tube technique requires 48 hours both for a negative or a presumptive positive test.
- Saving in work, certain supplies and glassware.

- This method gives direct results.
- Easy to use in laboratories, or even in the field if portable equipment is used.

dvantages:

- High turbidity caused by clay, algae, etc. prevents the filtration of a sufficient volume of water for analysis and it may also produce a deposit on the membrane which could interfere with bacterial growth.
- Presence of a relatively high non-coliform count may interfere with the determination of coliforms.
- Waters containing particular toxic substances which may be absorbed by the membranes can affect the growth of the coliforms.

STS FOR DETERMINATION OF INDICATOR ORGANISMS

) Multiple Tube Test

ılture Media:

ɔmmercially available dehydrated media simplify the preparation of culture broths, and are erefore recommended for laboratory work. Various manufactures produce these media as ɔwders, which can then be easily weighted out, dissolved in distilled water, and dispensed into ılture tubes prior to sterilization.

several different culture media are available for the presumptive test, for example: (i) lauryl yptose broth (LTB), (ii) MacConkey broth, and (iii) lactose broth. These three media are ɔmmon in use in many countries. The selectively of MacConkey broth and LTB depends espectively on the presence of bile salts and the surface-active agents, lauryl sulfate; lactose roth is a non-selective medium.

ıs a confirmatory medium for total coliforms (TC), brilliant green lactose bile broth (BGB) is nost widely used. To confirm the presence of fecal coliform (FC), either BGB broth or Escherichia coli (EC) broth is used.

Preparation of Media:

Media should be prepared in accordance with the manufacturer's instructions, as follows:

- Dissolve the stated amount of the dehydrated medium in distilled water to obtain the double-strength or single-strength presumptive medium (for confirmatory analysis, only singe-strength medium is used).
- Dispense the requisite volume into culture tubes containing an inverted Durham tube and cap the culture tubes.
- Sterilize in an autoclave or pressure cooker at 114°C for 10 minutes (or in accordance with the manufacture's specifications). It is particularly important that media containing disaccharides, e.g. lactose, are not autoclaved at higher temperatures.
- The sterilized medium should be stored at room temperature to maintain sterility. Since several dyes are light-sensitive, the solution should be protected from exposure to light.

Test Procedure:

Figure 1 shows the procedure involved in Multiple Tube (MT) Test of water and wastewater samples, together with the appropriate incubation times and temperatures. The procedure to be

used for testing relatively unpolluted water, such as treated water from waterworks, is described below.

(A) Remove the paper wrapping from the sample bottle.

(B) With the stopper in position, shake the bottle vigorously to achieve a homogeneous disoperation of bacteria. If the bottle is completely full of water, remove the stopper and discard about 20-30 ml of water, then replace the stopper and shake. This ensures thorough mixing.

(C) With a sterile 10 ml pipette, modulate 10 ml of the sample into each of five tubes containing 10 ml of presumptive broth (double strength). It is advisable to shake the tubes gently to distribute the sample uniformly throughout the medium.

(D) Incubated the tubes at 35°C or 37°C for 24 hours.

(E) At the end of the 24-hour incubation period, observe each tube for the presence of gas. Gas, if present, can be seen in the Durham tube; if none is visible, ge3ntly shake the tube. If any effervescence (foam, streams of tiny bubbles) is observed, the tube should be considered positive.

(F) Enter the number of positive tubes after 24 hours in table.

(G) Re-incubate negative tubes for a further 24-hour period. At the end of this period, check the tubes again for gas production as in (E) above. Gas production at the end of either 24 or 48 hours incubation is pressured to be due to the presence of coliform in the sample.

(H) Enter the number of positive tubes after 48 hours in the table.

(I) The confirmatory test should be carried out at the end of both the 24-hour and the 48-hour incubation. Using a loop, transfer one or two drops from each presumptive positive tube to a corresponding sterile confirmative 10-ml tube containing, e.g. BGB broth. Before each transfer, sterilize the inoculation loop by flaming and allow it to cool.

(J) If the presence of fecal coliforms is also to be investigated, subcultures in two tubes containing confirmative broth (e.g. BGB broth) should be prepared from each presumptive positive tube. EC medium is preferred in some areas for confirmation of fecal coliforms.

K) To confirm the presence of coliforms, incubate one subculture tube from each presumptive positive tube for 48 hours at 35°C or 37°C.

L) Check the tubes at the end of the 48 hour incubation period; the presence of gas coliforms that coliforms are present in the sample. Enter the results in the table.

M) To confirm the presence of fecal coliforms, incubate a second subculture tube from each presumptive positive tube for 24 hours at 44±0.5°C.

N) If, at the end of 24 hour incubation, gas is present in the tubes, the presence of fecal coliforms is confirmed.

Determination of MPN :

For treated water, where five 10 ml portions are portions are inoculated, the MPN can be found from the test results by means of Table 2. An example is given in Fig 2. To show how the test results are interpreted. It can be seen that three confirmed positive tubes have been obtained for the total coliform group. From Table 1, the corresponding value of the MPN can be seen to be 9.2 total coliforms per 100 ml sample. As regards the test for fecal coliforms, there was only one confirmed positive tube. Consequently, the value of the MPN for this group is 2.2 per 100 ml.

Table 2: MPN of various combinations of +ve and –ve results when five 10-ml portions are used

Number of the tubes giving positive reaction out of five 10-ml tubes	MPN
0	0
1	2.2
2	5.1
3	9.2
4	16.0
5	Intermediate

B) Membrane Filter Method:

Volume of water Sample for Filtration:

Since the filtration area is relatively small, it can only support the growth of a limited number of colonies. The optimum number is between 20 and 80 colonies with a maximum of 200. If this figure is exceeded, very small colonies or super imposed colonies may develop, or growth inhibition due to overpopulations may result. The choice of the volume of sample to be filtered will depend on the type of water. As a general rule, the following filtration volumes (Table 3) should be employed.

Table 3: Volume of water to be filtered for various types of water

Type of Water	Volume of sample to be filtered (ml)
Good quality treated water	50-100
Untreated Drinking Water	10-50
Surface Water	1-10

If the origin of the sample is unknown and its probable bacterial content is uncertain, water volumes differing by a factor of ten should be filtered in order to find the appropriate range for the analysis. If the volume to be filtered is less than 10 ml, at least 20 ml of sterile dilution water should be placed in the funnel prior to the filtration.