Water: Disinfection & Microbiological Analysis

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Abstract

Microbiological parameters supply unique information on water quality and public health risks from waterborne disease. Disinfection is the destruction of microorganisms capable of causing diseases. It is an essential and final barrier against human exposure to disease causing pathogenic microorganisms, including viruses, bacteria and protozoan parasites. The destruction of pathogens and parasites by disinfection helps considerable in the reduction of waterborne disease.

Keywords: *Water, Bacteriological Techniques, MPN, Disinfection Methods.*

INTRODUCTION

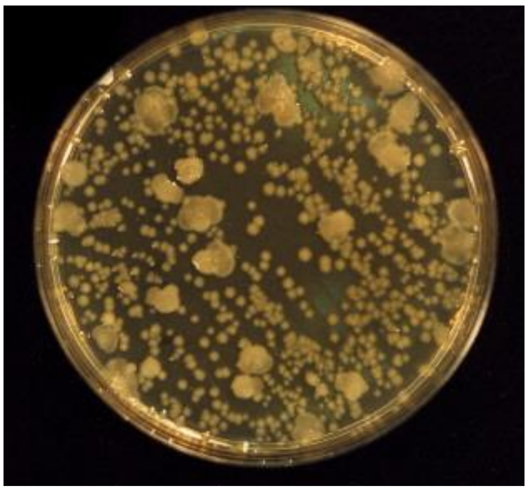
Increasing demand and shortage of clean water sources due to the rapid development of industrialization, population growth and long-term droughts have become an issue worldwide. It is estimated that around 4 billion people worldwide experience to have no or little access to clean water supply, and millions of people died of severe waterborne diseases annually. Microbiological parameters supply unique information on water quality and public health risks from waterborne disease. Potable water systems can become polluted with coliform and pathogenic bacteria from normal, diseased or carrier human and animal excrements. This can occur by cross connections between a water main and a sewer, especially when the pressure in the water main is lower than the atmospheric pressure, or from the entry of sewage water through leaks in damaged pipes. Also, deficiencies in water treatment may allow unharmed or injured organisms to escape. Pathogenic and toxigenic microbiological agents in drinking water have long been known to cause disease and health in consumers. The most common bacterial disease transmitted through water are cause by Shigella, Salmonella, enterotoxigenic, E.coli (ETEC), Camphlobacter jejuni, and Vibrio cholera. However, during the last 15 years the puglic health significance of viral and parasitic agents in diarrheal disease has been increasingly recognized. Disinfection is the destruction of microorganisms capable of causing diseases. It is an essential and final barrier against human exposure to disease causing pathogenic microorganisms, including viruses, bacteria and protozoan parasites.

Bacteriological Techniques

(1) Heterotrophic Plates Count (HPC)

Heterotrophic plate count was formerly known as standard plate count determination. The test provides as approximate enumeration of live heterotrophic bacteria. It yields useful information about bacterial quality and may provide supporting data for further investigations. Colonies may arise from pairs, chains clusters or single cells, all of which are included in the term colony forming units (CFU) which is used to report the result.

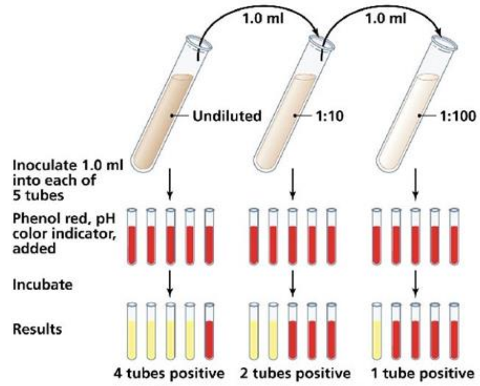
Colony counts are performed after plating aliquots of the water sample. Water of good quality is expected to give a low count, less than 100 per milliliter.



(2) Total Coliform Determination

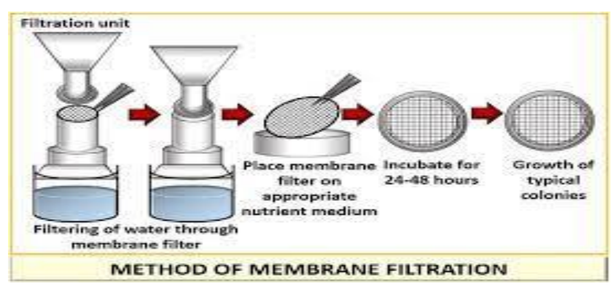
i. Multiple Tube Fermentation Method:

This is a technique for determining the most probable number (MPN) of bacteria in water. This method consists of inoculating known volume of water sample into tubes of selective lactose broth. The tubes are examined for growth and production of acid and gas after 24 to 48 hours of incubation. Positive tubes are inoculated into brilliant green lactose bile broth for confirmation of gas production. The resultant MPN is determined from the standard tables.



ii. Membrane Filter Method

In this technique a known volume of a water sample is passed through a nembrane filter that is then placed on m-Endo of LES-Endo agar for incubation. All organisms that produce red colonies with a metallic sheen within 24 hours of incubation are considered members of the coliform group. An advantage of the membrane filter method over the MPN technique is that results are available within 24 hours instead of the 3 to 4 days required for confirming MPN results, although verification of colonies observed after membrane filtration is advisable.



iii. Presence-Absence Coliform Test

Both MPN and membrane filter methods allow an evaluation of sanitary water quality and the effectiveness of treatment processes. However, according to the new regulations, 100 ml of drinking water should not contain any coliforms. Thus quantitative assessment of coliforms may not be necessary. Instead, a qualitative estimation using the presence-absence (P-A) of coliform concept will be allowed. In one P-A technique, 100 ml water samples are mixed with triple-strength P-A broth and incubated at 350 C. the samples are examined after 24 and 48 hours for the production of acid and gas.

iv. Rapid Detection of Coliforms

Similar to the membrane filter procedure, there is a method called seven hour test for faecal coliform. After filtering a water sample, the membrane filter is placed on M-7h FC agar medium and incubated at 41.50C. faecal coliforms colonies (yellow) arte enumerated after 7 hours of incubation. However, a compromise has to be made between sensitivity and speed in detecting and enumerating coliforms.

Another rapid method of faecal coliform detection, involves concentrating bacteria on a membrane filter which is placed in M-FC broth containing radiolabelled 14C-mannitol. The contents are incubated at 350C for 2 hours at 44.50C. the 14CO2 released is assayed by liquid scintillation spectrometry.

A test that requires only 1 hour of incubation for assessing microbiological quality of drinking water has also been reported. The test is based on the correlation of 32p uptake when a sample of water containing coliforms is incubated in a synthetic medium. The major drawback with these methods is related to the use of radioactive materials and to the need for specialized instruments.

Disinfection Methods

Disinfection is the destruction of microorganisms capable of causing diseases. It is an essential and final barrier against human exposure to disease causing pathogenic microorganisms, including viruses, bacteria and protozoan parasites. The destruction of pathogens and parasites by disinfection helps considerable in the reduction of waterborne disease.

A variety of chemical and physical agents may be used to carry out disinfection of water.

A. CHEMICAL METHODS

The common chemical disinfection used is:

(1) Chlorine and its compounds

(2) Chloramines

(3) Chlorine Dioxide

(4) Iodine

(5) Bromine

(6) Silver

(7) Potassium Permanganate

(8) Electro-chemical Activation (ECA)

(9) Activated Carbon

(1) CHLORINE AND ITS COMOUNDS

Chlorine (and its compounds) is widely used for the disinfection of water because:-

i. It is readily available as gas, liquid & powder.

ii. It is cheap.

iii. It is easy to apply due to relatively high solubility (7000mg/lit.)

iv. It leaves a residual is solution which provides protection in the distribution systems.

v. It is very toxic to most micro-organisms, stopping metabolic activities.

Chlorine is usually supplied under pressure in steel cylinders, drums or tanks – to which it is not corrosive in the dry states- as a mixture of about 85% liquid and 15% gas , although for small requirements chlorine is provided as sodium or calcium salts of hypochlorous acid. When chlorine is added in water, both, hydrolysis and disproportionation reaction occurs to create both chloride and hypochlorite ions. The quantities of each species present are governed by reaction concentrations, reaction rates & equilibria, pH and temperature.

Chlorine gas (C12) introduced into water hydrolyses according to the following equation:

$Cl_2+H_2O = HOCl + H^+ + Cl^-$

Hypochlorous acid

Hypochlorous acid dissociates in water –

$HOCl = H^+ + OCl^-$

Hypochrine ion

Both the undissociated hypochlorous acid and the hypochlorite ion are disinfecting agents. The hypochlorite ion is far less effective than the undissociated acid. The hypochlorite ion is only 1/80 as powerful a bacteriocide as the hypochloorous acid and only between 1/500 – 1/300 as effective as a cysticide. Hence the germicidal effect of chlorine in water is dramatically reduced as the pH rises above 7.

Free chlorine disrupts the integrity of the bacterial cell membrane, thus leading to loss of cell permeability and to the disruption of other cell function. Exposure to chlorine leads of leakage of protein, RNA and DNA.

Chlorine also damages the bacterial nucleic acids as well as enzymes (catalase).

One of the consequences of reduced catalase activity is inhibition by the accumulated H_2O_2 the protein coat is the target site for the viruses like rotavirus.

(2) CHLORAMINES

Disinfection of water with chloramines in lieu of free chlorine is known as chloramination. Chloramines do not react with organics to form trihalomethanes. Although they are less effective disinfectants than free chlorine, they appear to be more effective in controlling biofilm microorganisms because they interact poorly with capsular polysaccharide. In aqueous solutions, HOCl reacts with ammonia which forms three types of chloramines namely monochloramine (NH_2Cl), dichromine ($NHCl_2$) and trichloramine (NCl_3¬). Formation of these chloramines greatly depends on the pH of water.

Free chlorine inactivates enteric bacteria much faster than inorganic chloramines. Furthermore, the bactericidal activity of chloramines is found to increase with temperature and hydrogen ion concentration. Mycobacteria, viruses and protozoan cysts are more resistant (Ct value< 500) to chloramines than E. coli (Ct=113) and coliforms (Ct=8.5)

(3) CHLORINE DIOXIDE

The use of chlorine dioxide in water treatment is becoming increasing because it does not form trihalomethanes (THM) nor does not react with ammonia to form chloramines. It cannot be stored in compressed form in tanks and that is why generated at the site.

$2NaClO_2+Cl_2 = 2ClO_2+2NaCl$

In water it exists as a dissolved gas. In alkaline solutions, it forms chlorite and chlorate:

2ClO¬2+OH=ClO2+ClO3¬+H2O

In water treatment plants, chlorite is the predominant form generated. To reduce THM formation, ClO¬¬2 is used as a preoxidant and a primary disinfectant and is followed by the addition of chlorine to maintain the residual.

Dosages of ClO_2 employed in the wated industry are usually below 1.0 ppm. As a result of concern about suggested toxicity of chlorite and chlorate in drinking water, the USEPA in 1978 recommended a maximum combined concentration of 0.5 mg/lit. for chlorine dioxide and chlorite.

Chlorine dioxide is very effective microbial disinfectant and is equal or superior to chlorine in inactivating bacteria, viruses, and cysts of pathogenic protozoa.

(4) IODINE

Iodine is one of the oldest and most effective bactericidal agents and is unique in that it is effective against all kinds of bacteria. Long term use of iodinated water supply is not deleterious to health. In fact, iodine is required as nutrient and in iodine deficiency areas, people take supplementary iodine in the form of iodized salt containing 10-15 ppm iodine.

(5) BROMINE

A halogen like chlorine, bromine has similar disinfection properties and is sometimes used in recirculated water of swimming pools where the residual tends to be less irritating to the eyes than chlorine residuals. There is no wide distribution of liquid bromine or special equipment for its safe storage and application.

(6) SILVER

Silver is used as water disinfection for small amount of water. Colloid silver was used by Romans in storage jars in ancient time to preserve the quality of water. A concentration of 0.05 ppm of silver is sufficient to kill 99% bacteria. Silver impregnated gravel filter candles in small portable filter units to remove turbidity and provide disinfection.

(7) POTASSIUM

Potassium permanganate is a powerful oxidising agent and it has frequently been used for the disinfection of water. The commonly used used dosage is 0.5pm. mostly it is used for washing the storage water tanks at a particular intervals. Its use is not satisfactory and is not recommended for water disinfection.

(8) ELECTO-CHEMICAL ACTIVATION

Electro-chemical activation (ECA) enhances the chemical activity of the salts and constituents of the water. Redox potentials generated in anolyte and catholyte streams enhance the solution properties of the treated water as well providing direct sterilization. The Russians have used the ECA system successfully in a variety of areas. These include the space programmes, various military field activities, the sterilization of potable water systems and medical equipments, food processing and the treatment of industrial and agricultural effluents.

(9) ACTIVATED CARBON

Activated carbon has been used since antiquity but its microbiological aspects have been investigated recently. When water is filtered through activated carbon column, effective killing of microorganisms are observed. Activated carbon loaded with silver and crystalline iodine shows synergistic effect in bacterial inactivation.

B. PHYSICAL METHODS

The common methods used for disinfection of water are:

(1) Ozone

(2) Ultraviolet Light

(3) Gramma Radiation

(4) Solar Radiation

(5) Photodynamic Inactivation

(6) Filtration

(7) Ion-exchange System

i. OZONE

Ozone is an allotropic form of oxygen existing as the molecule O3 instead of O2. It is the most powerful oxidant employed as a water disinfection with an oxidation potential of 2.07 eV compared with that of 1.49 eV of hypochlorous acid, 0.75 eV of chloramines and 1.25 eV chlorine dioxide.

Disinfection is currently the wide-spread application of ozone in water treatment. Provided the water is of good quality in terms of colour, organics & turbidity, an ozone residual of 0.5 mg O3 per litre after 5 minutes contact time is considered sufficient for effective bacterial disinfection and viral inactivation as compared with a free residual inactivation as compared with a free residual chlorine concentration of 0.5 mg Cl2 per litre, with 30 minutes contect period at pH 7. Disinfection by ozone is not affected by the presence of ammonia or by the pH over the range from 606 to8.5, provide the water is of a good organic quality. However, in the presence of ozone, demand due to organics as evidenced by high COD, much larger dose of ozone with longer contact times are necessary for effective disinfection.

ii. ULTRAVIOLET LIGHT

Ultraviolet radiation (UVR) comprises a section of the electromagnetic spectrum with wavelength between 150-400 nm of which it is only the section between about 200-310 nm which is microbicidal, with a definite lethal intensity peak existing at about 255 nm. On either side of the peak, both at shorter and longer wavelengths, the germicidal potential of the radiation falls off quite dramatically.

Low pressure mercury vapour lamps which emit a narrow band of radiant energy at 253.7 nm are suggested for disinfection of water. Interference by physical and chemical constituents on disinfection of water stimulated interest in the improvement of disinfection with UVR. The method has some advantages in comparison with application of chemical oxidants. The physic-chemical and organoleptic features of the water are not changed and no toxic substances are introduced into water. The system for water disinfection with this method needs smaller area, is simple in use and maintenance and can be automated. The time period bactericidal radiation contact with water disinfected being short, upto 10 seconds, no holding tanks short, are required. Exposure to proper UVR dose causes efficient killing of vegetative and spore forming bacteria, viruses and other pathogenic microorganisms.

UV disinfection systems use low pressure mercury lamps enclosed in quartz tubes. The tubes are immersed in flowing water in a tank and allow passage of UV radiation at the germicidal wavelength. However, transmission of UV by quartz lamps must be regularly cleaned by mechanical, chemical and ultrasonic cleaning methods. Teflon has been proposed as an alternative to quartz but its transmission of UV radiation is lower than in quartz system.

UV radiation damages microbial DNA at a wavelength of approximately 260 nm. It causes thymine dimerization, which blocks DNA replication and effectively inactivates microorganisms. In viruses initial site is genome followed by structural damage to the virus coat.

iii. GAMMA RADIATION

Research is now focusing on the effectiveness of gamma radiation (Cesium-137 or Cobalt-60) in the disinfection of water as well as in the removal of organic matter (BOB and COD) from waste water. Disinfection of waste water with gamma radiation (Co-60) at a dose of 463 krad, resulted in 3 log inactivation of coliphage and 4-5 log inactivation for coliform and heterotrophic plate count.

iv. PHOTODYNAMIC INACTIVATION

Photodynamic inactivation (photochemical disinfection) consists of using visible light or sunlight as energy source. O2 and a sensitizer dye. Photochemical generation of singlet oxygen is simple and efficient. Sensitizers are well known for use as energy transfer agents. Dye sensitizers provide convenient means to generate singlet oxygen. The sensitizer is shifted from its electronic ground state to an excited triplet state. In second step, intersystem crossing leads to the reactive molecule in an excited triplet state. The energy transfer process occurs during the collision of triplet state sensitizer with ground state(triplet) oxygen. Among other factors, the efficiency of this water treatment method depends on the production rate of singlet oxygen in the aqueous solutions. The high oxidation strength of generated singlet oxygen is used for the killing of Microorganisms and destruction of organic pollutants.The heterogenous photocatalysis using TiO2 –based photocatalyst for disinfection of water is being used these days. Sun light or near – ultraviolet light combined with commercially available TiO2 inactivate the pathogenic microorganisms in water. Silver coated TiO¬2 shows synergistic effect in microbial inactivation.

v. FILTRATION

Filtration is one of the oldest process used for water purification. This process contribute greatly in the reduction of waterborne diseases such as typhoid fever and cholera. Slow and filtration, rapid sand filtration, diatomaceous earth filtration etc. are being used in water treatment plants where chlorination is done after filtration.

vi. ION-EXCHANGE SYSTEM

Strong base anion exchange resin exhibit no germicidal activity but microorganisms can be absorbed on resin surface. Ion exchange resins not only absorb bacteria better than conventional resin, but also hold the bacteria intact, the letter being killed due to cell lysis. These new generation resins are known as “Insoluble Polymeric Contact Disinfections” (IPCD). The IPCD resins have been shown to be bactericidal.

Conclusion

The primary objective of the microbiology of drinking water is to prevent waterborne disease outbreaks. A drinking water system can minimize the likelihood of such an outbreak by employing proper treatment and control practices, and by monitoring the effectiveness of these practices.The goal of disinfection of public water supplies is the elimination of the pathogens that are responsible for waterborne diseases. The transmission of diseases such as typhoid and paratyphoid fevers, cholera, salmonellosis, and shigellosis can be controlled with treatments that substantially reduce the total number of viable microorganisms in the water.

While the concentration of organisms in drinking water after effective disinfection may be exceedingly small, sterilization (i.e., killing all the microbes present) is not attempted. Sterilization is not only impractical; it cannot be maintained in the distribution system. Assessment of the reduction in microbes that is sufficient to protect against the transmission of pathogens in water is discussed below.

Chlorination is the most widely used method for disinfecting water supplies in the United States. The near universal adoption of this method can be attributed to its convenience and to its highly satisfactory performance as a disinfectant, which has been established by decades of use. It has been so successful that freedom from epidemics of waterborne diseases is now virtually taken for granted. As stated in Drinking Water and Health (National Academy of Sciences, 1977), "chlorination is the standard of disinfection against which others are compared."

However, the discovery that chlorination can result in the formation of trihalomethanes (THM's) and other halogenated hydrocarbons has prompted the reexamination of available disinfection methodology to determine alternative agents or procedures (Morris, 1975).

The method of choice for disinfecting water for human consumption depends on a variety of factors (Symons et al., 1977). These include:

- its efficacy against waterborne pathogens (bacteria, viruses, protozoa, and helminths);
- the accuracy with which the process can be monitored and controlled;
- its ability to produce a residual that provides an added measure of protection against possible post treatment contamination resulting from faults in the distribution system;
- the aesthetic quality of the treated water; and
- the availability of the technology for the adoption of the method on the scale that is required for public water supplies.

Economic factors will also play a part in the final decision; however, this study is confined to a discussion of the five factors listed above as they apply to various disinfectants.

The propensity of various disinfection methods to produce by-products having effects on health (other than those relating to the control of infectious diseases) and the possibility of eliminating or avoiding these undesirable by-products are also important factors to be weighed when making the final decisions about overall suitability of methods to disinfect drinking water.

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