

Chapter 11

Measurement, Analysis, and Remediation of Biological Pollutants in Water



Uthradevi Kannan, S. Krishna Prashanth and Shihabudheen M. Maliyekkal

Abstract Clean water is vital for supporting human life and the ecosystem. However, the laxity and mismanagement of water resources have endangered the availability of fresh water significantly. Water pollution and associated diseases claim around 2.1 million human lives every year. The outbreak of water-related microbial infections such as diarrhoea, typhoid, and cholera are the primary cause of the loss of lives. Though there has been remarkable progress in the control and prevention of infectious diseases, microbial risks remain a leading cause of human mortality in India, and the rest of the world and children are the worst affected. In this context, a comprehensive analysis of the source, occurrence, fate, and control of biological contaminants in drinking water is of utmost relevance. The rapid and early detection of the pathogenic organism is also of importance in mitigating the menace. This chapter elucidates the growing significance to address the issue of microbial contamination in drinking water and its associated health implications from the past to the present, recent developments in the technologies for the detection, analysis and the remediation of pathogens in the water.

Keywords Analysis · Biological pollutants · Disinfection · Measurement · Safe water

11.1 Introduction

11.1.1 Microbial Hazards: Growing Concern

Freshwater is a complex resource and is linked to almost everything in the world. Its adequate availability at the point of use is a precondition for the existence of humankind and the sustainability of the planet. Quality and quantity are the two significant attributes of this indispensable resource. Though the earth is covered with

U. Kannan · S. Krishna Prashanth · S. M. Maliyekkal (✉)
Department of Civil and Environmental Engineering, Indian Institute of Technology
Tirupati (IIT), Tirupati 517506, AP, India
e-mail: shihab@iittp.ac.in

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70% of the water, only 2.53% of the water is freshwater and in which <0.1% is available for human consumption (USBR 2017).

According to a United Nations (UN) report, the water consumption rate has increased twice than the population growth (Un, n.d.). The Global monitoring body, World Health Organization (WHO) and United Nations International Children’s Emergency Fund (UNICEF) joint monitoring programme for water supply, sanitation and Hygiene (JMP) has established reports on current scenario of issues in water supply, sanitation and hygiene, since 1990. The projections to the next few decades in context to water stress and scarcity across the globe is assessed and evaluated by these organizations (Fig. 11.1).

The 2017 update of WHO/UNICEF Joint Monitoring Programme for Water Supply, Sanitation and Hygiene (JMP) reports that in 2015, 2.1 billion individuals lacked “safely managed drinking water” as shown in Fig. 11.2 (UNICEF 2017). The figure also shows that 11% of the global population (844 million people) lacked even a basic drinking water service. Globally, there are about 423 million people collect water from unprotected groundwater, and 159 million people use surface water directly. Cities and towns pose a special and unique water challenge, as they are expected to be home for 66 percent of the world’s population by 2050 (UN 2018). The impact of poor water quality on people relying on these sources not only limits the access to safe water but also increases the threat to human health.

Globally, 80% of wastewater flows back into the ecosystem without being treated, and 1.8 billion people use untreated water supply as the source of drinking water, putting them at risk of contracting waterborne diseases (UNESCO 2017).

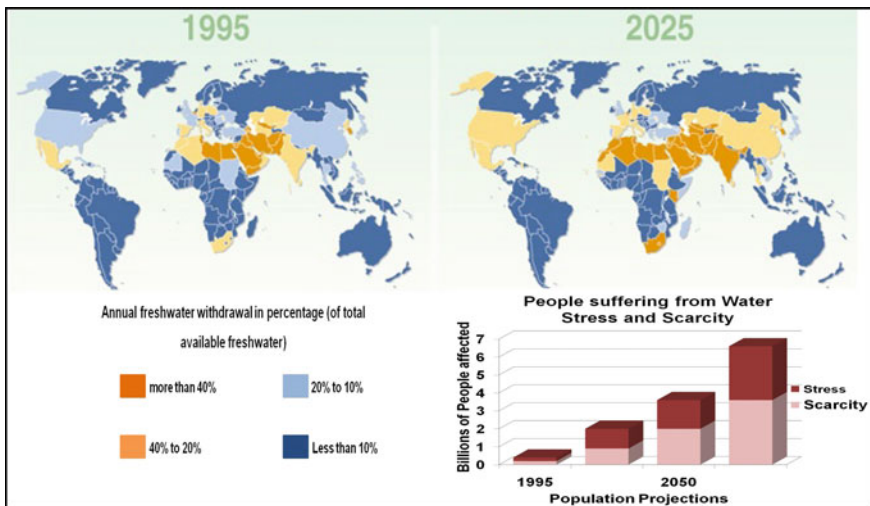


Fig. 11.1 Global water stress and scarcity (reprinted from the source Philippe Rekacewicz, UNEP/GRID-Arendal World Meteorological Organisation (WMO), Geneva, 1996; Global Environment Outlook 2000 (GEO), UNEP, Earthscan, London, 1999 with the permission of World Meteorological Organisation (WMO))

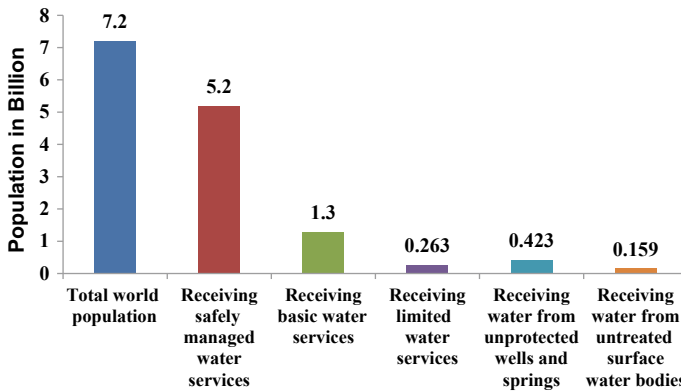


Fig. 11.2 Global drinking water coverage (adapted from the *Source* WHO/UNICEF joint monitoring programme report on water supply, sanitation and hygiene (JMP), 2017 with the permission of WHO)

Water-related illnesses including, dysentery, typhoid, cholera, and schistosomiasis, are prevalent across developing countries, and cholera contributes top of the list outbreaks in 132 countries (WHO 2016). According to a report published in 2015, the waterborne disease remains as an increasing threat among vulnerable and disadvantaged groups across the globe, especially in low-income nations, where 1 in 25 persons is affected due to diarrhoeal diseases. The estimates shows 60% of children under the age of five are affected (UNICEF 2016). Now, waterborne diseases stand as the leading cause of disease and death and accounting 3.4 million loss of lives worldwide (WHO 2001). WHO (2016) reports that diarrhoea is one of the top ten global reasons for death and the second leading cause of death in low-income countries. Around 1.8 million deaths occurred due to diarrhoeal diseases worldwide, and the crude death rate is 58 per 100,000 populations in low-income countries (WHO 2004). Every year around 1.3 to 4 million cholera cases and 21,000 to 143,000 associated deaths are reported worldwide (WHO 2016). According to Global Estimates, there are 20 million reported cases of hepatitis E virus (HEV) infections, which also includes 3.3 million symptomatic cases of hepatitis E infection. The HEV caused approximately 44,000 mortalities in the year 2015, out of which 3.3% were due to viral hepatitis (Rein et al. 2012). As per the Environment and Health Information System, around 13,548 children (0–14 years old) dies in Europe every year due to waterborne diseases (WHO 2007). These statistical facts demonstrate that presence of biological pollutants in water, among others, is a growing concern in both developing and developed countries and it requires adequate attention to meet the safe drinking water needs of the population.

Since the inception of identifying the reasons for human health deterioration due to microbial contamination in water, there have been various theories and subsequent experimental findings to understand the occurrence, health impact, and fate of pathogens in water. For the past few decades, several efforts have

been taken towards the development of robust, efficient, and affordable detection and remediation technology in curtailing the waterborne diseases caused by pathogens. Technology to mitigate the issue can be categorised into detection specific and remediation specific.

This chapter is composed primarily to enhance the knowledge on the importance of microbial contamination in drinking water by understanding the occurrences and sources of pathogens. A detailed review of literature is presented on the growing trends in the field of pathogenic detection techniques and remediation technologies from the past to the recent highlighting their principle, mechanism, applications, and limitations through illustrations and discussions thereof. The effort has also been taken to discern the real challenges in implementing these technologies, which may eventually be utilized in bridging the gap between the lab and the field.

11.1.2 Occurrences and Sources of Microbial Contaminants in Water

Presence of microbial pollutants such as bacteria, virus, protozoa, and helminths pose a severe threat to the quality of freshwater. The typical characteristics, source, and impact on human health of these organisms are summarised in Table 11.1. Their occurrences in water bodies vary depending on several factors. These include various chemical and physical characteristics of the catchment area, the intensity and extent of anthropogenic activities, and the domestic animal discharge. However, the human activities such as discharge of untreated or partially treated municipal wastewater, poor sanitation and hygiene, open defecation, industrial and agricultural wastes, and solid/semisolid refuse are the major sources of concern (Planning Commission 2002). A schematic illustration of routes of microbial contamination in water is shown in Fig. 11.3.

As per the 2002 Planning Commission report, there is a higher threat of waterborne diseases in rural areas caused by water contamination due to poorly maintained water and sewer networks, unscientific disposal of solid wastes, poor healthy sanitation and personal hygienic practices. The discharge of urban sewage is identified as a major source of contamination of Indian surface waters (Murty and Kumar 2011). The estimate shows 80% of surface water bodies in the country is polluted by domestic sewerage (Dey 2015). The Arya et al. (2019) report reveals that India produces 61,948 million litres per day (MLD) of urban sewage. The data also shows that more than 70% of the sewage is let out into the environment untreated (Arya et al. 2019).

Groundwater is a prominent source of drinking water to at least 50% of the population worldwide that also accounts for 43% of the water utilized for irrigation (Faures et al. 2001). Unlike surface water, the sub-surface water is considered less vulnerable to microbial pollution due to the barrier effects provided by the covering soil. There are higher chances of subsurface water contamination when these over-laying barriers are breached, allowing exposure to underground pollution sources,

Table 11.1 Causes, health symptoms, and transmission characteristics of some waterborne diseases of concern

S. No.	Disease caused	Causative agent	Predominant symptoms	Latency	Persistence	Infectivity	Ability to multiply
1.	Amoebiasis	Protozoan (<i>Entamoeba histolytica</i>)	Abdominal discomfort, fatigue, diarrhoea, weight loss, and flatulence	No	Low to medium	High	No
2.	Cholera	Bacterium (<i>Vibrio cholerae</i>)	Vomiting, occasional muscle cramps, and watery diarrhoea	No	Medium to high	Medium to low	Yes
3.	Cryptosporidiosis	Protozoan (<i>Cryptosporidium parvum</i>)	Abdominal discomfort and diarrhoea	No	Low to medium	High	No
4.	Hepatitis	Virus (Hepatitis A)	Abdominal discomfort, fever, chills, pain, dark urine jaundice	No	Low to medium	High	No
5.	Typhoid fever	Bacterium (<i>Salmonella typhi</i>)	Headache, fever, appetite loss, nausea, constipation, diarrhoea, vomiting, and appearance of abdominal rash	No	Medium to high	Medium to low	Yes
6.	Giardiasis	Protozoan (<i>Giardia lamblia</i>)	Diarrhoea, abdominal discomfort	No	Low to medium	High	No

Adapted from WHO (2011)

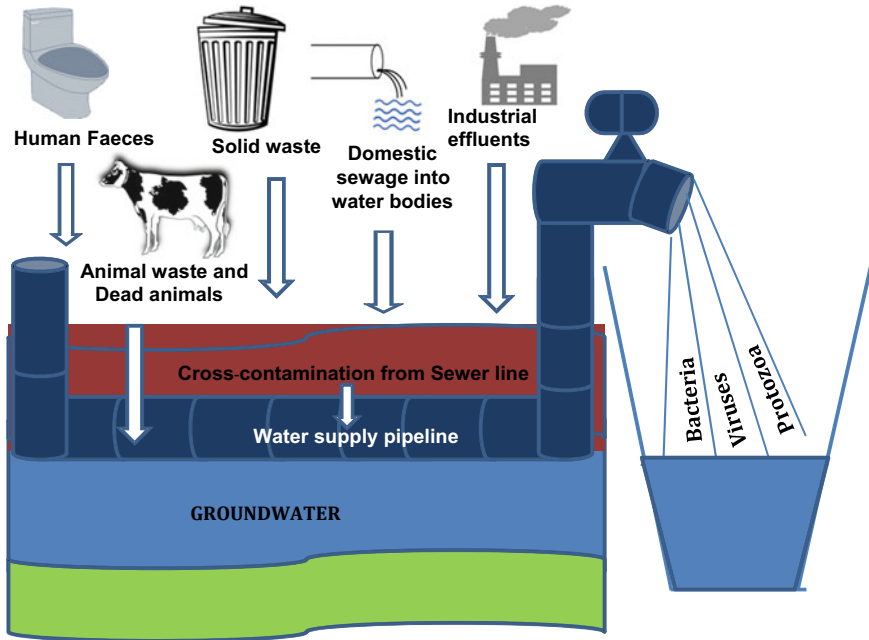


Fig. 11.3 Major sources of microbial contamination of drinking water

such as soak pits, toilets, and sewer lines containing municipal or commercial or industrial wastes. Though the typical presence of human enteric organism is less in groundwater, the pathogens of concern are faecal viruses, which has the potential to enter groundwater system through the porous soil matrices due to their relatively small size. The correlation of groundwater contamination to the global occurrences of the waterborne disease cannot be made typically, as there are various transmission routes. So the exposure-risk relationships are often unclear. However, it is established that several groundwater sources are contaminated with pathogenic organisms and is also responsible for waterborne diseases (Rivera-Jaimes et al. 2018). A study shows that water samples collected from the wells in proximity to the sources of untreated wastewater had higher counts of coliform and faecal coliform, making it unsuitable for both drinking and irrigation purposes (Blumenthal et al. 2000). Saha et al. (2018) and Dey et al. (2017) reported that in northwest Bangladesh, the shallow aquifers are microbiologically contaminated than deep aquifer (Saha et al. 2018; Dey et al. 2017). A study conducted in Kanpur, India documented waterborne disease at an incidence rate of 80.1 per 1000 population (Trivedi et al. 1971). Amongst the shallow wells used by the residents as a source of drinking water, 70% were found to be contaminated by pathogens.

Rural areas of the developing countries using groundwater as a source for drinking are more vulnerable to waterborne diseases than the ones using piped water

supplies. The bacteriological quality analysis including Total Coliform, Faecal Coliform, and Faecal *streptococci* showed that the collected groundwater samples from Triffa aquifer basin, Eastern Morocco were contaminated due to unprotected septic tanks and the wastewater dumped in the upstream end of river Cheraa wadi (Yahya et al. 2017). An experimental investigation made by Venkatesan et al. (2014) to study the impact of flooding on microbial contamination in groundwater at Chennai, India after a major flood event revealed higher counts of coliform in subsurface water sources at most affected areas. The rapid escalation in the microbial growth was attributed to the contaminated storm water runoff entering into Adyar River, Tamil Nadu, India (Gowrisankar et al. 2017). The residences with on-site septic systems were likely more affected due to the infiltration of the contaminated river into the groundwater sources (Jamieson et al. 2003).

The increase in levels of pathogenic contamination in estuaries and marine environment is also poses a threat to public health. In context to marine water, the prominent reasons for microbial contamination are failures in septic systems, discharges of sewage from shoreline outfalls, farm animal wastes, and runoff from naturally vegetated areas. The storm water runoffs from urban, commercial, and industrial lands, the practice of open defecation near coastal areas are also responsible for contamination of marine waters (Pandey et al. 2014). World Ocean Network reports that around 90% of wastewater and 70% of industrial waste is being discharged into oceans by the developing countries (Vandeweerd et al. 2002). Estuaries located adjacent to residential areas, when used as a mode of transportation and for recreational activities can cause a significant impact on pathogen levels (Schriewer et al. 2010). The pathogens, including *Salmonella*, *Vibrio cholerae*, *Cryptosporidium*, *Giardia*, and *Campylobacter spp.* are reported in estuaries (Rhodes and Kator 1990).

The other potential source of pollution is due to the regrowth of microbes in the water distribution network (Shaheed et al. 2014). In the United States, around 10% of outbreaks are caused by contaminated water due to the improper water distribution network (Craun et al. 2010). The corrosion and poor surface finish in the water supply pipelines enables enhanced colonization of microorganisms and the formation of biofilms (Rakić 2018). These biofilms can act as a short or long-term habitats for pathogenic organisms, such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Legionella spp.*, *Campylobacter spp.*, noroviruses, adenoviruses, rotaviruses, and parasitic protozoa (*Cryptosporidium parvum*) (Wingender and Flemming 2011). The emergence of new pathogens, mutants of the existing pathogens, and the presence of multi-drug resistance species are also reasons for concern.

11.1.3 Transmission of Waterborne Diseases

It is a fact that microbial hazard is a principal cause of human mortality in the developing world. There are various groups of pathogenic microorganisms, and they have different modes of transmission, as is shown in Fig. 11.4. Drinking water is

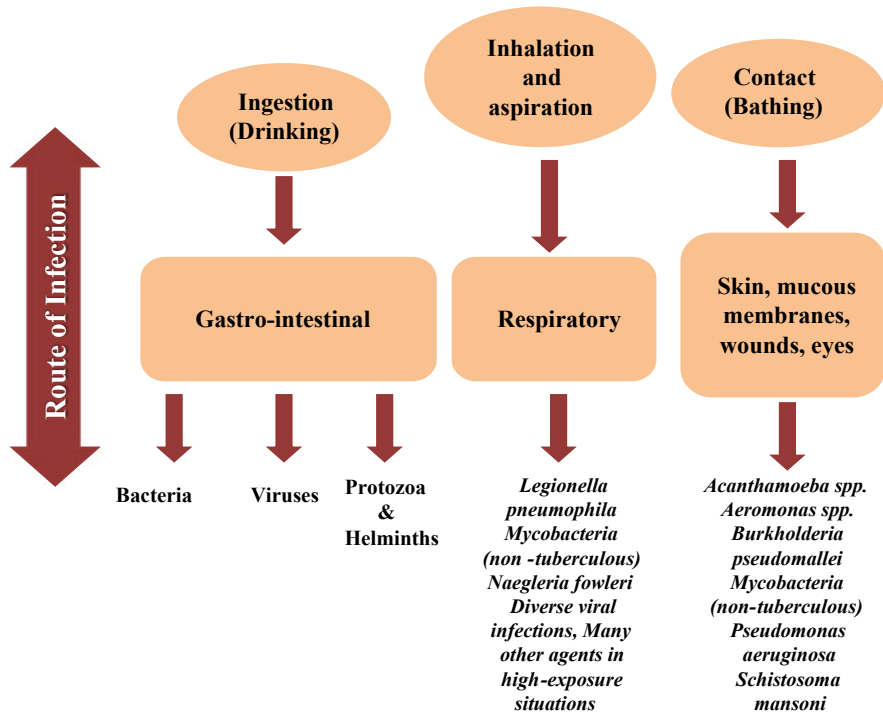


Fig. 11.4 Schematic representation of transmission pathways of waterborne microorganism with examples. Adapted from the source Guidelines for drinking-water quality, 4th edition, with the permission of WHO

observed to be the only carrier of the faecal-oral route of pathogenic transmission (WHO 2011).

The transmission characteristics of pathogens can be categorized based on latency, persistence, infectivity, and the ability to multiply. Latency is the lag time between excretion of a pathogen and the stage at which it becomes infective to a new host. Typically, protozoa, bacteria, and enteric viruses have no latent period (Feachem et al. 1983). The most of helminths require a different latent period either for eggs to progress to the transmittable stage or to pass through an intermediate form to complete their life cycles (Cotruvo et al. 2004). Persistence is determined by the span of time that a pathogenic organism exists in the environment outside a human host in viable condition. Persistent microbes can travel through a prolonged route, viz., through a sewage treatment system and can still be infectious to human living away from the original host. In general, persistence increases in the order: bacteria > protozoa > viruses > helminths, whose persistence is measured in months. The infective dose refers to the microbial concentration that can cause infection upon ingestion. Usually, the minimum infective doses for viruses and protozoa are less than that of bacteria.

A summary of various waterborne diseases linked to the protozoa and helminths, its relative infectivity, and persistence in water are discussed in Table 11.1. The pathogens that are not listed in Table 11.1 can also transmit by water, and the list is not complete.

11.1.4 Drinking Water Safety Guidelines

The purpose of the disseminated WHO standard guidelines is to enable countries and regions to develop their own standards conforming to the regulation. It suggests that immediate action must be taken if *E. coli* is detected in drinking water. Monitoring the levels of *E. coli* and faecal coliforms is a common method in the quantification of the pathogen loads in water bodies (Feachem et al. 1983). For decades, public health experts and scientists have assessed water quality in rivers, estuaries, and coastal waters in terms of faecal coliforms and *E. coli* (Pandey and Soupir 2013). However, *E. coli* cannot predict the existence of all pathogenic organisms. For example, *Cryptosporidium* oocysts may survive chlorine disinfection and may be present in the absence of *E. coli*, showing the limitation of using *E. coli* as a potential indicator for faecal contamination. However, *E. coli* is the designated WHO indicator for reliable diagnosis of microbial quality of the water (WHO 2011). The guideline values for assessing the microbial quality are given in Table 11.2.

Despite of establishing definite standard regulations for safe supply of water, the concern of detecting and monitoring these pathogens in water samples collected from various sources have been still a challenging task for both developed and developing countries. The following discussions will describe the existing methods of monitoring and removing pathogenic organisms in water.

Table 11.2 Guideline values for verification of microbial quality

Organisms	Guideline value
All water directly intended for drinking <i>E. coli</i> or thermo-tolerant coliform bacteria	Must not be detectable in any 100-ml sample
Treated water entering the distribution system <i>E. coli</i> or thermo-tolerant coliform bacteria	Must not be detectable in any 100-ml sample
Treated water in the distribution system	Must not be detectable in any 100-ml sample

Adapted from the source Guidelines of Microbial quality, WHO (2011)

11.2 Detection and Analysis of Pathogenic Organisms in Water

Efficient testing and fast detection of pathogenic organisms are vital in the management of water-borne illness. It is the main checkpoint in eliminating the pathogens in drinking water, food, and other biological samples. It also plays a significant role in diagnosing and preventing diseases (Vidic et al. 2017). A typical detection technique should be sensitive, rapid, and affordable. There are several routes, such as culture/growth, optical, molecular, and bio-sensing based are used to detect the pathogenic organism in these samples. The major methods under each detection technique are briefly discussed below. Table 11.3 presents a summary of the testing methods.

11.2.1 Culture/Growth Based Method

Culture or growth based technique is a traditional method employed for the detection of pathogenic organisms in the water. The majority of the testing for bacteria detection is done through this conventional approach. It involves growing and isolation of organisms on Petri-plates containing growth-media, followed by biochemical tests to confirm the presence of pathogenic microorganisms. It is a time-consuming technique, and typically take 5–7 days to obtain the results (Rajapaksha et al. 2019). It is not suitable to detect organisms which are viable but present in the non-culturable state. However, the traditional culture-dependent method is regarded as the standard method for the detection of pathogens, and it is still being used as a regulatory requirement by water treatment companies and laboratories to monitor the microbial quality of drinking water (American Public Health Association, American Water Works Association 1989). The estimation of the most probable number (MPN) or multiple fermentation is a commonly practiced growth-based method to find the concentration of viable microorganisms in the water sample. It is a statistical method that relies on the principle of extinction dilution for testing the quality of water and assesses its suitability for human consumption. The technique typically identifies the presence of an indicator organism of faecal origin to establish the existence of pathogenic microorganisms (Munoz and Silverman 1979). It works based on the principle of fermentation of lactose to produce the acid as well as gas. The presence of coliform is showed by the colour change of the medium, by a change in pH, or by the collection of gas in inverted Durham's kept in the test medium. The total coliforms can be determined by counting the number of tubes showing both colour change and production of gas (Fung and Miller 1970). The MPN analysis is usually performed in 3 steps, including presumptive, confirmatory, and completed test. The presumptive test is the first step and is carried out to identify the presence or absence of the coliform organism in the water sample. If this screening test is negative, the

water sample is considered free from pathogens. If the test is positive, further confirmatory analysis is required to ratify the faecal origin of the coliform organisms. The completed test is performed to check and eliminate the false positive test. These steps are illustrated in Fig. 11.5.

The method is time-consuming and takes normally up to 72 h for obtaining the results. The development of Membrane Filtration (MF) technique shortens the process and reduces the completion time to 24 h. In this technique, the sample is allowed to pass through the membrane filter (pore size of 0.45 μm), and the membrane containing the trapped bacteria is transferred on to a Petri-plate containing the nutrient agar medium. The results were obtained by counting the bacterial colonies, which is grown on the incubated Petri-plates. The number of bacterial colonies grown on the agar medium is counted and is represented as CFU/ml.

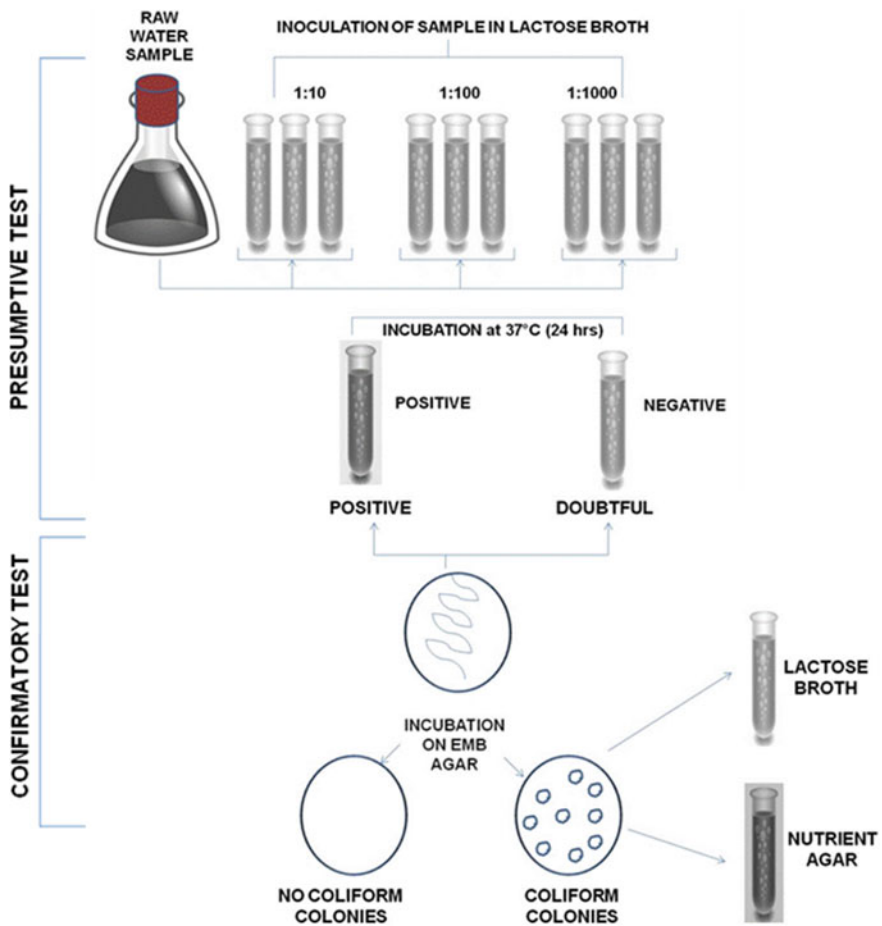


Fig. 11.5 Schematic representation of MPN test

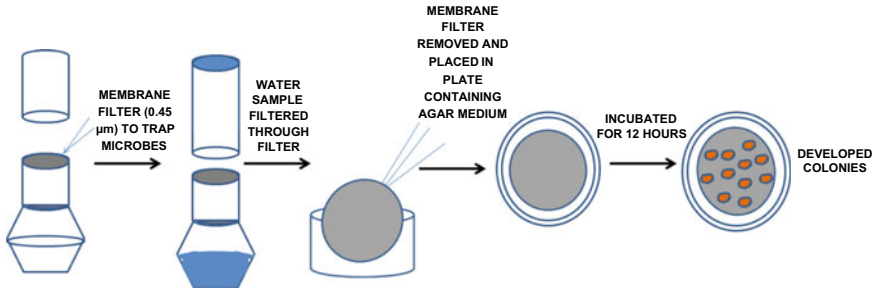


Fig. 11.6 Schematic representation of membrane filtration method for the detection of pathogens

The technique is highly effective for assessing the performance of chlorination as it removes the bactericidal agents through filters (Tankeshwar 2010). The process flow diagram is presented in Fig. 11.6. Apart from standard nutrient agar medium, chromogenic agar medium such as Eosin Methylene blue agar (Leininger et al. 2001) and Macconkey agar (March and Ratnam 1986) is also used for differentiating coliforms from faecal coliforms and isolation of members of family Enterobacteriaceae, respectively. Furthermore, agar medium is available with chromogenic and fluorogenic substrates that helps in the fast and real-time detection of total coliforms and *E. coli* (Manafi and Kneifel 1989).

Pathogenic viruses can also be detected using the culture-based method. The method involves inoculation of virus stock aliquots onto the medium containing susceptible cell monolayers followed by incubation. The inoculated virus gets attach to the cells, and these infected cells release progeny of virus that forms the circular zone of infected cells over the medium called plaque. The result is expressed in plaque forming units (PFU) per ml (Dulbecco and Vogt 1953). Though culture based method are widely used, the technique is time-consuming and requires a lot of resources, including various laboratory equipment and a skilled workforce. The safety concern and less sensitivity of the test may limit the use of the said method in some cases. Therefore, there is a need for the development of rapid and easy to use techniques.

In drinking water treatment and distribution systems, the bio-stability of the water is assessed by assimilable organic carbon (AOC), which represents the dissolved organic carbon that is assimilated by the microbes present in the drinking water (Kooij 1992). In water distribution system, AOC can be correlated with the presence of biofilm and regrowth of microorganisms. Typically, AOC is analysed by the standard method, and it is explained in brief as follows. The AOC determination involves two steps: (i) culturing of microbes and (ii) enumeration of microbes. In the first step, the water sample is inoculated with test microorganisms such as *Pseudomonas sp.* (P-17) and *Spirillum sp* (NOX) and incubated at 15 °C for 9 days. Once the microbial growth attains the stationary phase, the cells are enumerated using the plate count on the agar medium (Tang et al. 2018; Hammes and Egli 2005). The net microbial growth is related to the growth of test organism on acetate (P-17) and oxalate (NOX). The results are represented as acetate-C equivalents. The bio-stable water should have an

AOC concentration of 10 $\mu\text{g/L}$ acetate-C equivalents, which depends on the available chlorine in the water (Kooij 1992).

11.2.2 *Optical-Based Methods*

The culture-based technique is not suitable for non-culturable organisms. The method is time intensive and laborious. On the contrary, the optical-based methods are nonculture-based technique and is simple, fast, and less costly. The optical-based microscopic techniques are used to visualize the size and morphology of the bacterial cells. However, most of the microbes lack colour and contrast which makes its visualization more difficult. In such cases, the incorporation of fluorescent dyes and stains can help in overcoming the above-said limitations (Claus 1992). The stains are made of salts containing positive or negative ion depends on the chromophore. Typically, the negatively charged bacterial cell wall sticks to the positively charged chromophores, which makes the visualization of microbes under light microscopy easier. The commonly used dyes are safranin, methylene blue, malachite green, eosin, eosin, fuchsin, rose bengal, and crystal violet (Microbiology L.I 2019). The traditional light microscope uses light (400–700 nm) for the illumination to magnify the bacterial cells in the sample. On the other hand, the fluorescence microscope uses much higher light intensity to excite the sample of interest that contains fluorescent dyes. Here, the fluorescent microscope contains the filter cube set that allows the radiation of a wavelength which matches with the fluorescing compounds (Bradburry 1996). The fluorescent microscope enables the real-time detection of dead and live bacterial cells using DEAD/LIVE bacterial viability kit, which contains the fluorescent stains (Boulos et al. 1999). The refinement in the field of microscopes paved the way for the development of differential interference and phase contrast microscope (Keevil 2003), confocal laser scanning microscopes (CLSM), and total internal reflection fluorescence microscopes (TIRF) for the visualization of microorganisms. The phase contrast microscopes are used to visualize the microbes without staining (Keevil 2003). The CLSM (Sheppard et al. 1997) and TIRF (Axelrod 2001) are mainly used to image the structural components of cells, genetic material present inside the cells, and the specific cells within major.

Recently, adenosine triphosphate (ATP), an indicator of the presence of microbial growth, based optical detection method has been developed a potential (Selan et al. 1992). The bioluminescence, a light emission due to chemical reactions in the organism, forms the basis of this detection technique. In this method, a buffering agent that lyses the cell wall of the bacteria is added to the water sample, which is concentrated by membrane filtration. This buffering agent releases the ATP, and the concentration of ATP is measured by light emission intensity (580 nm) produced via luciferin-luciferase assay. The activity of the assay is standardized against known concentrations of ATP, and the results are represented as Relative Light Unit/ml (RLU/ml) (Turner et al. 2010). This ATP based measurement was also used to check

the efficiency of the treatment processes, such as ozonation, UV treatment, and chlorination with respect to bio-stability of treated water. This method also acts as the best surrogate for measuring the growth of biomass and for determining the biomass production potential (van der Wielen and van der Kooij 2010). Though the culture-based methods are convenient, simple to perform, low-cost, and rapid, the limited representation of microbial communities is a limitation.

Recently, flow cytometry (FCM), an optical based detection method, is used to identify the individual microbial cells presents in a complex microbial community (Basiji et al. 2007). The working mechanism of FCM is given in the following steps: (i) the microbes present in the suspension is allowed to pass through a laser beam, (ii) the cells present in the suspension are scattered by light, and the fluorogenic substrates are excited to produce emission. The scattered light is captured at a low angle (forward scattering) and high angle (sideward scattering). The fluorescence is detected by using the selective wavelength filters. The method can be used to find the size, shape, and the number of microorganisms present in the sample. The schematic representation is shown in Fig. 11.7. Unlike fluorescence microscopy, FCM does not produce the images, rather the characteristic feature of each cell are presented as histograms or dot plots. The FCM is highly sensitive (<100 cells/ml) and rapid technique (<3 min per sample) (Hammes and Egli 2010). Along with the bacteria, yeast cells (Díaz et al. 2010), algae (Dubelaar and Gerritzen 2000), viruses (Brussaard

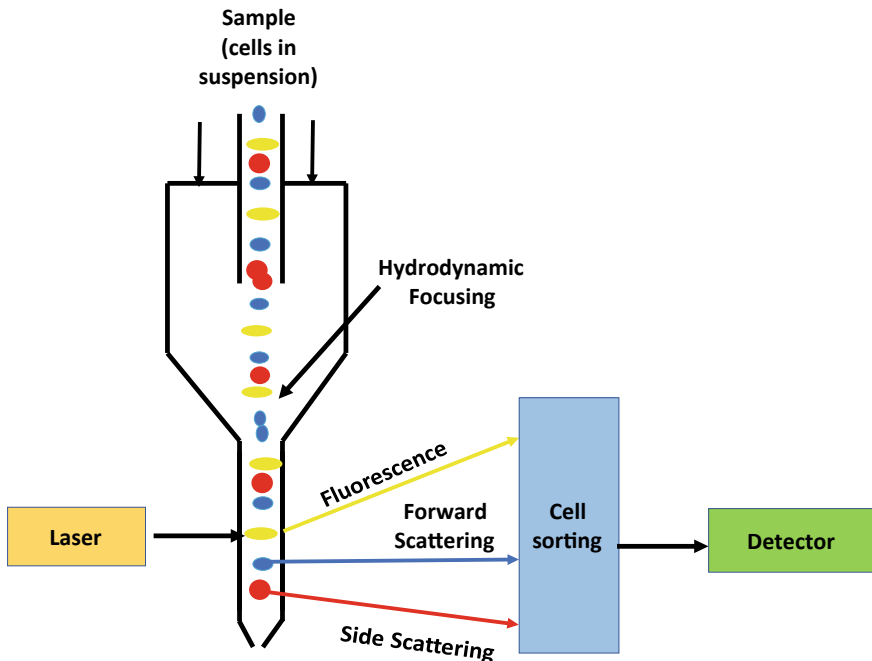


Fig. 11.7 Schematic representation of flow cytometry for the detection of pathogens

et al. 2000), and protozoa (Vesey et al. 1994) present in the water samples can also be detected by FCM (Ambriz-Aviña et al. 2014). The FCM is a single cell technique, and it cannot be used for analysing biofilms present in the drinking water sample. Though there are improvements in the field of optical based detection techniques for the identification and enumeration of the pathogens, the disparity in the order of magnitude of the total number of cells between microscopic counting and plate counting methods needs further attention.

11.2.3 *Molecular Based Methods*

The molecular-based methods are more sensitive, reliable, robust, and yield conclusive results (Derveaux et al. 2010). The technique is suitable for the detection of a broad spectrum of microorganisms, including emerging pathogens. Unlike conventional techniques, this process functions by detecting specific ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) sequences in the target organism (Garibyan and Avashia 2013; Law et al. 2015). The method allows for simultaneous detection and identification of pathogenic microorganisms.

Polymerase chain reaction (PCR) is one of the popular molecular-based detection ways in practice. It was invented in the late 20th century by Kary Mullis, and he was awarded Nobel Prize in chemistry for the invention in 1993 (McPherson and Møller 2000). It is very sensitive and can detect single bacterial pathogen (Velusamy et al. 2010). It involves amplification of a primer mediated enzymatic DNA and creation of specific DNA fragments (Valones et al. 2009). The amplification typically occurs in a cyclic three-step process (Law et al. 2015). These include DNA melting, annealing, and extension. In DNA melting or DNA denaturation, a double-stranded DNA is physically separated to two-pieces of single-stranded DNA at elevated temperatures (90–97 °C). In the next step, the oligonucleotides or specific primers anneal (50–60 °C) and bind to the complementary sequences of DNA. The two DNA strands then form a template for DNA polymerase to synthesise a new DNA strand. In the final step, the new DNA strand is used as a template to create the duplicate copies, and the original DNA template is amplified exponentially through a chain reaction.

The developments in the PCR based detection techniques include cold PCR (Milbury et al. 2011), heat pulse extension-PCR (HPE-PCR) (Orpana et al. 2013), and nanoparticle-PCR (Ma et al. 2013). But the limitations with PCR is that it cannot differentiate between live or dead cells, and it will produce false results if there is any contamination in the sample. So, the PCR technique may not be useful for the detection of pathogens present in the wastewater sample. To eliminate the lack of differentiation between live and dead cells, the reverse transcriptase PCR (RT-PCR) was developed (Cangelosi and Meschke 2014). The pathogenic viruses can also be determined by RT-PCR and real-time PCR (Mattison and Bidawid 2009). Recently, multiplex PCR assay has been developed by the researchers to detect 10 viruses in a single tube (Pham et al. 2010; Wolf et al. 2008). Furthermore, advanced molecular methods such as usage of DNA based fluorescent probes and Enzyme-Linked

Immunosorbent Assay (ELISA) could be used for the identifying pathogenic species (Kittigul et al. 2001).

11.2.4 Bio-sensing Based Methods

There is an growing demand for a versatile and sensitive technique that detects pathogens in a rapid manner. Biosensors are devices that work based on the detection of signals produced by the interaction between the bio-recognition elements, such as enzymes, antibodies, aptamers, oligonucleotides probes, nucleic acids, and cell-surface molecules (Rider et al. 2003), and the target analyte species (Zourob et al. 2008).

Here, the biological response is converted to electrical signals, and it is recorded by a detector (Brindha et al. 2018). The schematic diagram showing the working principle of bio-sensors is given in Fig. 11.8. A comparative analysis of various types of biosensors is presented in Table 11.3. In addition to the existing bio-recognition elements, functional nanomaterials are also being used for the detection of pathogens (Krishnan et al. 2019). The method has a few limitations. The credibility and sensitivity of bio-sensors are affected due to the interference caused by organic/inorganic molecules and other contaminants, such as humic substances with the microbes. The natural receptors such as antibodies and enzymes that are immobilized at the transducer surface are prone to the degeneration, which results in loss of selectivity and

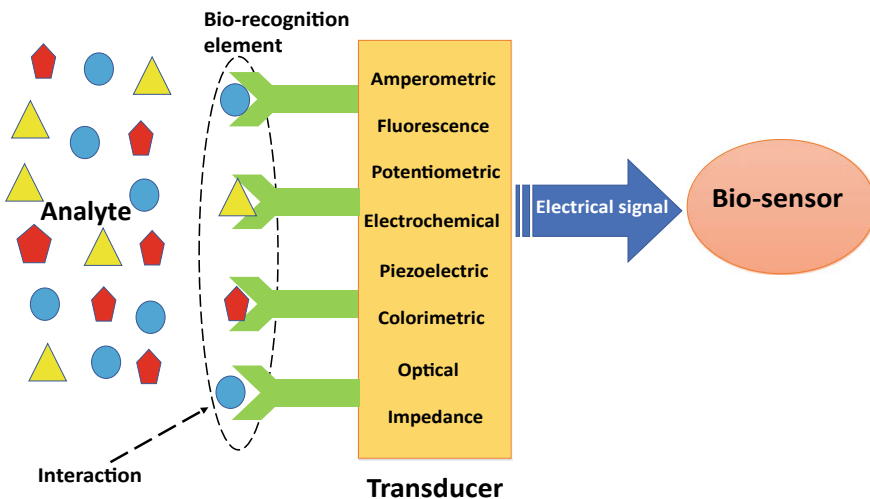


Fig. 11.8 Schematic representation of working mechanism of bio-sensor (courtesy of: <http://agscientific.com/blog/2016/01/biosensors-and-diabetes-trending-applications>)

Table 11.3 Summary of various detection technologies

Technology/methodologies to detect pathogens	Advantages	Disadvantages
<i>Optical based methods</i>		
Microscopic methods	Can differentiate the pathogens based on the structure; Faster technique	Requires sophisticated instruments
Flow cytometry	Simultaneous analysis of physical and characteristics of cells up to 1000 particles/se, rapid analysis, provides the finger print of microbial community in water sample	Expensive
ATP measurements	Fast: low-cost kits are available	Requires sophisticated instruments
<i>Culture/growth based methods</i>		
Heterotrophic plate count	Reliable results	Time consuming; require well equipped laboratories; expensive; viable but non culturable bacteria can yield false negatives
Assimilable organic carbon	Widely used in drinking water research; can assess the potential growth of biofilms	Quantify the nutrients instead of bacteria, it assumes the growth of bacteria is limited by organic carbon, not applicable for all types of bacteria
Enzyme catalysed multiple tube fermentation or most probable number (MPN) technique	Easy interpretation; effective method for analysing samples with high turbidity	Time consuming; not very accurate; requires a of resources
<i>Molecular method</i>		
Quantitative PCR (Q-PCR) or Real Time PCR (RT-PCR)	Rapid; high sensitive and quantitative; accurate gene quantification; target specific	Difficult to obtain good quality RNA in RT-PCR
<i>Bio sensing method</i>		
Bio sensing based Method	Rapid, sensitive	Costly, antibodies are so sensitive, limited stability of fluorophores

Table 11.4 Comparison of bio-sensing technologies

S. No	Biosensing techniques	Pathogens	Detection limit	Detection time	Reference
1.	Electrochemical biosensor	<i>E. coli</i> O157:H7	10 to 10 ⁶ CFU/mL	45 min	Wang and Alotilja (2015)
2.	Electrochemical genosensor	<i>S. Typhi</i>	50 pM	Not given	Das et al. (2014)
3.	Potentiometric biosensor	(i) <i>C. Parvum</i>	5 × 10 ² oocysts/mL	60 min	Laczka et al. (2013)
		(ii) <i>V. Cholera</i>	10 × 10 ⁻⁹ to 10 × 10 ⁻⁶ M		
4.	Amperometric biosensor	<i>E.coli</i> O157:H7	10 ² CFU/mL	–	Xu et al. (2016)
5.	Impedance based biosensors	<i>L. Monocytogenes</i>	30 CFU/mL	–	Chen et al. (2015)
6.	IR and Raman spectroscopy based biosensor	<i>Pseudomonas aeruginosa</i> and <i>E. coli</i>	10 ¹ to 10 ⁶ PFU/ml		Al-Qadiri et al. (2008)
		<i>Polio virus, Staphylococcca, and enterotoxin B</i>	1.3 pg/ml	6 h post infection (hpi)	Lee-Montiel et al. (2011)
7.	Fluorescence based biosensor	<i>E. coli</i> and <i>S. aureus</i>	10 ³ cells/ml	–	Wang et al. (2016a, b)
		<i>E. coli, E. aergens, E. dissolvens, S. aureus</i>	30 CFU/ml	<120 min	Dogan et al. (2016)
8.	Colorimetric biosensor	<i>Salmonella enteritidis</i>			
		<i>E. coli</i> and <i>S. aureus</i>	30 CFU/ml for <i>E. coli</i> & 200 CFU/ml for <i>S. aureus</i>	–	Thakur et al. (2015), Safavieh et al. (2014)
9.	Piezo-electric biosensors	<i>E.coli</i> O157: H7	2 × 10 ³ CFU/ml 10 ⁷ to 10 ⁹ CFU/ml	–	Wang et al. (2008)
		<i>S. typhimurium</i>	10 ⁶ to 10 ¹⁰ CFU/ml	<30 min	Babacan et al. (2000)

sensitivity. These challenges may be addressed by replacing the natural bio recognition elements with bio-mimetic elements, such as aptamers, peptides, and molecular imprinted polymer that can enhance the sensitivity of the detection (Kumar et al. 2018).

11.3 Remediation of Microbial Contaminated Water

11.3.1 A Brief Review of Disinfection Technologies

Access to clean water is a fundamental human right and is essential for a healthy life. The relationship between the quality of water and health are well documented. One of the primary reasons for the loss of human lives in most of the countries is the consumption of contaminated water. However, providing safe water to every person is a challenging task due to the increasing presence of pollutants and the growing gap between demands and supply. There are several classes of pollutants reported in drinking water and are presented in Fig. 11.9. Among these microbial pollutants requires special attention due to their widespread occurrence and potential to cause adverse ill effects on human health. A brief review of various aspects of the treatment of pathogens is presented below.

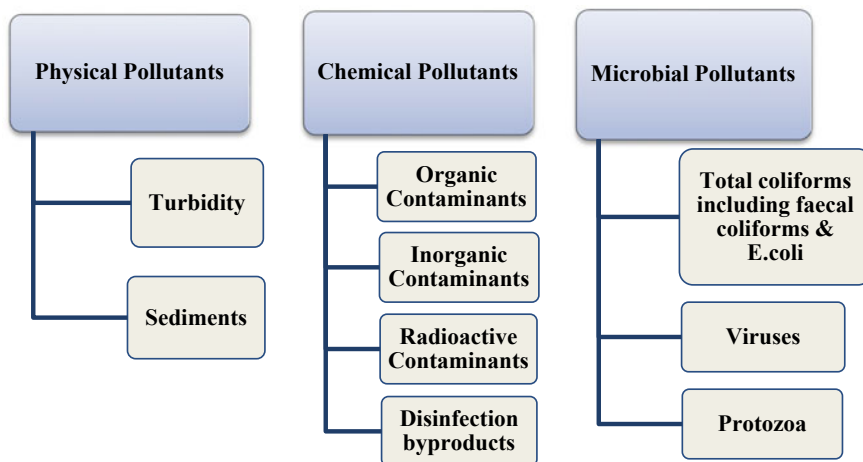


Fig. 11.9 Classification of various pollutants in drinking water

11.3.2 *Centralised Versus Point-of-Use Technologies*

The goal of disinfection is to alleviate the pathogens responsible for waterborne diseases. Several treatment technologies have been employed to achieve the goal. However, several factors govern the selection of disinfection technologies. These include the ability of the disinfectant to kill a broad spectrum of pathogenic organism, the capacity to provide residual disinfection activity, affordability, the formation of disinfection by-products, and the aesthetic quality of the treated water (National Research Council (US) Safe Drinking Water Committee 1980). The scale of operation is another critical parameter that decides the success of the treatment process. Treatment may be done at a small scale in decentralized plants or can be done at community level at a centralized facility. However, establishing large community water treatment systems is challenging for developing nations. The challenges include capital investment, skilled labour, and governance, access to appropriate technologies, piped water supply networks, water scarcity, maintenance, and recontamination. The poor success in overcoming these challenges has increased the popularity of decentralized or point-of-use (POU) water treatment systems. In developing countries where only limited households have piped water (Kanungo et al. 2010), POU interventions seem to be a sustainable way of providing safe drinking water.

Though technologically advanced countries can afford to use a complex system to meet the stringent regulations, their public water system is also not completely free from pathogenic organisms. Several incidences of the pathogenic microorganism in piped water are reported from developed countries (EPA 1996). Studies show that despite keeping adequate disinfectant residual, there is a significant deterioration of water quality due to the proliferation of microbes in the bio-films attached to the distribution pipes (Machell et al. 2010; Szewzyk et al. 2000; Simoes and Simões 2013; Douterelo et al. 2014). The data further support the inefficiency of the public water supply system to contain waterborne outbreaks. In this context, there is a need for an affordable and efficient alternate disinfection system. A well designed and maintained POU disinfection seems to be an attractive option (Vagliasindi et al. 1998; Sobsey et al. 2008).

Several point-of-use water purification technologies are developed to disinfect water. Among the available technologies, chlorination with safe storage, combined coagulant-chlorine disinfection, SODIS (solar UV radiation + with thermal effects), ceramic filter, bio-sand filter are well documented and capable of reducing waterborne infectious disease (Sobsey et al. 2008; Rose et al. 2006). State-of-the-art literature reviews show that POU household interventions contribute to a 30–40% reduction in diarrheal diseases (Clasen et al. 2007; Fewtrell et al. 2005). According to a recent review, over 18 million people use POU water treatment systems, with 12.8 million using chlorination, 2.1 million using SODIS, 0.934 million using flocculation/chlorination, 0.7 million using bio-sand filtration, and 0.35 million using ceramic filtration (Sobsey et al. 2008; Clasen 2008) have compared these widely promoted and used POU systems for performance and sustainability and identified ceramic and bio-sand based systems are most effective. However, Lantagne et al.

(2008) pointed out the flaws in the comparison and strongly commented that the comparison is biased. The role of participant motivation in reducing dysentery and non-dysentery diarrhoea by disinfection using SODIS among children (0.5 to 6 years) living in peri-urban communities in South Africa was studied (Du Preez et al. 2010). After comparing 383 children in 297 houses using SODIS with 335 children in 267 families with no intervention, the authors concluded that the motivation of participants is also an essential factor for measurable health gain.

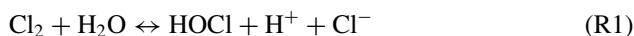
Despite of obtaining promising results with the technologies validated in the laboratory and field, except boiling, the large-scale deployment of the technologies has hindered. Some of the reasons highlighted for the failure are (Sobsey et al. 2008): (i) Inability to provide adequate safe water, (ii) difficulty in operation and maintenance, (iii) large user time to treat water, (iv) affordability, (v) weak supply chain for needed replacement of units or parts, (vi) objectionable taste and odour, and (vii) bio fouling. Therefore, addressing these issues is vital for the successful implementation of POU treatment systems. Moreover, public participation, socio-economic considerations, local water quality, and consumer preference also need to be considered as sustainability criteria for developing POU water treatment systems.

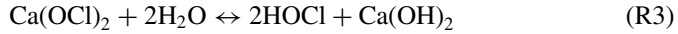
11.3.3 Convectional Disinfection Technologies

The microbial quality of water can be improved through physical, chemical, or biological methods. There are several options available under each method. However, commonly practiced techniques involve chlorination, ozonation, filtration, UV irradiation, boiling, and SODIS process. A brief description of each technology is discussed below.

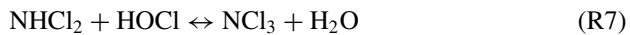
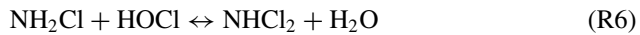
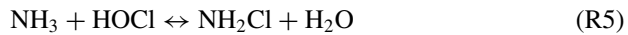
Disinfection with Chlorine: Chlorination is a popular disinfection processes and is achieved by introducing chlorine gas or its derivatives such as sodium hypochlorite (NaOCl) or calcium hypochlorite (Ca(OCl)₂) into water. The chlorine gas was first discovered in 1774 by Karl W. Scheele. Later, Humphrey Davy recognized it as a disinfectant in 1810 (Pradeep 2009). The continuous chlorination of public water process was first introduced in 1904 by Sir Alexander Houston of the London. In 1908, the application of calcium hypochlorite to Bubbly Creek water supply of the city of Chicago was initiated in the US (Logan and Savell 1940).

Upon addition of chlorine or its derivative to water, the chlorine agent undergoes hydrolysis and results in the formation of free-chlorine species (HOCl and OCl⁻). These species are responsible for disinfection of water. The reactions involved in the chlorination process are given below.





Among the free chlorine species, HOCl is the more powerful oxidizing agent (Metcalf 2003). The relative concentration of these species will vary according to the pH of the water. The chlorine can also combine with ammonia present in water and form chloramines. The critical reactions involved in the formations of chloramines are given below.



The formation of chloramines is dependent on the relative concentration of ammonia and chlorine, pH, contact time, and temperature (Metcalf 2003). Though chloramines are less effective compared to free-chlorine species, they are unlikely to produce disinfecting by products (DBPs).

Disinfection with Ozone: Ozonation is the second most widely used disinfection process after chlorination. In 1906, France reported the first use of ozone as a disinfectant (Pradeep 2009). Ozone is generated on-site and is typically produced through electrical discharge method. A schematic diagram showing the generation of ozone is shown in Fig. 11.10. The freed radicals (HO and HO₂) formed as a result of the decomposition are probably responsible for the defection (Metcalf 2003). Unlike chlorine and chloramines, ozone is effective against a broad spectrum of the organisms, including *Giardia lamblia* and *Cryptosporidium parvum*. It is also found to be effective against spores and cysts (Budu-Amoako et al. 2011). However,

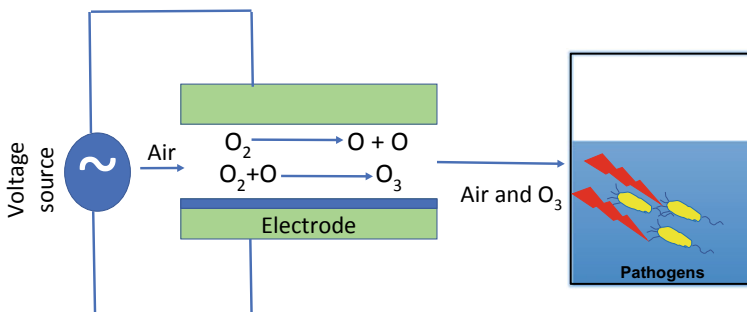


Fig. 11.10 Schematic representation of ozonation (courtesy of: chemistry of ozone disinfection in wastewater, environmental protection agency EPA 2016)

it does not maintain residual ozone concentration and less effective in preventing recontamination of water (EPA 2011).

Disinfection with UV: The first use of UV treatment in municipal water supplies was reported in 1916. Presently, the use of this technology is used in several applications. Unlike ozone and chlorine, UV light is a physical disinfecting agent, and hence, it is free from taste, odour, and harmful by-products even at high dose (EPA 2011). UV radiation at the right wavelength (255–265 nm) has shown active bactericidal and virucidal properties. The schematic diagram of the UV disinfection process is presented in Fig. 11.11b. A comprehensive review of UV based disinfection system is given elsewhere (Nyangaresi et al. 2018; Li et al. 2018).

Disinfection with Solar Radiation: Solar disinfection, popularly known as SODIS process, works based on the germicidal property of UV radiation and thermal heating.

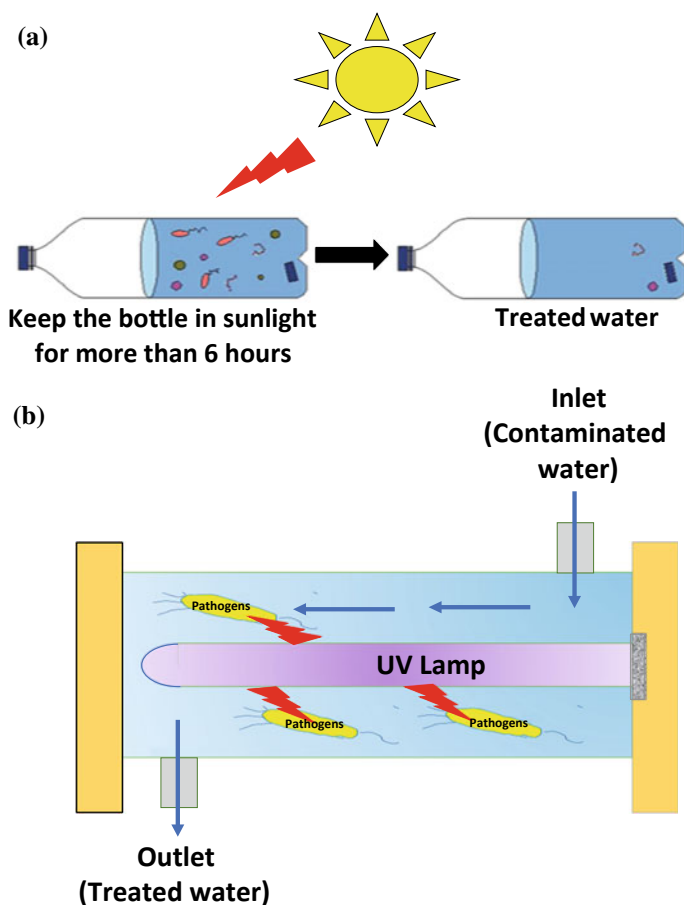


Fig. 11.11 Schematic representation of **a** solar disinfection (SODIS) (courtesy of www.billionbottleproject.org). **b** UV irradiation

In the SODIS process, the water is exposed to natural sunlight instead of light from a UV lamp. The conventional UV system uses UV-C (200–280 nm) radiations, whereas the SODIS process uses UV-A (320–400 nm) radiations. The interaction of UV-A with water generates reactive oxygen species (ROS) and ROS damage the DNA and deactivate the germs in the water. However, the SODIS process is not as effective as conventional UV treatment because UV light constitutes only <5% of the total solar spectrum (McGuigan et al. 2012). The schematic diagram of SODIS process is presented in Fig. 11.11a. Some of the viruses, protozoan species exhibits resistance to chlorination. So, we are in necessity to find the alternative technology that can kill various kinds of pathogenic species.

11.3.4 Nanotechnology Enabled Disinfection Process

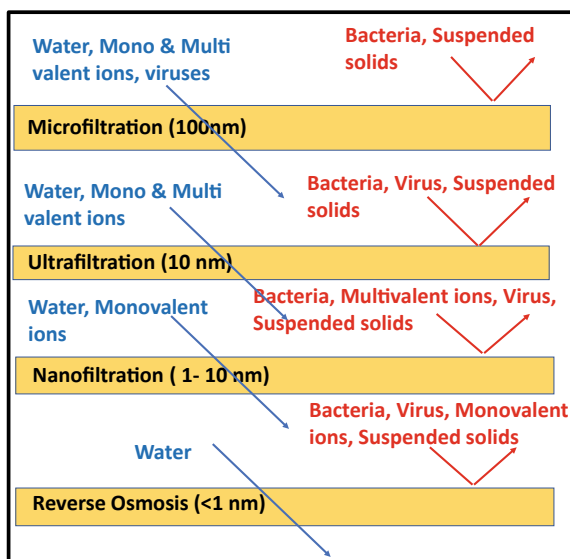
Nanotechnology-enabled water purifiers may hold the key for safe drinking water in the future (Shannon et al. 2010; Hossain et al. 2014). The technology uses engineered nanoscale material to improve microbial quality of the water. In comparison to conventional chemical disinfectants, nanoscale materials are not strong oxidants and hence unlikely to produce harmful DBPs. Several natural and engineered nanomaterials are available for disinfection. These include photo catalytic TiO₂ (Dimitroula et al. 2012), silver nanoparticles (Sankar et al. 2013), MgO (Stoimenov et al. 2002; Ganguly et al. 2011), zero-valent iron (Crane and Scott 2012) and so on. Among these disinfectants, the silver-based system is more matured and used in the field level application. The silver nanoparticles are active disinfectant and work for a broad spectrum of bacteria and viruses (Karumuri et al. 2013; De Gusseme et al. 2010; Loo et al. 2013). Though the research and development in this area is not fully matured, the current advancement in nanotechnology may prove to be of significant interest to both developed and developing countries in addressing the problem of safe drinking water.

11.3.5 Membrane Based Pathogens Control Technologies

The use of membrane filtration system has increased significantly over the last two decades. It has become one of the popular methods of purification of water now. The process of membrane includes micro-filtration, ultra-filtration, nano-filtration, and Reverse Osmosis (RO). The classification is based on the pore sizes of the membrane. Among the membranes, RO can efficiently remove bacteria, viruses, and other suspended solids present in the water. It can control the disinfection by-products as well (Van der Bruggen et al. 2003). However, the large amount of rejects, clogging of the membrane, and high energy consumption needs further attention.

Recent years, the use of biomimetic membranes is gaining interest in water treatment. In this technology, aquaporin, a bio-inspired membrane is used. Aquaporin

Fig. 11.12 Schematic representation of membrane filtration (courtesy of: <https://www.logisticson.com/en/technologies/membrane-filtration>)



acts as a water channel and allows only the passage of water through it. This method may reduce the cost of filtration by 30% of the conventional membranes (Wah 2016). The schematic representation of the membrane filtration technique is given in the Fig. 11.12.

11.3.6 Disinfection by Advanced Oxidation Process

The advanced oxidation process (AOP) is a promising technology in the field of water purification. The process typically uses ozone (O₃), UV light, hydrogen peroxide (H₂O₂), or combination thereof. The OH radicals formed during the processes are mainly responsible for the destruction of pathogens. The use of UV/TiO₂ (Matsunaga et al. 1985), UV/H₂O₂ (Gassie et al. 2016), ozone-UV (Crittenden et al. 2012), and photo-Fenton (Rossi et al. 2009) are also studied as next-generation disinfectants. In photo-catalysis, a light source of a specific wavelength is used to excite the electron from valence band to conduction band. The ability of the catalyst to produce the electron-hole pairs decides the efficiency of the process. The free radicals formed during the reaction destroy the pathogens present in the water (Giannakis et al. 2018).



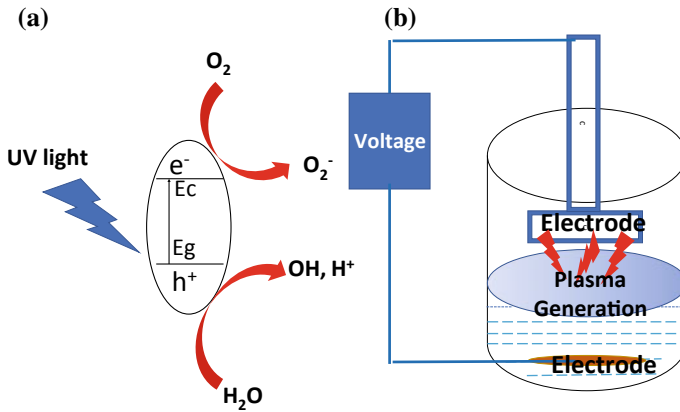
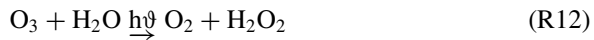


Fig. 11.13 Schematic representation of **a** photo-catalysis, **b** plasma treatment



Equations R8–R10 explain the radical formation in the UV/TiO₂ process, whereas Eqs. R11 and R12 show the radical formation in UV/H₂O₂ and ozone-UV process, respectively. Though AOPs are highly effective against the pathogens, the presence of natural organic matter such as fulvic acid, humic acid, and other ions affects its performance (Keane et al. 2014).

Plasma-based treatment technology is an emerging field for the removal of pathogens present in the drinking water (Rossi et al. 2009; Roth et al. 2010). In plasma technology, the Pulsed power technique (PPP) is found to be an effective method to disinfect the pathogen in short span of time (<6 min) (Singh et al. 2017). The schematic representation of UV photo-catalysis, and plasma techniques are given in Fig. 11.13a, b respectively.

11.4 Conclusion

Potable water is an absolute necessity for humankind as long as life exists. The pursuit of safe drinking water has been the highest priority for humans over centuries. The massive rise in the global population, poorly-managed water systems, and pollution have made the search more challenging. A large number of freshwater bodies across the world are contaminated and has become a major risk to human health and the

ecosystem. Realizations on the link that exist between water and health, proving safely managed drinking water has become a criteria agenda in every framework of the developmental organization.

Among the contaminants identified in drinking water, the pathogen causes significant threat due to their widespread occurrence and potential to cause diseases. Though several technologies have been developed to detect and control the pathogens, the lack of reliable and affordable detection and control technologies hindered access to such interventions for a large population, especially from developing world. The emergence of disinfectant and antibiotic-resistant microorganisms also have become a cause of concern for the process of control and detection. There is a need for safe, affordable, and reliable point-of-use disinfection and detections system. There is also scope for developing hybrid systems, which facilitates both detection and removal of pathogens in drinking water. As to move forward, the technologies with above-mentioned features should be made available to the public at affordable cost, thereby promoting sustainable, healthy, and productive living condition.

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