Chapter 12 Pathogens and Their Removal

INTRODUCTION

Microbial contamination is the most critical risk factor in drinking water quality with the potential for widespread waterborne disease. Illness derived from chemical contamination of drinking water supplies is negligible when compared to the number due to microbial pathogens (Galbraith *et al.*, 1987; Herwaldt *et al.*, 1992).

CLASSIFICATION OF PATHOGENS

The term pathogenic is applied to those organisms that either produce or are involved in the production of a disease. Three different groups of pathogenic microorganisms can be transmitted via drinking water to humans; these are bacteria, viruses and protozoans. All are transmitted by the faecal–oral route, so largely arise either directly or indirectly by contamination of water resources and supplies by sewage or animal faeces. It is theoretically possible, although unlikely, that other pathogenic organisms, such as nematodes (roundworm or hookworm) and cestodes (tapeworm), may also be transmitted via drinking water.

Diseases closely associated with water are classified according to their mode of transmission and the form of infection into four different categories: waterborne, water-washed, water-based and water-related diseases.

1. *Waterborne diseases*. These diseases occur where a pathogen is transmitted by ingestion of contaminated water. The classical waterborne diseases are mainly low-infective dose infections, such as cholera and typhoid, with all the other diseases high-infective dose infections that include infectious hepatitis and bacillary dysentery. All waterborne diseases can also be transmitted by other routes, which permits faecal material to be ingested.
2. *Water-washed diseases*. These include faecal–orally spread disease or disease spread from one person to another facilitated by a lack of an adequate supply of

water for washing. The incidence of all these diseases will fall if adequate supplies of washing water, regardless of microbial quality, are provided. These are diseases of mainly tropical areas, and include infections of the intestinal tract, the skin and eyes. Most of the intestinal infections are diarrhoeal diseases responsible for the high mortality rates among infants in hot climates. The infections of the skin and mucous membranes are non-faecal in origin, and include bacterial skin sepsis, scabies and cutaneous fungal infections (such as ringworm). Diseases spread by fleas, ticks and lice are also included in this category, such as epidemic typhus, rickettsial typhus and louse-borne fever.

3. *Water-based infections*. These diseases are caused by pathogenic organisms which spend part of their life cycle in aquatic organisms. All these diseases are caused by parasitic worms, with the severity of the infection depending on the number of worms infesting the host. The two commonest water-based diseases are schistosomiasis due to the trematode *Schistosoma* spp. and guineaworm, which is the nematode *Dracunculus medimensis*. *Schistosoma* worms use aquatic snails as intermediate hosts and are estimated as infecting as many as 200 million people, while the guineaworm uses the small crustacean *Cyclops* spp. as its intermediate host.
4. *Water-related diseases*. These are caused by pathogens carried by insects that act as mechanical vectors and which live near water, all these diseases are very severe and the control of the insect vectors is extremely difficult. The most important water-related diseases include two viral diseases: yellow fever transmitted by the mosquito *Aedes* spp. and dengue carried by the mosquito *Aedes aegypti* which breed in water. Gambian sleeping sickness, trypanosomiasis, is caused by a protozoan transmitted by the riverine tsetse fly (*Glossina* sp.) which bites near water, while malaria is caused by another protozoan (*Plasmodium* sp.) which is transmitted by the mosquito *Anopheles* sp. that breeds in water (Bradley, 1993). Climate change will have a significant effect on the distribution of these diseases, especially those in Category 4 (Section 23.2).

STRATEGIES FOR CONTROLLING PATHOGEN TRANSFER

A barrier approach is the key strategy for controlling the health risks posed by microbes in drinking waters. This involves the treatment of wastewaters to remove pathogens as well as the treatment of raw waters, which includes disinfection (Fig. 12.1). Monitoring water supplies for the presence of specific pathogens is difficult and largely impractical, so a more indirect approach is adopted where water is examined for indicator bacteria whose presence in water implies some degree of contamination. The use of indicator organisms, in particular the coliform group, as a means of controlling the possible presence of pathogens has been paramount in the approach to assessing water quality adopted by the World Health Organization (WHO), US

Environmental Protection Agency (USEPA) and the European Union (EU) (Section 8.1). This approach is based on the assumption that there is a quantifiable relationship between indicator density and the potential health risks involved. A water quality guideline is established (Fig. 12.2) which is a suggested upper limit for the density of an indicator organism above which there is an unacceptable risk (Cabelli, 1978). An

Source	Faecal coliforms (FC)	
Human faecal coliform discharges	1 950 000 000 FC/person/day	
	Faecal coliforms/100 ml	
Municipal raw sewage	8 260 000 FC cells/100 ml	
Sewage treatment reductions		
	Cumulative reduction (%)	FC surviving
Primary	50	4 130 000
Secondary	80	1 652 000
Tertiary	98	165 200
Disinfection	99.99	800
Self-purification and effluent dilution 10–15%		
Water supply treatment		
	Cumulative reduction (%)	FC surviving
Raw water storage	50	200–350
Coagulation–sedimentation	60	80–140
Filtration	99.9	0.8–1.4
Disinfection	99.9999	0.000 08–0.000 14

Figure 12.1 The use of barriers is vital in the control of pathogens in water supplies. (Reproduced from Geldreich (1991) with permission of John Wiley and Sons Inc., New York.)

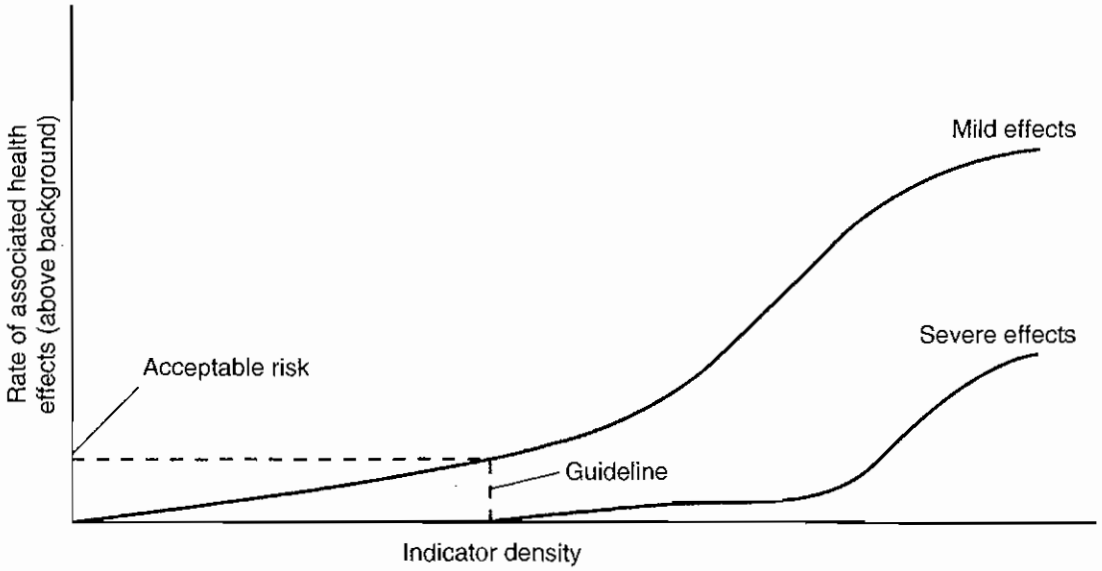

Figure 12.2 The desired water quality criteria and the development of guidelines from them. (Reproduced from Cabelli (1978) with permission of Wiley-Interscience, New York.)

example is given for determining the risk of infection from bathing waters in Section 8.2 (Fig. 8.1). Ideally water destined for human consumption should be free from micro-organisms; however, in practice this is an unattainable goal.

The extent to which strategies, such as the barrier approach and the establishment of allowable limits for bacteria in water, have been successful in maintaining water quality is seen by the dramatic decline in epidemic and endemic waterborne bacterial diseases, such as typhoid fever and cholera, in the more developed regions of the world (Sobsey *et al.*, 1993).

12.1 Waterborne Pathogens

The major waterborne disease-causing pathogens are summarized in Table 12.1 and are considered below.

12.1.1 PRIMARY BACTERIAL PATHOGENS

Salmonella

The various serotypes that make up the genus *Salmonella* are the most important group of bacteria affecting the public health of both humans and animals in Western Europe; wild, domestic and farm animals often acting as reservoirs of human salmonellosis. Water resources can become contaminated by raw or treated wastewater, as

Table 12.1 Bacterial, viral and protozoan diseases generally transmitted by contaminated drinking water.

Agent	*Disease*	*Incubation time*
Bacteria		
Shigella spp.	Shigellosis	1–7 days
Salmonella spp.		
S. typhimurium	Salmonellosis	6–72 h
S. typhi	Typhoid fever	1–3 days
Enterotoxigenic *Escherichia coli*	Diarrhoea	12–72 h
Campylobacter spp.	Gastroenteritis	1–7 days
Vibrio cholerae	Gastroenteritis	1–3 days
Viruses		
Hepatitis A	Hepatitis	15–45 days
Norwalk-like agent	Gastroenteritis	1–7 days
Virus-like particles <27 nm	Gastroenteritis	1–7 days
Rotavirus	Gastroenteritis	1–2 days
Protozoa		
Giardia lamblia	Giardiasis	7–10 days
Entamoeba histolytica	Ameobiasis	2–4 weeks
Cryptosporidium	Cryptosporidiosis	5–10 days

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well as by effluents from abattoirs and animal-processing plants (Gray, 2004). It is commonly present in raw waters but only occasionally isolated from finished waters, chlorination being highly effective at controlling the bacteria. Typical symptoms of salmonellosis are acute gastroenteritis with diarrhoea, and can also be associated with abdominal cramps, fever, nausea, vomiting, headache and, in severe cases, even collapse and possible death. The most serious diseases associated with specific species are typhoid fever (*Salmonella typhi, S. typhi*) and paratyphoid (*S. paratyphi* and *S. schottmuelleri*). Salmonellosis carries a significant mortality amongst those with acquired immuno deficiency syndrome (AIDS) and poses significant problems in its management.

Shigella

Shigella causes bacterial dysentery or shigellosis and is one of the most frequently diagnosed cause of diarrhoea in the US. Shigellosis is a problem of both developed and developing countries, with the eastern Mediterranean countries considered as an endemic region for the disease. The species of this bacterial genus are rather similar in their epidemiology to that of *Salmonella* except they rarely infect animals and do not survive quite so well in the environment. When the disease is present as an epidemic it appears to be spread mainly by person-to-person contact, especially between children, shigellosis being a typical institutional disease occurring in over-crowded conditions. There has been a significant increase in the number of outbreaks arising from poor quality drinking water contaminated by sewage. Of the large number of species (>40), only *S. dysenteriae, Shigella sonnei, S. flexneri* and *S. boydii* are able to cause gastrointestinal disease. *S. sonnei* and *S. flexneri* account for >90% of isolates, although it is *S. dysenteriae* type 1 which causes the most severe symptoms due to the production of the shiga toxin.

Cholera

While the disease in now extremely rare in the developed world, major waterborne outbreaks occur in developing countries, war zones and disaster areas. It is still endemic in many areas of the world especially those which do not have adequate sanitation and in particular situations where the water supplies are continuously contaminated by sewage. However, over the past 15–20 years the incidence and spread of the disease has been causing concern which has been linked to the increasing mobility of travellers and the speed of travel. An infected person or symptomless carrier of the disease excretes up to 10^{13} bacteria daily, enough to theoretically infect 10^7 people! Up to 10^6–10^7 organisms are required to cause the illness; hence cholera is not normally spread by person-to-person contact. It is transmitted primarily by drinking contaminated water, but also by eating food handled by a carrier, or which has been washed with contaminated water, and is regularly isolated from surface waters in the UK and the US. It is an intestinal disease with characteristic symptoms: that is,

sudden diarrhoea with copious watery faeces, vomiting, suppression of urine, rapid dehydration, lowered temperature and blood pressure, and complete collapse. Without immediate medical therapy the disease has a >60% mortality rate, the patient dying within a few hours of first showing the symptoms, although with suitable treatment the mortality rate can be reduced to <1%. *Vibrio cholerae* are natural inhabitants of brackish and saline waters, and are rapidly inactivated under unfavourable conditions, such as high-acidity or high-organic-matter content of the water, although in cool unpolluted waters the bacterium will survive for up to 2 weeks. Survival is even greater in estuarine and coastal waters.

12.1.2 OPPORTUNISTIC BACTERIAL PATHOGENS

Opportunistic bacteria are usually found as part of the normal heterotrophic bacterial flora of aquatic systems and may also exist as part of the normal body microflora (Table 12.2) (Reasoner, 1992). These organisms are normally not a threat to healthy individuals but under certain circumstances they can lead to infection in certain segments of the community, in particular newborn babies, the elderly and the immuno-compromised. It is thought that numerous hospital-acquired infections are attributable to such organisms (Du Zuane, 1990). Some of the organisms listed in Table 12.2 are also considered as primary pathogens, meaning that they are also

TABLE 12.2 Typical opportunistic bacterial pathogens that are isolated from drinking water

Acinetobacter spp.
Achromobacter xylosoxidans
Aeromonas hydrophila
Bacillus spp.
Campylobacter spp.[a]
Citrobacter spp.
Enterobacter aerogenes
Enterobacter agglomerans
Enterobacter cloacae
Flavobacterium meningusepticum
Hafnia alvei
Klebsiella pneumoniae[a]
Legionella pneumopila[a]
Moraxella spp.
Mycobacterium spp.
Pseudomonas spp. (non-aeruginosa)[a]
Serratia fonticola
S. liquefaciens
S. marcescens
Staphylococcus spp.[a]
Vibrio fluvialis[a]

[a]Indicates that the organism may be a primary pathogen.
Reproduced from Reasoner (1992) with permission of US Environmental Protection Agency, Washington, DC.

capable of being primary disease-causing agents rather than secondary invaders. Of these organisms, those of particular concern at present include *Campylobacter* spp., enterotoxigenic *Escherichia coli* (*E. coli*), *Mycobacteria* spp., *Legionella pneumophila* and aeromonads.

Campylobacter

Campylobacter is a major cause of gastroenteritis, being more common than *Salmonella*. In the US, the annual incidence of this organism is between 30 and 60 per 100 000 of the population (Skirrow and Blaser, 1992). In developing countries, outbreaks of *Campylobacter* enteritis are a major cause of morbidity and mortality in the first 2 years of life. While *Campylobacter* enteritis is essentially a foodborne disease, with the most important reservoirs of the bacterium being meat, in particular poultry, and unpasturized milk, waterborne transmission has been implicated in several large outbreaks. Waterborne transmission of *Campylobacter* occurs in untreated, contaminated waters; in situations where faulty disinfection has occurred or where waters have been contaminated by sewage or animal wastes (Tauxe, 1992). Household pets, farm animals and birds are all known to be the carriers of the disease. There is a definite seasonal variation in numbers of *Campylobacters* in river water, with greatest numbers occurring in the autumn and winter. *Campylobacter jejuni* (*C. jejuni*) serotypes are common in human infections and especially common downstream of sewage effluent sites, confirming sewage effluents as important sources of *C. jejuni* in the aquatic environment. Gulls are known carriers and can contaminate water supply reservoirs while they roost. Dog faeces in particular are rich in the bacterium, making contamination of surface waters used for abstraction by surface (urban) run-off an important source of contamination. *Campylobacter* can remain viable for extended periods in streams and groundwaters. Survival of the bacterium decreases with increasing temperature but with survival at 4°C in excess of 12 months.

Escherichia coli 0157:H7

E. coli 0157:H7 causes haemorrhagic colitis, haemolytic–uraemic syndrome and is a major cause of kidney disease in children. Like *C. jejuni*, this organism is generally associated with food, in particular beef and milk, but in recent years has been implicated in a number of waterborne outbreaks. The number of organisms required to initiate infection is thought to be <100.

Mycobacteria

Mycobacteria spp. are now recognized as being opportunistic pathogens of considerable significance that cause a wide range of diseases in humans, including pulmonary disease, cervical lymphadenopathy as well as localized and soft tissue infections. It is well

established that *Mycobacteria* are commonplace in all types of aquatic environments, including estuaries, ocean water, groundwater, surface water and distribution systems (Jenkins, 1991). The majority of waterborne mycobacterial outbreaks are attributable to treatment deficiencies, such as inadequate or interrupted chlorination; but other factors may also influence the growth of this organism in water supplies, such as pitting and encrustations found inside old water pipes which protect bacteria from exposure to free chlorine. *Mycobacteria* can also colonize areas where water is moving slowly, as in water distribution systems in large buildings, such as blocks of flats, offices and hospitals, thus continuously seeding the system (Du Moulin and Stottmeier, 1986). Disease associated with these bacteria is steadily rising, particularly among patients with AIDS.

Aeromonads

Aeromonas spp. have been implicated as the causative agent in a number of waterborne outbreaks and are considered to be an important, and often fatal, cause of non-gastrointestinal illness in immuno-compromised individuals. Aeromonads have been isolated from both chlorinated and unchlorinated drinking water supplies occurring in greatest numbers during the summer months. They have also been isolated in waters containing no *E. coli* and few total coliforms (Schbert, 1991).

Legionella pneumophila

The bacterium *Legionella pneumophila* has been associated with domestic water systems, especially hot water stored at between 20°C and 50°C. Heat exchangers, condensers in air conditioning units, cooling towers and shower heads have all been found to be havens for the bacteria leading to human infection. Infection is by inhalation of aerosols from contaminated appliances. The bacteria survive and grow within phagocytic cells, multiplying in the lungs causing bronchopneumonia and tissue damage. The bacterium is widespread in natural waters so any water supply can become contaminated. *Legionella* are commonly found in hospital water systems, and hospital-acquired (nosocomial) Legionnaires' disease is now a major health problem (Joseph *et al.*, 1994).

It appears that a long retention time, and the presence of key nutrients, such as iron, provides ideal conditions for the bacterium to develop. Therefore iron storage cisterns subject to corrosion are particularly susceptible. In addition, water pipes that are installed alongside hot water pipes or other sources of heat may also permit the bacteria to develop. Prevention of *Legionella* infection is normally achieved by either hyperchlorination or thermal eradication of infected pipework, although these methods are not always successful or with a permanent effect. The only effective preventative method has been shown to be the use of ultraviolet (UV) sterilizers as close to the point of use as possible.

TABLE 12.3 Some human enteric viruses and the diseases they cause

Virus group	*Serotypes*	*Some diseases caused*
Enteroviruses		
Polioviruses	3	Paralysis, aseptic meningitis
Coxsackievirus		
A	23	Herpangia, aseptic meningitis, respiratory illness, paralysis, fever
B	6	Pleurodynia, aseptic meningitis, pericarditis, congenital heart disease anomalies, nephritis, fever
Echovirus	34	Respiratory infection, aseptic meningitis, diarrhoea, pericarditis, myocarditis, fever and rash
Enteroviruses (68–71)	4	Meningitis, respiratory illness
Hepatitis A virus (HAV)		Infectious hepatitis
Reoviruses	3	Respiratory disease
Rotaviruses	4	Gastroenteritis
Adenoviruses	41	Respiratory disease, acute conjunctivitis, gastroenteritis
Norwalk agent (calcivirus)	1	Gastroenteritis
Astroviruses	5	Gastroenteritis

Reproduced from Bitton (1994) with permission of Wiley-Liss Inc., New York.

12.1.3 VIRUSES

There are over 120 distinct known types of human pathogenic viruses. Of most concern for drinking water are those which cause gastrointestinal illness (enteric viruses) which includes enteroviruses, rotaviruses, astroviruses, calciviruses, Hepatitis A virus, Norwalk virus and other 'small round' viruses (West, 1991). However, the health risks presented by these viruses are not just restricted to gastroenteritis (Table 12.3).

Viral contamination arises when sewage containing pathogenic viruses contaminates surface and groundwaters that are subsequently used as sources of drinking waters. Most viruses are able to remain viable for several weeks in water at low temperatures, so long as there is some organic matter present. Of most concern in Britain and the US is viral hepatitis. There are three subgroups, Hepatitis A which is transmitted by water, Hepatitis B which is spread by personal contact or inoculation and which is endemic in certain countries such as Greece, and Hepatitis C which is a non-A- or B-type hepatitis virus. Hepatitis A is spread by faecal contamination of food, drinking water and areas, which are used for bathing and swimming. Epidemics have been linked to all these sources, and it appears that swimming pools and coastal areas used for bathing which receive large quantities of sewage are particular sources of infection. There is no treatment for Hepatitis A, with the only effective protection being good personal hygiene, and the proper protection and treatment of drinking water

(Section 13.6). Symptoms develop 15–45 days after exposure, and include nausea, vomiting, muscle ache and jaundice. Hepatitis A virus accounts for 87% of all viral waterborne disease outbreaks in the US (Craun, 1986).

Two viruses which have caused recent outbreaks of illness due to drinking water contamination are Norwalk virus and rotavirus (Cubitt, 1991). Norwalk virus results in severe diarrhoea and vomiting. It is of particular worry to the water industry in that it appears not to be affected by normal chlorination levels. Also it seems that infection by the virus only gives rise to short-term immunity while life-long immunity is conferred by most other enteric viruses. Rotavirus is a major contributor to child diarrhoea syndrome. This causes the death of some 6 million children in developing countries each year. This is not a serious problem in developed countries due to better hygiene, nutrition and health care. Outbreaks do occur occasionally in hospitals, and although associated with child diarrhoea, it can be much more serious if contracted by an adult.

Viruses are usually excreted in numbers several orders of magnitude lower than that of coliforms (APHA, 1992). Because they only multiply within living susceptible cells, their numbers cannot increase once excreted. Once in a cell-free state, their survival and infectivity in the aquatic environment depends on a variety of biotic (i.e. type of virus, bacterial and algal activity, and predation by protozoa) and abiotic factors (i.e. temperature, suspended matter, pH, salinity, UV light penetration, organic compounds, adsorption to suspended matter and aggregation). Temperature is considered to be the most important factor influencing viral destruction outside the host cell, being rapidly inactivated once exposed to temperatures in excess of 50°C. Suspended solids provide a certain degree of protection for viruses. Adsorption onto organic matter can prevent inactivation by UV light. Once adsorbed the viruses can settle from suspension and survive for long periods in sediments to become resuspended if the water becomes turbulent.

In practice viruses generally pass unaffected through wastewater treatment plants and so will be found in surface waters receiving both treated and untreated sewage (Gray, 2004). Therefore effective water treatment disinfection is critical to prevent viruses entering potable water supplies. Enteric viruses have been isolated from drinking waters that have been treated by chlorination or other processes, such as ozonation and chemical coagulation. Such drinking waters contain chlorine levels originally thought to be virucidal (0.1–0.3 $mg\,l^{-1}$). Initially the ability to survive chlorination was thought to be due to a lack of contact time with chlorine. It is now well established that some enteric viruses are more resistant to chlorination than coliforms. Exposure to over 2 $mg\,l^{-1}$ of chlorine for 30 min is required to inactivate the infectivity of the Hepatitis A virus, while exposure to as much as 5–6 $mg\,l^{-1}$ chlorine for 30 min may be required to destroy the infectivity of the Norwalk virus. It has been suggested that such resistance may be due to the protective effect of viral aggregation (West, 1991).

12.1.4 PROTOZOA

Protozoan pathogens of humans are almost exclusively confined to tropical and sub-tropical areas, which is why the increased occurrence of *Cryptosporidium* and *Giardia* cysts in temperate areas is causing so much concern. However, with the increase in travel, carriers of all diseases are now found worldwide, and cysts of all the major protozoan pathogens occur in European sewages from time to time. Two other protozoan parasites which occur occasionally in the UK are *Entamoeba histolytica* (*E. histolytica*), which causes amoebic dysentery, and *Naegteria fowleri*, which causes the fatal disease amoebic meningoencephalitis.

Giardia lamblia

Giardia lamblia is a flagellated protozoan that is a significant cause of gastroenteritis ranging from mild to severe and disabilitating disease with a worldwide distribution being significantly more common in children than in adults. Cysts are passed from the body in the stools and are ovoid, 14–16 μm long, 6–12 μm wide and are quadrinucleate. *Giardia* cysts are relatively resistant to environmental conditions and are capable of survival once excreted for long periods, especially in winter.

Transmission of *Giardia* cysts may be by faecal contamination of either hands, food or water supplies. The disease is a zoonosis and cysts from a human source can be infective to about 40 different species of animal including pets and vice versa. Cysts are therefore widely distributed in the environment entering waterways through sewage or storm water discharges or via the droppings of infected animals. Giardiasis is now established to be one of the most common causes of waterborne diseases in the developed world. Most *Giardia* outbreaks occur in waters where chlorination is the only form of water treatment. This resistance to disinfection levels typically used in water treatment indicates the need for additional treatment barriers. In recognition of this, recent amendments to the US Safe Drinking Water Act now requires that all surface waters intended for human consumption must undergo filtration to specifically remove cysts and sufficient disinfection to destroy *Giardia* and prevent disease transmission. To date no such provisions exist in European legislation. There is no way of preventing infection except by adequate water treatment and resource protection. Current disinfection practices are generally inadequate as the sole barrier to prevent outbreaks. Boiling water for 20 min will kill cysts, while the use of 1 μm pore cartridge filters to treat drinking water at the point of use are also effective.

Cysts are generally present in very low densities and so samples must be concentrated first. This is achieved by using ultra-filtration cassettes or finely wound polypropylene cartridges (APHA, 1992). As cyst numbers cannot be amplified by *in vitro* cultivation, they are generally detected by immuno-fluorescence with poly- or monoclonal antibodies or by direct phase contrast microscopy (Table 12.4).

TABLE 12.4 The standard methods associated with the sampling, isolation and enumeration of pathogens in drinking water, recreational waters and sewage sludge in the UK. These are available online from the Environment Agency

Drinking water

The Microbiology of Drinking Water 2002 – Part 1: Water quality and public health. MDW2002(1). Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Drinking Water 2002 – Part 2: Practices and procedures for sampling. MDW2002(2). Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Drinking Water 2002 – Part 3: Practices and procedures for laboratories. MDW2002(3). Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Drinking Water 2002 – Part 4: Practices and procedures for laboratories. MDW2002(3). Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Drinking Water 2002 – Part 5: Isolation and enumeration of enterococci by membrane filtration. MDW2002(5). Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Drinking Water 2004 – Part 6: Methods for the isolation and enumeration of sulphite-reducing clostridia and Clostridium perfringens *by membrane filtration.* Blue Book No. 192. Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Drinking Water 2002 – Part 7: The enumeration of heterotrophic bacteria by pour and spread plate techniques. MDW2002(7). Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Drinking Water 2002 – Part: Methods for the isolation and enumeration of Aeromonas and Pseudomonas aeruginosa by membrane filtration. MDW2002(8). Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Drinking Water 2004 – Part 9: Methods for the isolation and enumeration of Salmonella and Shigella by selective enrichment, membrane filtration and multiple tube most probable number techniques. Blue Book No. 194. Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Drinking Water 2002 – Part 10: Methods for the isolation of Yersinia, Vibrio and Campylobacter by selective enrichment. MDW2002(10). Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Drinking Water 2004 – Part 12: Methods for the isolation and enumeration of micro-organisms associated with taste, odour and related aesthetic problems. Blue Book No. 197. Standing Committee of Analysts, Environment Agency, London.

Recreational water

The Microbiology of recreational and environmental waters 2000. Blue Book No. 175. Standing Committee of Analysts, Environment Agency, London.

Sewage sludge

The Microbiology of Sewage Sludge 2003 – Part 1: An overview of the treatment and use in agriculture of sewage sludge in relation to its impact on the environment and public health. Blue Book No. 188. Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Sewage Sludge 2003 – Part 2: Practices and procedures for sampling and sample preparation. Blue Book No. 189. Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Sewage Sludge 2003 – Part 3: Methods for the isolation and enumeration of Escherichia coli, *including verocytotoxigenic* Escherichia coli. Blue Book No. 190. Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Sewage Sludge 2003 – Part 3: Methods for the isolation and enumeration of Salmonellae. Blue Book No. 191. Standing Committee of Analysts, Environment Agency, London.

The Microbiology of Sewage Sludge 2004 – Part 4: Methods for the detection, isolation and enumeration of Salmonellae. Blue Book No. 195. Standing Committee of Analysts, Environment Agency, London.

Cryptosporidium parvum

Cryptosporidium parvum is a coccidian protozoan parasite and while the first case of human infection was not recorded until 1976, cryptosporidiosis is acquired by ingesting viable oocysts that are ovoid, between 4 and 6 μm in diameter, and generally occur in low numbers in water. Clinical symptoms of cryptosporidiosis include an influenza-like illness, diarrhoea, malaise, abdominal pain, anorexia, nausea, flatulence, malabsorption, vomiting, mild fever and weight loss. Generally this disease is not fatal among healthy individuals. However, in young malnourished children, it can cause severe dehydration and sometimes death (Smith, 1992). In the immunocompromised, including those with AIDS or those receiving immuno-suppressive drugs, and also those with severe malnutrition, cryptosporidiosis can become a life-threatening condition causing profuse intractable diarrhoea with severe dehydration, malabsorption and wasting. Sometimes the disease spreads to other organs. These symptoms can persist unabated until the patient eventually dies. In the UK, cryptosporidiosis is currently the fourth most commonly identified cause of diarrhoea in which a parasitic, bacterial or viral cause was established (Department of the Environment and Department of Health, 1990) and is particularly difficult to treat. Little is known of the exact infectious dose size, but a single oocyst may be enough to cause infection, although outbreaks of cryptosporidiosis are usually associated with gross contamination. The organism is not host specific and is capable of infecting many species of mammal, bird and reptile. Oocysts from humans are infective for numerous mammals.

Studies of water resources in the UK and the US have found that oocysts commonly occurred in all types of surface water (lakes, reservoirs, streams and rivers) including pristine waters with densities ranging from 0.006 to 2.5 oocysts l^{-1} (Department of the Environment and Department of Health, 1990; Le Chevallier *et al.*, 1991). Significantly higher numbers of oocysts are found in water resources receiving untreated or treated wastewaters, while oocysts tend to occur much less frequently in groundwaters. Oocysts can survive for up to 18 months depending on the temperature. Most of the oocysts found in both surface and groundwaters are derived from agricultural sources. Cattle and infected humans can excrete up to 10^{10} oocysts during the course of infection, so that cattle slurry, wastewater from marts and sewage should all be considered potential sources of the pathogen. The most important outbreak of cryptosporidia in the US in recent years occurred in April 1993 in Milawakee. The water distribution system serving 800 000 people was contaminated by raw water from a river swollen by spring run-off. In all 370 000 people became ill, 4400 were admitted to hospital and approximately 40 died (Jones, 1994).

It has been established that chlorination at levels used in water treatment is ineffective against oocysts while ozone can achieve 90% inactivation at 1 ppm of ozone for 5 min. The critical factor in water treatment is the recycling of the backwash water from rapid sand filtration. This water can contain up to 10 000 oocysts l^{-1} with a very

high chance of resultant break through if recyeled. In practice well-operated treatment processes with proper filtration and disposal of filter backwash water should be capable of achieving 99% reduction in oocyst concentration (Section 10.1.7). Their detection in water samples relies on filtration of large volumes of water (100–500 l) to remove oocysts and examination of the concentrate by microscopy. Most methods available for oocyst detection are adaptations of those used for *Giardia* detection (Table 12.4).

12.1.5 CONCLUSION

Human enteric viruses and protozoan parasites possess certain traits, which aid waterborne transmission and which have contributed to their increase in recent years (West, 1991). These include:

(a) an ability to be excreted in faeces in large numbers during illness;
(b) failure of conventional sewage treatment to remove them;
(c) they can survive as an environmentally robust form or they demonstrate resilience to inactivation whilst in an aquatic environment;
(d) they are largely resistant to common disinfectants used in drinking water treatment;
(e) most importantly, they only require low numbers to elicit infection in hosts consuming or exposed to water.

These factors are compounded by the difficulty of isolating and accurately detecting these pathogens in both treated and untreated effluents, as well as in both surface and groundwater resources. For both viruses and protozoa, large sample volumes must be examined in order to detect small numbers of organisms. Propagation and identification may take several days. In addition, by the time the outbreak is eventually recognized, it is usually long after the initial contamination event and that water is no longer available for examination. This is particularly the case with protozoan pathogens when a minor operational error during backwashing sand filters may result in the breakthrough of cysts and oocysts into the treated water.

12.2 Microbial Assessment of Drinking Water Quality

In order to prevent pathogen transfer to consumers via drinking water, microbial analysis is required. Chemical analysis can only be used for the assessment of water treatment efficiency and to monitor compliance to legal standards. Biological examination of water is used to detect the presence of algae and animals that may affect treatment or water quality, and to identify possible defects in the distribution network.

12.2.1 MONITORING PATHOGENS

Routine monitoring of pathogenic micro-organisms in drinking water is extremely difficult because:

(a) pathogens are outnumbered by the normal commensal bacterial flora in both human and animal intestines;
(b) large volumes of water ($\leqslant$2 l) must be filtered to ensure pathogens are recovered;
(c) isolation of pathogens require specific and complicated tests often using special equipment;
(d) positive identification may require further biochemical, serological or other tests.

It is therefore impracticable to examine all water samples on a routine basis for the presence or absence (P-A) of all pathogens. In order to routinely examine water supplies a rapid and preferably a single test is required. The theory being it is more effective to examine a water supply frequently using a simple general test, as most cases of contamination of water supplies occur infrequently, than only occasionally by a series of more complicated tests. This has led to the development of the use of indicator organisms to determine the likelihood of contamination by faeces.

The use of indicator organisms is a widely accepted practice, with legal standards based on such organisms. The main criteria for selection of an indicator organism are:

(a) they should be a member of the normal intestinal flora of healthy people;
(b) they should be exclusively intestinal in habit and therefore exclusively faecal in origin if found outside the intestine;
(c) ideally they should only be found in humans;
(d) they should be present when faecal pathogens are present and only when faecal pathogens are expected to be present;
(e) they should be present in greater numbers than the pathogen they are intended to indicate;
(f) they should be unable to grow outside the intestine with a die-off rate slightly less than the pathogenic organisms;
(g) they should be resistant to natural environmental conditions and to water and wastewater treatment processes in a manner equal to or greater than the pathogens of interest;
(h) they should be easy to isolate, identify and enumerate;
(i) they should be non-pathogenic.

While no organism can meet all these conditions consistently, in temperate regions *E. coli* fulfils most of these requirements with other coliform organisms, faecal streptococci and *Clostridium perfringens* also widely used. The EU Drinking Water Directive (Section 8.1) specifies numerical standards for *E. coli* and enterococci

(faecal streptococci) in drinking water; *E. coli*, enterococci, *Pseudomonas aeruginosa* and total viable counts of heterotrophic bacteria at 22°C and 37°C in bottled waters (Table 8.6). Routine (indicator) monitoring is restricted to coliforms, *Clostridium perfringens* and heterotrophic bacteria at 22°C only (Table 8.7).

These three groups are able to survive for different periods of time in the aquatic environment. Faecal streptococci die fairly quickly outside the host and their presence is an indication of recent pollution. *E. coli* (faecal coliforms) can survive for several weeks under ideal conditions and are far more easily detected than the other indicator bacteria. Because of this it is the most widely used test although the others are often used to confirm faecal contamination if *E. coli* is not detected. Sulphate-reducing clostridia (*Clostridium perfringens*) can exist indefinitely in water. When *E. coli* and faecal streptococci are absent, its presence indicates remote or intermittent pollution. It is especially useful for testing lakes and reservoirs, although the spores do eventually settle out of suspension. The spores are more resistant to industrial pollutants than the other indicators and it is especially useful in waters receiving both domestic and industrial wastewaters. It is assumed that these indicator organisms do not grow outside the host and, in general, this is true. However, in tropical regions *E. coli* in particular is known to multiply in warm waters and there is increasing evidence that *E. coli* is able to reproduce in enriched waters generally, thus indicating an elevated health risk. Therefore, great care must be taken in the interpretation of results from tropical areas, so the use of bacteriological standards designed for temperate climates are inappropriate for those areas. The most widely used identification and enumeration techniques for indicator micro-organisms in drinking waters are summarized below. However, these methods are constantly being refined and updated with the latest UK standard methods listed in Table 12.4.

12.2.2 HETEROTROPHIC PLATE COUNT BACTERIA

Heterotrophic plate counts (HPCs) represent the aerobic and facultatively anaerobic bacteria that derive their carbon and energy from organic compounds (Table 12.5). Certain HPC organisms are considered to be opportunistic pathogens (Table 12.2) and have been implemented in gastrointestinal illness (Section 12.1.2).

Heterotrophic bacteria are commonly isolated from raw waters and are widespread in soil and vegetation, and can survive for long periods in water and rapidly multiply, especially at summer temperatures. There is also concern that these organisms can rapidly multiple in bottled waters, especially if not stored properly once opened (Gray, 1994). The EU Drinking Water Directive requires that there is no significant increase from background levels of HPC bacteria in either tap or bottled waters. While HPC bacteria are not a direct indicator of faecal contamination, they do indicate variation in water quality and the potential for pathogen survival and regrowth.

TABLE 12.5 Example of the HPC bacteria isolated from distribution and raw waters

Organism	*Distribution water*		*Raw water*	
	Total	*Percentage of total*	*Total*	*Percentage of total*
Actinomycete	37	10.7	0	0
Arthrobacter spp.	8	2.3	2	1.3
Bacillus spp.	17	4.9	1	0.6
Corynebacterium spp.	31	8.9	3	1.9
Micrococcus luteus	12	3.5	5	3.2
Staphylococcus aureus	2	0.6	0	0
S. epidermidis	18	5.2	8	5.1
Acinetobacter spp.	19	5.5	17	10.8
Alcaligenes spp.	13	3.7	1	0.6
F. meningosepticum	7	2.0	0	0
Group IVe	4	1.2	0	0
Group MS	9	2.6	2	1.3
Group M4	8	2.3	2	1.3
Moraxella spp.	1	0.3	1	0.6
Pseudomonas alcaligenes	24	6.9	4	2.5
P. cepacia	4	1.2	0	0
P. fluorescens	2	0.6	0	0
P. mallei	5	1.4	0	0
P. maltophilia	4	1.2	9	5.7
Pseudomonas spp.	10	2.9	0	0
Aeromonas spp.	33	9.5	25	15.9
Citrobacter freundii	6	1.7	8	5.1
Enterobacter agglomerans	4	1.2	18	11.5
Escherichia coli	1	0.3	0	0
Yersinia enterocolitica	3	0.9	10	6.4
Group IIk biotype I	0	0	1	0.6
Hafnia alvei	0	0	9	5.7
Enterobacter aerogenes	0	0	1	0.6
Enterobacter cloacae	0	0	1	0.6
Klebsiella pneumoniae	0	0	0	0
Serratia liquefaciens	0	0	1	0.6
Unidentified	65	18.7	28	17.8
Total	347	100	157	99.7

Reproduced from Bitton (1994) with permission of Wiley-Liss Inc., New York.

The HPC organisms typically found in raw waters and within water supply distribution systems are listed in Table 12.5. HPCs are carried out normally using the spread plate method using yeast extract agar (YEA) and incubated at 22°C for 72 h and 37 °C for 24 h, respectively. Results are expressed as colony forming units (cfu) per ml. Counts at 37°C are especially useful as they can provide rapid information of possible contamination of water supplies (Department of the Environment, 1994).

HPCs have long been employed to evaluate water quality (Table 12.6) although less importance is currently placed on HPCs for assessing the potability of drinking water. It is considered that their value lies mainly in indicating the efficiency of various water

TABLE 12.6 Sanitary quality of water based on HPC

Quality	*Number of bacteria/ml*
Exeessively pure	<10
Very pure	10–100
Pure	100–1000
Mediocre	1000–10 000
Impure	10 000–100 000
Very impure	>100 000

treatment processes including disinfection, as well as the cleanliness and integrity of the distribution system. They are more useful in assessing the quality of bottled waters, which may be stored for long periods before being sold for consumption. The US National Primary Drinking Water Regulations now include maximum contaminant levels (MCLs) of no >500 cfu ml^{-1} for HPCs (USEPA, 1990) although this is primarily to reduce possible interference with the detection of coliforms.

12.2.3 ENUMERATION TECHNIQUES

The exact methods employed to enumerate indicator bacteria are specified by the legal standards used. Two techniques are principally used, membrane filtration and the multiple tube methods. The EU Drinking Water Directive specifies that *E. coli*, enterococci (faecal streptococci), *Pseudomonas aeruginosa* and *Clostridium perfringens* must all be isolated using the membrane filtration method (Table 12.4), although the multiple tube method is still widely used for clostridia because of the need to incubate under anaerobic conditions.

Coliforms do not only occur in faeces, they are normal inhabitants of water and soil. The presence of coliforms in a water sample does not necessarily indicate faecal contamination, although in practice it must be assumed that they are of faecal origin unless proved otherwise. The total coliform count measures all the coliforms present in the sample. However, only *E. coli* is exclusively faecal in origin with numbers in excess of $10^8 g^{-1}$ of fresh faeces. So it is important to confirm *E. coli* is present. Routine coliform testing comprises of two tests giving the total coliform count and faecal coliform (*E. coli*) count.

Membrane filtration

The membrane filtration method is now widely used for all coliform testing. Known volumes of water are passed through a sterile membrane filter with a pore size of just 0.45 μm (Fig. 12.3). This retains all the bacteria present. The membrane filter is placed onto a special growth medium, which allows the individual bacteria to grow into colonies. Special media, which only allow specific bacteria to grow, are used.

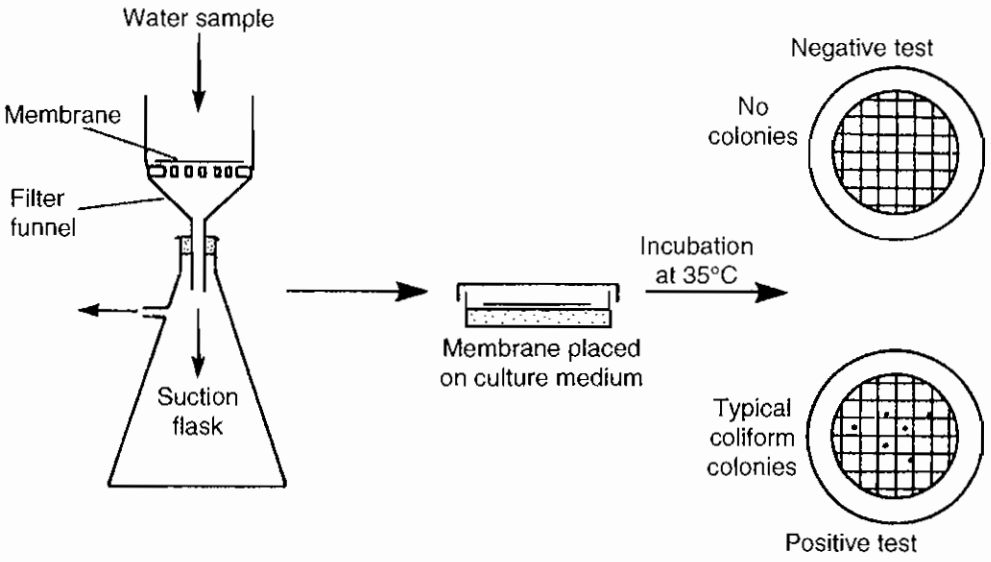

FIGURE 12.3 Major steps in coliform testing by the membrane filtration technique.

For total coliforms the membrane filter is placed onto membrane-enriched teepol medium, which contains the detergent teepol to inhibit non-intestinal bacteria from growing. A different medium (m-ENDO) is normally used in the US (APHA, 1992). The membrane filter on the culture medium is incubated. During this incubation period, the nutrients diffuse from the culture medium through the membrane and the coliform bacteria are able to multiply and form recognizable colonies that can then be counted. Membranes are incubated at 30°C for 4 h followed by 14 h at 37°C for total coliforms or 14 h at 44°C for *E. coli*, the colonies of which are a distinctive yellow colour. Faecal streptococci are determined using the same technique except that different culture media and incubation conditions are required (i.e. membrane *Enterococcus* agar medium and incubated for 4 h at 37°C followed by 44 h at 45°C with colonies appearing either red or maroon in colour) (Table 12.4).

Multiple tube

The principle of the test is that various volumes of sample water are inoculated into a series of tubes containing a liquid medium that is selective for coliform bacteria (Fig. 12.4). From the pattern of positive and negative growth responses in the dilution series, a statistical estimate of the number of coliforms and subsequently *E. coli* can be made. This is called the most probable number (MPN) estimate, which is calculated by reference to probability tables. The MPN is expressed as the number of cells per 100 ml of sample. The technique is carried out in two discrete stages. The first estimates the number of coliforms present on the assumption that all the tubes, which show acid and gas productions contain coliform organisms. Because this assumption is made, this is known widely as the presumptive coliform count. The second stage tests for the presence of *E. coli*. The first stage is completed over 48 h, while the second takes a further 24 h.

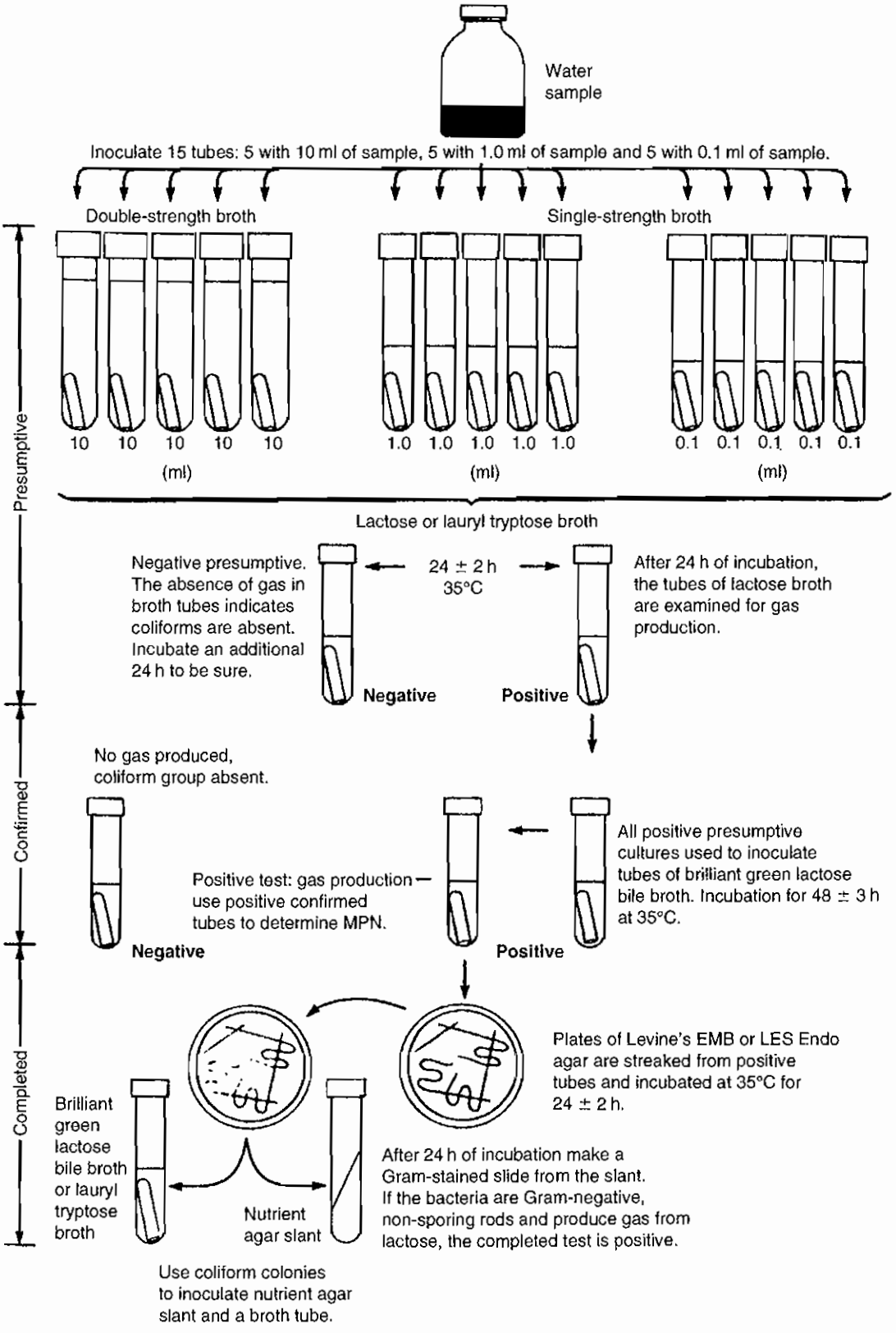

FIGURE 12.4 Summary of the multiple tube fermentation test for coliforms. (Reproduced from Prescott *et al.* (1993) with permission of McGraw-Hill, New York.)

The membrane filtration technique is considered to have many advantages over the multiple tube method for water testing. These include:

(a) presumptive coliform counts are available in a shorter time (18 h);
(b) it is a simpler test with less steps;

(c) there is considerable saving in the laboratory in the amounts of culture media, labour and glassware required;
(d) larger volumes of sample may be processed;
(e) it is possible to carry out filtration in the field;
(f) false negative results due to the development of aerobic and anaerobic spore bearing organisms are unlikely to occur.

Details of all microbial methods are given in *Standard Methods* for the US (APHA, 1992) and for the UK in *Report on Public Health and Medical Subjects No. 71* (Department of the Environment, 1994), although the latter is now regularly updated online by the Environment Agency (Table 12.4).

12.2.4 EMERGING TECHNOLOGIES

There are many emerging new technologies for microbial water testing, primarily to detect coliforms. These are focused on complex biochemical techniques, such as hybridization and polymerase chain reaction (PCR), gene probe technology and monoclonal antibody methods, which allow single bacterial cells to be detected (Gleeson and Gray, 1997). Enzyme detection methods are now widely in use, especially in field situations. Total coliform detection is based on the presence of β-galactosidase, an enzyme that catalyses the breakdown of lactose into galactose and glucose; while *E. coli* is based on the detection of β-glucuronidase activity. These tests are known as ONPG and MUG methods, respectively, after the substrates used in the tests and are now accepted standard monitoring methods in the US. These ONPG–MUG tests can be used to give an MPN value or simply indicate the presence or absence (P-A) of coliforms or *E. coli*. These tests will become increasingly important as there is a general swing away from standards based on microbial density to those simply based on P-A of coliforms in a sample. The best-known commercial ONPG–MUG preparations are currently Colilert® (Access Analytical, Branford, CT), Coliquick® (Hach Co., Loveland, CO) and Colisure® (Millipore Co., Bedford, MA). The ingredients for these new tests come in powder form (in test tubes for the quantitative MPN method and in containers for P-A analysis). A measured amount of water is added to each tube or container and the powder dissolves into a colourless solution. The tubes are placed in an incubator for 24 h at 35°C. The solution in tubes with total coliforms will be yellow which is then exposed to a hand-held fluorescent light. If the tube contains *E. coli* the solution will fluoresce brightly. The specificity of this method eliminates the need for confirmatory and completed tests.

The coliform test is still widely considered the most reliable indicator for potable water. However, in recent years there has been growing dissatisfaction with the use of coliforms as indicator organisms. Recent years have seen increasing reports of waterborne outbreaks largely as a result of protozoan and viral agents in waters considered safe to drink under current legislation, which relies largely on the coliform

test. The major deficiencies identified with the use of coliforms as indicators for drinking water quality assessment are:

(a) the regrowth of coliforms in aquatic environments;
(b) the regrowth of coliforms in distribution networks;
(c) suppression by high background bacterial growth;
(d) they are not directly indicative of a health threat;
(e) a lack of correlation between coliforms and pathogen numbers;
(f) no relationship between either protozoan or viral numbers;
(g) the occurrence of false positive and false negative results.

This has been reviewed by Gleeson and Gray (1997).

12.2.5 STANDARDS

EU legislation

In December 1998 the newly revised Drinking Water Directive was published (Section 8.1). As well as complying with new fixed parameters, water supplied under the Directive will have to be free of pathogenic micro-organisms and parasites in numbers constituting a danger to public health (Table 8.6). Although as before there are no guide values given for specific viruses, protozoans or bacterial pathogens. The New Directive gives separate maximum permissible concentrations for the microbial parameters for both tap and bottled waters, except natural mineral waters that have their own Directive. For tap water maximum permissible concentrations are given for *E. coli* (0 per 100 ml) and enterococci (0 per 100 ml). For bottled waters the maximum permissible concentrations are stricter than previously with *E. coli* (0 per 250 ml) and enterococci (0 per 50 ml). *Pseudomonas aeruginosa* has also been included for the first time with a maximum permissible concentration of 0 per 250 ml. Maximum colony counts at 22°C and 37°C are 100 and 20 ml^{-1}, respectively. The microbial parameters are listed in a new section, Part A, of the Directive. Part B contains chemical parameters and Part C indicator parameters. Included in Part C are total coliforms with a maximum permissible concentration of 0 per 100 ml for tap waters and 0 per 250 ml for bottled waters; and also included are total bacterial counts (22°C) which must not show any abnormal change with a maximum permissible value of 0 per ml in tap waters and sulphite-reducing clostridia at 0 per 100 ml (Table 8.7). Specific and detailed notes are given on analysis, including the composition of all recommended media. The recommended analyses in the proposed Directive are given in Table 12.7.

US legislation

The USEPA set a MCL based on the P-A concept for coliforms. This revised MCL came into force on 31 December 1990. The recommended sampling frequency of water supplies is dependent on the population served. For systems requiring >40

TABLE 12.7 The recommended microbial methods in the EU Drinking Water Directive (98/83/EEC)

Total coliforms Membrane filtration followed by incubation on membrane lauryl broth for 4 h at 30°C followed by 14 h at 37°C. All yellow colonies are counted regardless of size.
E. coli Membrane filtration followed by incubation on membrane lauryl broth for 4 h at 30°C followed by 14 h at 44°C. All yellow colonies are counted regardless of size.
Faecal streptococci Membrane filtration followed by incubation on membrane *Enterococcus* agar for 48 h at 37°C. All pink, red or maroon colonies which are smooth and convex are counted.
Sulphite-reducing clostridia Maintain the sample at 75°C for 10 min prior to membrane filtration. Incubate on tryptose–sulphite–cycloserine agar at 37°C under anaerobic conditions. Count all black colonies after 24 and 48 h incubation.
Pseudomonas aeruginosa Membrane filtration followed by incubation in a closed container at 37°C on modified Kings A broth for 48 h. Count all colonies which contain green, blue or reddish-brown pigment and those that fluoresce.
Total bacteria counts Incubation in a YEA for 72 h at 22°C and for 24 h at 37°C. All colonies to be counted.

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samples per month, <5% of samples must be total coliform positive. For systems where the frequency of analysis is <40 samples per month, then no >1 sample per month may be total coliform positive. If a sample is found to be total coliform positive then repeat samples must be taken within 24 h. Repeat samples must be taken at the same tap and also at adjacent taps within five service connections (Section 10.2), both up- and downstream of the original sample point. If these repeated samples are also total coliform positive, then the samples must be immediately tested for faecal coliforms or *E. coli*. If these prove positive then the public must be informed. A full detail of the revised coliform rule is given by Berger (1992).

A 100 ml sample bottle must be used in analysing total coliforms using one of the following techniques: 10-tube multiple tube fermentation technique, the membrane filtration technique, the P-A coliform test or the minimal-media ONPG–MUG test. This P-A concept has a number of potential advantages:

(a) Sensitivity is improved because it is more accurate to detect coliform presence than to make quantitative determinations.
(b) The concept is not affected by changes in coliform density during storage.
(c) Data manipulation is much improved.

The introduction of new and rapid testing systems will most likely see a similar P-A coliform test introduced throughout Europe.

WHO recommendations

The WHO (2004) guidelines for drinking water are perhaps the most important standards relating to water quality (Section 8.1). Standards are based solely on the presence of *E. coli* or thermotolerant coliforms, which must not be detectable in any 100 ml sample of water entering or within the distribution system, or in the drinking water delivered to the consumer (Table 8.8). This simple P-A approach makes water safety easier to manage with the presence of either *E. coli* or thermotolerant coliforms indicative that faecal contamination is able to enter the supply system. There are no specific guideline values for other bacteria, or for viruses or parasites. This is because the analytical methods for these organisms are too costly, complex and time consuming for routine laboratory use. Instead, guideline criteria are outlined based on the likely viral content of source waters and the degree of treatment necessary to ensure that even large volumes of water have a negligible risk of containing viruses. It is considered that 'the attainment of the bacteriological criteria and the application of treatment for virulogical reduction should ensure the water presents a negligible health risk' (WHO, 2004).

12.3 Removal of Pathogenic Organisms

Efficient wastewater treatment is critical for the prevention of waterborne disease. Conventional treatment can only remove up to 80–90% of bacterial pathogens with tertiary treatment increasing this to ≥98%. While wastewater disinfection can increase this to >99.99%, there will still be significant numbers of pathogens present in the final effluent. Dilution and the effects of natural biotic and abiotic factors in surface waters will reduce the density of pathogens further, although all water resources can become contaminated from other sources, such as diffuse agricultural pollution, surface (urban) run-off, and contamination from septic tank systems and landfill leachate. Also, with the emphasis of discharge standards for wastewater treatment plants based largely on environmental criteria, efficiency of pathogen removal is often ignored and usually poor. This places an ever-increasing burden on the final barrier, water treatment. With increasing concerns over the transfer of antibiotic resistance between bacteria in biological wastewater treatment systems and the increasing occurrence of chlorine-resistant pathogens, microbial discharge standards for sewage treatment plants are likely, making wastewater disinfection inevitable. The effectiveness of water and wastewater treatment in the removal of faecal coliforms is summarized in Fig. 12.1.

12.3.1 ENVIRONMENTAL FACTORS OF SURVIVAL

All pathogens are able to survive for at least a short period of time in natural waters, both fresh and saline, and generally this period is extended at cooler temperatures and if organic pollution is present. In raw sewage 50–75% of the coliforms are associated

with particles with settling velocities $>0.05\,cm\,s^{-1}$, so in conventional primary sedimentation at a treatment plant significant removals of enteric bacteria are achievable. Some idea of the effect of time on the survival of pathogenic micro-organisms can be obtained by examining the removal efficiency of storage lagoons which are used at some waterworks to improve water quality prior to treatment and supply. Up to 99.9% reduction of enteric bacteria can be obtained by storage, although this is dependent on temperature, retention time and the level of pollution of the water. For example, a 99.9% reduction in pathogenic viruses by storage requires a retention time of 20 days at 20–27°C but up to 75 days at 4.8°C. Viruses can survive longer than bacteria in natural waters, with some viruses surviving for up to twice as long as certain indicator bacteria (Table 12.8). Temperature is an important factor in the survival of viruses and at low temperatures (4–8°C) survival time will increase.

Both UV radiation and short-wave visible light is lethal to bacteria with the rate of death related to light intensity, clarity of the water and depth. In the dark the death rate of coliforms follows first-order kinetics over the initial period; however, predation by protozoa causes a departure from the log-linear relationship. The death rate is measured as the time for 90% mortality to occur (T_{90}). In the dark at 20°C the T_{90} for coliforms is 49 h while under mid-day sunlight the T_{90} is reduced to 0.3 h. The death rate is also temperature dependent, increasing by a factor of 1.97 for each 10°C rise in temperature, and is proportional to the total radiation received regardless if continuous or intermittent. The amount of radiation required to kill 90% (S_{90}) of coliforms is estimated as 23 cal. cm^{-2} (11.3 cal. cm^{-2} for *E. coli*). The S_{90} ratio of faecal streptococci to coliforms varied from 10 to 40 indicating that the die-off rate for faecal streptococci is appreciably slower than for total coliforms. The effect of temperature is far less marked under light conditions and, while variations in salinity have no effect on death rate, it is substantially slower in fresh and brackish waters. The daily solar radiation in southern Britain is between 50 to 660 cal. cm^{-2} so there appears to be ample radiation to inactivate all faecal coliforms. Wavelength is also important with about 50% of the lethal effect of solar radiation attributable to wavelengths below 370 nm, 25% to near visible UV (370–400 nm) and 25% to the blue-green

Table 12.8 Effects of temperature on the time (t) required for 99.9% inactivation of enteric micro-organisms during storage

Species	*Temperature (°C)*	*Time (t)*	*Temperature (°C)*	*Time (t)*
Poliovirus	4	27	20	20
Echovirus	4	26	20	16
E. coli	4	10	20	7
S. faecalis	4	17	20	8
Poliovirus type 1, 2, 3	4–8	27–75	20–27	4–20
Coxsackie virus type A2, A9	4–8	12–16	20–27	4–8

Reproduced from Kool (1979) with permission of John Wiley and Sons Ltd, Chichester.

region of the visible spectrum (400–500 nm). The effect of wavelengths >500 nm is negligible. As this die-off rate for coliforms is proportional to the light intensity and is therefore essentially a first-order relationship, it can be expressed as:

$$\frac{dC}{dt} = -kI_o \cdot e^{-az}C \tag{12.1}$$

where C is the coliform concentration at time t at a depth z, and k is a proportionality coefficient, I_o the light intensity just below the water surface and a the effective attenuation coefficient.

12.3.2 WASTEWATER TREATMENT

The removal of pathogenic micro-organisms is brought about by a combination of physical, chemical and biological processes. Physically pathogens are removed by adsorption and settlement, while the overall concentration is reduced by dilution. The chemical nature of the wastewater will determine whether the environmental conditions are suitable for the survival or even the growth of pathogens; however, factors such as hardness, pH, ammonia concentration, temperature and the presence of toxic substances can all increase the mortality rate of the micro-organisms. Biologically, death of pathogens can occur due to a number of reasons including starvation, although predation by other micro-organisms and grazing by macro-invertebrates are important removal mechanisms. During treatment of sewage the microbial flora changes from predominately faecal in character to that found in enriched freshwaters.

It is convenient to look at the wastewater treatment plant as an enclosed system with inputs and outputs. It is a continuous system so the outputs, in the form of sludge and a final effluent, will also be continuous. While a comparison of the number of pathogens in the influent with the final effluent will provide an estimate of overall removal efficiency, it will not give any clues as to the mechanism of removal. Essentially pathogens are either killed within the treatment unit, discharged in the final effluent or concentrated in the sludge which will result in secondary contamination problems if disposed either to agricultural land or into coastal waters. An estimation of the specific death rate ($-\mu$) of a pathogen can be calculated by accurately measuring all the inputs and outputs of the viable organism (Pike and Carrington, 1979). All the individuals of the population of the pathogen (x), which are assumed to be randomly dispersed within the reactor, have the same chance of dying within a specific time interval ($t - t_o$). Under steady-state conditions, the causes of death can be assumed to remain constant in terms of concentration (in the case of a toxic substance) or number (of a predator), so the rate of death will be proportional to the number of survivors (x_t) of the original population (x_o). This can be represented by a first-order, exponential 'death' equation:

$$x_t = x_o \cdot e^{-\mu(t-t_o)} \tag{12.2}$$

The problem with using Equation (12.2) in practice is obtaining accurate estimates of the viable micro-organism present in the outputs, especially if the cells have flocculated or are attached to film debris. The type of reactor is also important in the estimation of the death rate. In an ideal plug-flow system (e.g. percolating filter) in which first-order kinetics apply, the fraction of pathogens surviving (x_t/x_0) can be related to the dilution rate or the reciprocal of the retention time (ϕ) according to Equation (12.3):

$$\frac{x_t}{x_0} = \frac{-\mu}{\phi} \tag{12.3}$$

In a continuous-stirred tank reactor (CSTR) the specific death rate is calculated by assuming the rate of change in pathogen concentration within the reactor (dx/dt) equals the input concentration (x_0) minus the output concentration (t), minus those dying within the reactor ($-\mu$). So by assuming that the ratc of change is zero within the reactor under steady-state conditions, then:

$$\frac{dx}{dt} = \phi x_0 - \phi x_t - \mu x \tag{12.4}$$

Primary sedimentation

Bacteria and viruses are significantly reduced by primary settlement, with settled sludge containing the whole range of pathogens found in raw sewage. Ova (eggs) and cysts of parasites are only significantly removed at this stage in wastewater treatment plants. Settlement efficiency is dependent on the size and density of the ova and cysts, and as their free-falling settling velocities are not much greater than the theoretical upflow velocity, near quiescent conditions are required for optimum removal. The larger, denser ova of *Ascaris lumbricoides* and *Taenia saginata* are more efficiently removed than the smaller cysts of *Entamoeba* spp. The settling velocity of *T. saginata* is about 0.6–0.9 m h^{-1}, although much less if detergents are present, resulting in 68% removal after 2 h and 89% after 3 h settlcment.

Activated sludge

The activated sludge process is highly efficient in the removal of pathogenic bacteria and viruses, achieving a 90% removal efficiency or more (Geldreich, 1972). The major removal mechanism of bacteria in the activated sludge process is predation by a variety of amoebae, ciliate protozoa and rotifers. The ciliate protozoa and rotifers feed only on the freely suspended bacteria and not on flocculated forms (Curds *et al.*, 1968) (Fig. 12.5). Amoebae occur in similar numbers to ciliates and have similar yield coefficient biomass and generation times. They also play a significant role in the removal of bacteria by predation and are able to feed on flocculated forms as well as the freely

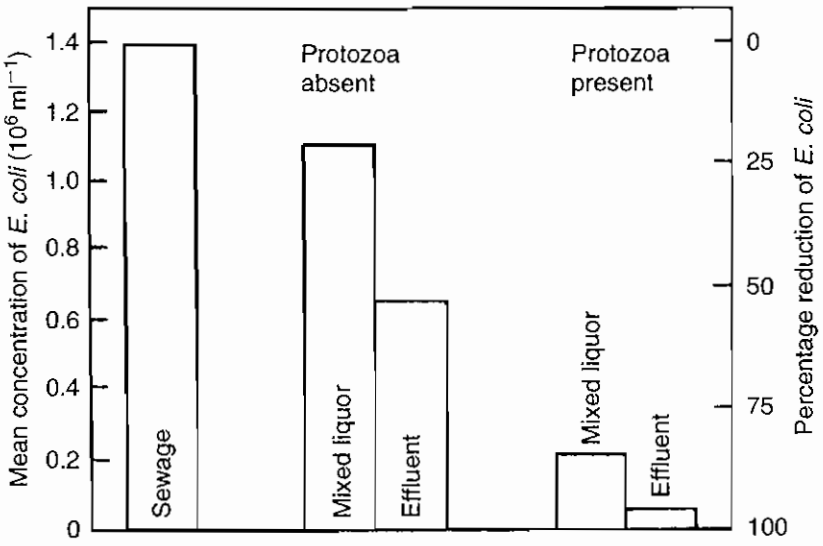

Figure 12.5 Removal of *E. coli* in experimental-activated sludge plants operating in the absence or presence of ciliated protozoa. (Reproduced from Curds and Fey (1969) with permission of Elsevier Science Ltd, Oxford.)

suspended bacteria. The estimated specific growth rate of *E. coli* is $-7.9\ \text{day}^{-1}$ in the presence of protozoa compared with $+0.12\ \text{day}^{-1}$ in the absence of protozoa, suggesting that *E. coli* are capable of very slow growth in sewage when ciliates are absent. In general terms the percentage removal of coliform bacteria is directly related to the specific sludge wastage rate, with 90% removal at a sludge wastage rate of $0.65\ \text{day}^{-1}$ which rapidly decreases as the wastage rate increases (Pike and Carrington, 1979).

The prime removal method of viruses is adsorption onto sludge flocs, predation having a negligible effect on concentration in the liquid phase (Section 20.6). Both viruses and bacteria are adsorbed according to the empirical Freundlich adsorption isotherm, where the count of particles adsorbed per unit mass of sludge (Y/m) is proportional to a power (n) of the count of particles (x) in the liquor at equilibrium:

$$\frac{Y}{m} = kx^n \tag{12.5}$$

This adsorption model is for unreactive sites whereas Michaelis–Menten or Monod kinetics are for reactive sites. However, as Pike and Carrington (1979) point out, the successful fitting of an adsorption model to data may not demonstrate that adsorption is the only factor operating since, for example, protozoa attached to the activated sludge flocs and feeding off freely suspended bacteria will quantitatively behave as a continual adsorption site. Upwards of 90% of enteroviruses are removed by the activated sludge process (Geldreich, 1972). Ova and cysts of parasites are able to survive the activated sludge process and are not effectively removed.

Fixed-film reactors

Percolating filters are extremely effective in removing pathogenic bacteria with normal removal efficiencies of >95%. Removal is achieved by similar mechanisms as in the activated sludge process, except that filters are plug-flow systems with a fixed and

not a mixed microbial biomass, so that opportunities for contact between pathogens and adsorption sites in the biomass are reduced.

Removal of bacteria is directly related to the bacterial count of the sewage and at low-rate loadings to the surface area of the filter medium. Removal efficiency falls off during winter that suggests that maximum removal of pathogens occurs when the film is most actively growing and under maximum grazing pressure, which is when maximum availability of adsorption sites will occur (Gray, 2004). Ciliate protozoa, rotifers and nematodes can ingest pathogenic bacteria. However, in percolating filters there is a much larger range of macro-invertebrate grazers feeding directly on the film and so indirectly feeding on the pathogens. Once pathogens have been adsorbed onto the film they are essentially 'removed' and their subsequent ingestion by a grazing organism may not be significant. The major limitation of percolating filtration in the removal of pathogens is their physical adsorption from suspension. Rotating biological contactors are also extremely efficient in the removal of pathogenic bacteria, with median removal of *E. coli* normally >99.5%. Percolating filtration is not very effective in the removal of parasite ova and cysts although the nature of the film does allow some retention of ova. Geldreich (1972) in a review on waterborne pathogens quotes removal rates of 18–70% for tapeworm ova and 88–99% for cysts of *E. histolytica*.

Waste stabilization ponds

All stabilization ponds and lagoons are extremely effective in removing pathogenic bacteria, viruses and the other parasites. Removal mechanisms include settlement, predation, inactivation due to solar radiation which is also linked with temperature, increase in pH due to day-time assimilation of carbon dioxide which can reach in excess of pH 9.0, and finally antibacterial toxins produced by algae. It is most appropriate to consider ponds as CSTRs, so the survival of pathogens (x_t/x_o) can be calculated as:

$$\frac{x_t}{x_o} = \frac{1}{[1 - (\mu/\phi)]} \tag{12.6}$$

Ponds are normally in series, so if they have similar dilution rates the survival of pathogens can be calculated using the following equation:

$$\frac{x_n}{x_o} = \frac{1}{[1 - (\mu/\phi)]^n} \tag{12.7}$$

where n is the number of ponds. A problem arises if the number of ponds in series is large (i.e. >5) because then the system behaves more like a plug-flow reactor. In this case the relationship becomes:

$$\frac{x_n}{x_o} = \exp - (\mu/\phi_n) \tag{12.8}$$

where ϕ_n is the dilution rate of the complete system.

Solar radiation is a major removal mechanism of bacteria and viruses in maturation ponds. In the UK at a retention time of 3.5 days the removal rate of *E. coli* was >90% in the summer but fell to 40% in the winter (60% for faecal streptococci) due to the seasonal difference in light intensity (Toms *et al.*, 1975). In the British situation the algal density is rarely sufficient to significantly shift the pH in order to kill the pathogens, in fact the algae can reduce mortality of bacteria and viruses by reducing light intensity by shading. The depth is also an important factor with removal rates reduced with depth.

In tropical and sub-tropical countries the removal of pathogens is as important as biochemical oxygen demand (BOD) removal is in temperate zones, and in many countries more so. Facultative ponds are very efficient in the removal of pathogens (Gloyna, 1971) with removal rates of coliform and streptococci bacteria >99% and viruses inactivated by light so that >90% removals are achieved. The greater efficiency of facultative ponds compared with other systems is due to the much longer retention times, so ponds in series achieve greater removals than single ponds. Major removal mechanisms are the high pH values created by photosynthesis and the higher zooplankton predation rate which is recorded. Anaerobic treatment followed by facultative treatment gives a higher die-off rate of pathogenic bacteria than a facultative pond followed by a maturation pond in series. Pond systems appear extremely effective in the removal of parasites due to the long retention time allowing maximum settlement. Cysts of the protozoans *E. histolytica* and *Giardia lamblia* are almost completely removed, while the helminth parasites, such as *Schistosoma, Ascaris, Enterobius, Ancylostoma* and *Trichuris* are also effectively removed (Gloyna, 1971). Maximum removal occurs in the first pond and as the nematode eggs in particular are highly resistant, extreme care must be taken with the disposal of the raw sludge from the pond if contamination is to be prevented (Section 18.2).

The removal of helminth (nematode) eggs in stabilization ponds is dependent on retention (ϕ). So for ponds in series (including anaerobic, facultative and maturation) the number of eggs in the final effluent (E_f) is calculated using Equation (12.9):

$$E_f = E_i \left[1 - \left(\frac{R_1}{100}\right)\right]\left[1 - \left(\frac{R_2}{100}\right)\right]\left[1 - \left(\frac{R_3}{100}\right)\right] \tag{12.9}$$

where E_i is the number of eggs in the raw sewage (l^{-1}). Values of R are obtained from Table 12.9. If E_f is >1 then an extra maturation pond is required. Maturation pond design is based on the removal of *E. coli* or faecal coliforms (Mara, 1996).

Example 12.1 Assuming an egg density of $2000 l^{-1}$ in the raw sewage calculate the final density of eggs in the final effluent after anaerobic lagoon, facultative pond and maturation pond treatment in series. The retention times in the various ponds are 5.0, 3.6 and 8.5 days, respectively.

TABLE 12.9 Estimated values of percentage removal (R) of intestinal nematode eggs in waste stabilization ponds at various retention times (θ) in days

θ	R	θ	R	θ	R
1.0	74.67	4.0	93.38	9.0	99.01
1.2	76.95	4.2	93.66	9.5	99.16
1.4	79.01	4.4	93.40	10	99.29
1.6	80.87	4.6	94.85	10.5	99.39
1.8	82.55	4.8	95.25	11	99.48
2.0	84.08	5.0	95.62	12	99.61
2.2	85.46	5.5	96.42	13	99.70
2.4	87.72	6.0	97.06	14	99.77
2.6	87.85	6.5	97.57	15	99.82
2.8	88.89	7.0	97.99	16	99.86
3.0	89.82	7.5	98.32	17	99.88
3.2	90.68	8.0	98.60	18	99.90
3.4	91.45	8.5	98.82	19	99.92
3.6	92.16			20	99.93
3.8	92.80				

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Using Table 12.9 to calculate R for each pond and Equation (12.9) then:

$$E_f = 2000\left[1 - \left(\frac{95.62}{100}\right)\right]\left[1 - \left(\frac{92.16}{100}\right)\right]\left[1 - \left(\frac{98.82}{100}\right)\right]$$

$$= 2000(0.0438)(0.0784)(0.0118)$$

$$E_f = 0.081^{-1}$$

Digestion

There are very conflicting results relating to the efficiency of anaerobic digestion and composting to remove pathogens from sewage sludge. Anaerobic digestion certainly reduces the numbers of pathogens considerably but not always completely. While many bacteria, fungi and viruses are rapidly killed by air drying such organisms can survive anaerobic digestion at 20 or 30°C for long periods. For example, *S. typhi* can survive digestion for 12 days at 20°C or 10 days at 30°C, *Ascaris* ova 90 days at 30°C and hookworm ova 64 days at 20°C or 41 days at 30°C. Both the latter ova can also withstand air drying. Complete destruction of pathogens is only possible by heating the sludge to 55°C for 2 h or treating with lime.

Sterilization and disinfection methods

Sterilization and disinfection of final effluents to remove any disease-causing organisms remaining in effluents is not widely practised in Europe, but is common in the

TABLE 12.10 Typical chlorine dosages to achieve disinfection of various wastewaters

Wastewater	*Chlorine dose (mg l^{-1})*
Raw sewage	8–15
Settled sewage	5–10
Secondary treated effluent	2–8
Tertiary treated effluent	1–5
Septic tank effluent	12–30

USA. The increasing need to reuse water for supply after wastewater treatment will mean that the introduction of such methods to prevent the spread of diseases via the water supply is inevitable. The two processes are distinct form each other. Sterilization is the destruction of all the organisms in the final effluent regardless of whether they are pathogenic or not, while disinfection is the selective destruction of disease-causing organisms. There are three target groups of organism, the viruses, bacteria and protozoan cysts, and each is more susceptible to a particular disinfection process than the other. The main methods of sterilization and disinfection are either chemical or physical in action. The factors affecting the efficiency of chemical disinfectants are contact time, concentration and type of chemical agent, temperature, types and numbers of organisms to be removed, and the chemical nature of the wastewater. Typical chlorine doses for wastewaters are given in Table 12.10. Viruses require free chlorine to destroy them and at higher doses compared with enteric bacteria. Chemical disinfection has been discussed in Section 10.1. The commonest physical method for removing pathogens is UV radiation, which acts on the cellular nucleic acids destroying bacteria, viruses and any other organisms present. Although expensive in terms of energy, such systems can be highly effective and do not affect receiving water quality. The greatest effect occurs at a wavelength of 265 nm, with low-pressure mercury arc lamps (254 nm) most widely used. The major operational problem is to obtain maximum penetration of the rays to ensure that even turbid effluents are fully sterilized. Numerous systems have been evaluated to obtain maximum exposure of wastewater to the radiation, but the most effective system to date is the use of thin-film irradiation (<5 mm). UV irradiation is particularly useful in preventing contamination of lakes and coastal waters with pathogens, which are popular for bathing. Filtration is also used to remove pathogens from effluents that have received tertiary treatment (Section 20.8).

12.3.3 WATER TREATMENT

Storage

Bacteria and viruses are significantly reduced when water is stored in reservoirs. During the spring and summer, sunlight, increased temperature and biological factors ensure that between 90% and 99.8% reductions of *E. coli* occur. The percentage

reduction is less during the autumn and winter due to the main removal mechanisms being less effective, so expected reductions fall to between 75% and 98%. Lowest reductions occur when reservoirs are mixed to prevent stratification. The greatest decline in *E. coli* and *Salmonella* bacteria occurs over the first week, although the longer the water is stored the greater the overall reduction. Temperature is an important factor. For example, poliovirus was reduced by 99.8% in <15 days at 15–16°C compared with 9 weeks for a similar reduction at 5–6°C. For optimum removal of all micro-organisms of faecal origin then 10 days retention should achieve between 75% and 99% reduction regardless of temperature. Protozoan cysts are not effectively removed by storage because of their small size and density. For example, the settling velocity of *Cryptosporidium* oocysts is $0.5\,\mu m\,s^{-1}$, compared with $5.5\,\mu m\,s^{-1}$ for *Giardia* cysts. So while it would be feasible in a large storage reservoir to remove a percentage of the *Giardia* cysts, if the retention time was in excess of 6 weeks, and mixing and currents were minimal, *Cryptosporidium* oocysts would remain in suspension (Denny, 1991). *Salmonella*, faecal streptococci and *E. coli* are excreted in large numbers by gulls. The presence of gulls, especially in high numbers, on storage reservoirs may pose a serious problem either from direct faecal discharge and/or from rainfall run-off along contaminated banks.

Unit processes

Bacteria and viruses are removed by a number of unit processes in water treatment, especially coagulation, sand filtration and activated carbon filtration (Section 10.1). Coagulation using alum removes about 90% of faecal indicator bacteria, 95–99% of all viruses and about 60–70% of the total plate count bacteria, although these figures vary widely from treatment plant to treatment plant. Other coagulants, such as ferric chloride and ferric sulphate, are not quite as efficient. The use of polyelectrolytes as coagulant aids does not improve removal of viruses. Rapid sand filtration is largely ineffective in removing viruses and bacteria unless the water has been coagulated prior to filtration. In contrast, slow sand filtration is able to remove up to 99.5% of coliforms and 97–99.8% of viral particles from water, although performance is generally worse in the winter (Denny, 1991). Activated carbon can remove viruses, which are adsorbed onto the carbon. This is by electrostatic attraction between positively charged amino groups on the virus and negatively charged carboxyl groups on the surface of the carbon. Removal efficiency is very variable and depends on pH (maximum removal occurs at pH 4.5), the concentration of organic compounds in the water, and the time the filter has been in operation. Removal rates of between 70% and 85% are common. However, these filters can become heavily colonized by heterotrophic micro-organisms. Minute fragments are constantly breaking off the granular-activated carbon and each is heavily coated with micro-organisms. These micro-organisms are not affected to any great extent by disinfection, and so can introduce large numbers of micro-organisms including pathogens into the distribution system (Le Chevallier and McFeters, 1990).

Cysts should be removed effectively by coagulation and the addition of polyelectrolyte coagulant aids should also enhance removal. Using optimum coagulant conditions (as determined by the jar test) then 90–95% removal should be possible. Rapid sand filtration is not an effective barrier for cysts unless the water is coagulated prior to filtration. When used after coagulation then very effective removal of *Giardia* is achieved (99.0–99.9%). The only proven effective method of removing both *Cryptosporidium* and *Giardia* cysts is by slow sand filtration, with 99.98% and 99.99% of cysts removed, respectively (Hibler and Hancock, 1990).

Disinfection

Disinfection is absolutely vital to ensure that any micro-organisms arising from faecal contamination of the raw water are destroyed (Section 10.1.8). Chlorination is by far the most effective disinfectant for bacteria and viruses because of the residual disinfection effect that can last throughout the water's journey through the distribution network to the consumer's tap. The most effective treatment plant design to remove pathogens is rapid and slow sand filtration, followed by chlorination or treatment by pre-chlorination followed by coagulation, sedimentation, rapid sand filtration and post-chlorination. Both of these systems give >99.99% removal of bacterial pathogens including *C. perfringens*. Chlorine and monochloramine have been found to be ineffective against *Cryptosporidium* oocysts. Ozone at a concentration of about 2 mg l^{-1} is able to achieve a mean reduction in viability of oocysts of between 95% and 96% over a 10-min exposure period.

The disinfection rate determines the level of destruction of pathogens for a given period of contact. The death of micro-organisms is a first-order reaction in respect of time for a given disinfectant and concentration. This is Chick's law and is used to estimate the destruction of pathogens by a disinfectant as a function of time.

$$\frac{dN}{dt} = -kN \tag{12.10}$$

This can be integrated as:

$$N = N_0 e^{-kt} \tag{12.11}$$

or

$$\ln \frac{N}{N_0} = -kt \tag{12.12}$$

where N is the concentration of viable micro-organisms at time t, N_0 the initial concentration of viable micro-organisms and k the first-order decay rate. The negative sign indicates that the number of organisms decreases over time. The persistence of

pathogens and non-pathogenic micro-organisms in aquatic environments approximate to Chick's law which when plotted as $\ln(N/N_0)$ versus t is a straight line (Fig. 12.6) with the slope being $-k$. If $-\ln(N/N_0)$ is plotted against t instead, then the straight line intercepts 0 and the slope is k. Temperature and salinity particularly affect the decay constant k for any specific micro-organism. This can be adjusted by using Equation (12.13) for temperature or Equation (12.14) for both:

$$k_T = k_{20} \times 1.047^{T-20} \tag{12.13}$$

$$k_T = k_{20} \times (0.006 \times \% \text{ seawater}) \times 1.047^{T-20} \tag{12.14}$$

The rate coefficient k is redefined to incorporate the effect of a specific disinfectant at concentration C:

$$k = k'C^n \tag{12.15}$$

where n is an empirical constant known as the coefficient of dilution. Equation (12.12) can be rewritten as:

$$\ln \frac{N}{N_0} = -k'C^n t \tag{12.16}$$

or

$$\log \frac{N}{N_0} = \frac{-k'C^n t}{2.3} \tag{12.17}$$

So to determine the level of $C^n t$ to achieve the objective (N/N_0):

$$C^n t = \frac{-1}{k'} \ln\left(\frac{N}{N_0}\right) = \frac{-2.3}{k'} \log\left(\frac{N}{N_0}\right) \tag{12.18}$$

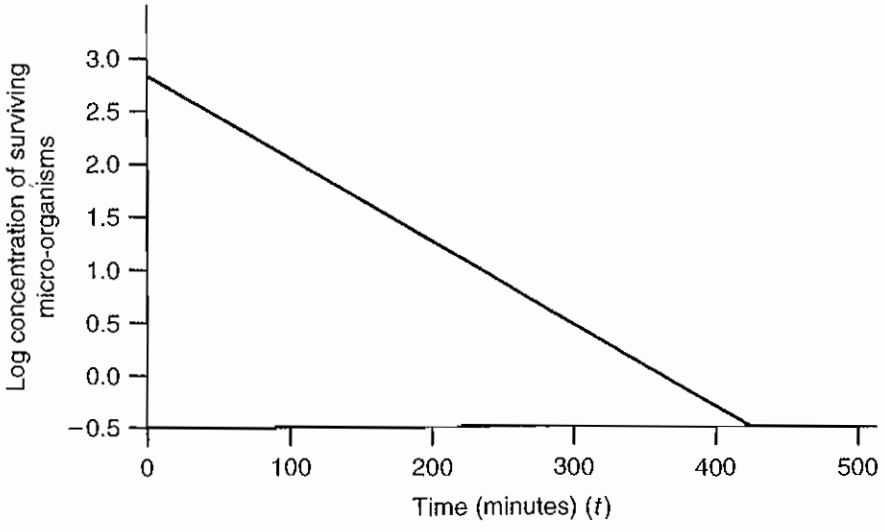

FIGURE 12.6 Destruction of pathogens according to Chick's law.

From Equation (12.18) variations in $\log(N/N_0)$ can be related to variations in $C^n t$ which is the basis of disinfection practices of drinking water. Where $n = 1$ then $\log(N/N_0)$ is proportional to Ct so individual rates for C and t need not be specified. However, where n is not equal to 1 then individual values of C and t are required. Where $n < 1$ reaction rate (t) is the most important factor for pathogen inactivation, while when $n > 1$ disinfection concentration (C) is the major factor (Bryant *et al.*, 1992).

It is a fact that conventional water treatment cannot guarantee the safety of drinking water supplies at all times. Outbreaks of waterborne diseases can and do happen, although very infrequently. With correct operation the chances of pathogenic micro-organisms causing problems to consumers will be reduced even further. The greatest risks come from private supplies that are not treated. The installation of a small UV water sterilizer unit installed under the sink on the rising main directly to the kitchen tap ensures microbially safe water. However, the only effective way to destroy protozoan cysts is to physically remove them. This is achieved by using a 1 μm pore-sized fibre cartridge filter that is placed upstream of the UV sterilizer. The filter will also increase the efficiency of the sterilization by removing any particulate matter that may harbour or shield pathogens from the UV radiation.

Wash water and water-treatment sludge contain all the pathogens removed from the water, and so must be handled as carefully as any other microbiologically hazardous waste. It is important that the sludge is not disposed of in such a way as to recontaminate the raw water source (Section 10.1.11).

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