Occurrence of Microorganisms in Treated Wastewater Effluent and Their Impact on the Surface and Groundwater Resources

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Abstract

Microorganisms are typically present in all media and they pose a greater threat to environmental sources and general health. The most significant part that has been affected by microorganisms is water since it plays a major role in all vital bodily functions. A sufficient water supply is a basic need for humanity. Yet, many nations around the world do not have access to safe and potable water, and many people die because of waterborne pathogens. For this reason, the immediate actions that many countries have adopted are the construction of water and wastewater treatment plants (WWTPs) to reduce dangers related to these pathogens. Nevertheless, pathogens can escape when the processing of wastewater is not applied correctly. Therefore, pathogens can find their way into sludge, effluent, agricultural land, and ultimately water bodies. Considering all points mentioned above, this review briefly discusses the effects of microorganisms in water resources and displays dangers related to human health, and subsequently focuses on the survival and transport of microorganisms. Conventional and advanced processes for pathogen removal from wastewater are also discussed.

Keywords: Water resources, WWTPs, microorganisms, wastewater, effluents, human health, surviving, transport.
DOI: 10.7176/CER/14-5-02
Publication date: August 31st 2022

1. Introduction

The microorganisms present in wastewater include bacteria, viruses, protozoa, and parasites among others. Due to the prevalence of human and animal diseases water may become unsafe for consumption once discharged into surface and groundwater. Ultimately, if the pathogenic concentration is extremely high, the water also may become dangerous for other recreational activities such as fishing and swimming. Bacteria remain the most common water-contaminating pathogens. Subsequently, water analysis is undertaken to assess the suitability of water for consumption. Researchers have adopted novel techniques presently used for the isolation and identification of pathogens of interest including numerous pathogenic bacteria. The following techniques are frequently used in microbial water quality monitoring programs: Most Probable Number Technique (MPN), Filtration Technique, Centrifugation, Flow Cytometry, Cultivation Techniques, Immunocapture, Chromogenic Media-Based Detection Methods, Emerging Procedures (DNA – Chip Array, Laser Scanning Analysis, and Biosensors), and other sophisticated techniques (PCR, Molecular Methods Targeting Nucleic Acids, Hydro-extraction, and Fluorescence *in Situ* Hybridisation (Köster *et al.* 2003, Huq *et al.* 2006). In this context, analysis of water for all known pathogens would be very expensive and time-consuming. Hence, specific microorganisms are not verified except if they are suspected. Rapid indicator organisms, for instance, fecal bacteria (*E. coli*, *Pseudomonas aeruginosa or Enterococci)* are used (Jung *et al.* 2014). Such indicator bacteria should not occur in drinking water supplies. Although human coliform bacteria do not cause disease, their presence is associated with pathogens and indicates that the water resource has been exposed to contamination with human fecal waste. Such bacteria occurrence is always related to fecal pollution and enteric pathogens including *Salmonella* spp., *Yersinia* spp., *Shigella* spp, and others. These enteric bacteria are accountable for many sicknesses (Rodríguez-Chueca *et al.* 2014). The water quality assessment requires water sampling and measurement of the bacteria concentration in a sample. If the bacteria of concern exceed the standard value, certain tests might be conducted to investigate the presence of specific pathogens. The ratio of total fecal coliforms to fecal streptococci (FC/FS ratio) can give information on the source of contamination. Due to the risk of microbial contamination to public health and environmental resources, there are some processes to disinfect wastewater effluents. These techniques comprise chemical (ozonation, chlorination/dichlorination), physical (membrane filtration), and biological (aerobic treatment processes decrease pathogens, yet it may not be adequate to consider as a disinfection procedure). Recently, advanced oxidation processes like UV light, hydrogen peroxide (H_2O_2)/solar irradiation, electrokinetic and photo-Fenton processes are being applied to remove pathogens from effluents (Rodríguez-Chueca *et al.* 2014, Rodríguez-Chueca *et al.* 2015). The purpose of this review is to identify the microorganisms in wastewater, particularly pathogens risk to human health. The questions this manuscript aims to answer are:

(i) Do microorganisms survive in sewage treatment plants, effluent, and sludge?
(ii) How microorganisms can reach surface water and groundwater?

(iii) Are conventional/existing treatment processes efficient and effective in pathogen removal?

2. Wastewater Reuse

Wastewater reuse is a promising alternative source of water supply considering the water shortage caused by climate change. Nevertheless, if not effectively treated, wastewater embodies a source of bacteriological health risk. For instance, the reuse of wastewater in agriculture may also represent a potential health risk related to aerosol production. Indeed, some irrigation techniques (e.g., spray irrigation) can influence the dispersion of aerosols containing pathogens such as *Legionella*, which is correlated with occupational risk. Even though wastewater is known to be a significant source of *Legionella*, only a few studies have been involved in the occurrence of this bacterium in WWT systems. Several studies have underlined the significance of *Legionella* monitoring in wastewater-treated effluents. The existence of *Legionella spp.* was documented in reclaimed wastewater applied for various purposes (e.g., agricultural use, toilet flushing … etc.) in the USA, Australia, and France (Elektorowicz *et al.* 2016).

To increase the microbial quality of wastewater for reuse purposes, several approaches and techniques can be employed (Adam and Elektorowicz 2017). For example, it is feasible to introduce new technologies in the secondary steps of the wastewater treatment plant (e.g., membrane bioreactor, MBR) (Lagum 2019). It is also possible to introduce new disinfection technologies or optimize the performance of the final disinfection steps (e.g., type and quantity of disinfectant). Membrane bioreactor (MBR) is a very sophisticated technology that has made the reuse of wastewater a realistic possibility (Adam and Elektorowicz 2018). In the MBR system, biological degradation of waste material takes place by activated sludge followed by a direct liquid/solid separation via membrane filtration.

MBRs could be appropriate for wastewater reuse applications as the MBRs technology appears to have a greater capability to eliminate viruses (e.g., coliphage) or bacterial indicators (e.g., fecal coliforms) compared to conventional activated sludge processes (ASPs). Microfiltration (MF) and ultrafiltration (UF) membranes (pore size 0.05 to 0.4 μm) have been extensively employed for various wastewater treatment and reuse (Adam and Elektorowicz; 2018, Lagum 2021).

Despite these promising findings, additional investigation is needed to gain a better understanding of the MBRs, because different factors can influence MBR performance. Factors influencing the membrane bioreactor performance and the permeability of membranes include activated sludge components and properties (i.e., mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), particle size, floc structure, sludge volume index (SVI), sludge disintegration, and organic/inorganic ratio), ambient conditions, microbial by-products (i.e., soluble microbial products (SMP) and extracellular polymeric substances (EPS), membrane characteristics (i.e., membrane configuration, pore size, porosity, material and hydrophobicity), and critical operating parameters (i.e., solids retention time (SRT), hydraulic retention time (HRT), transmembrane pressure (TMP) and flux (J), dissolved oxygen (DO), pH, food to microorganism (F/M), cross-flow velocity (CFV), steady-state condition, and aeration). However, extensive studies were conducted at Concordia University (Montreal, QC, Canada) to develop and establish a novel technology so-called membrane electro–bioreactor (MEBR) that could overcome the challenges relevant to traditional MBR applications (Elektorowicz *et al.* 2016, Adam and Elektorowicz 2018, Adam; and Elektorowicz; 2018). This technology is anticipated to replace the traditional MBR in the near future since it solves the major MBR problems (Lagum 2019, Lagum and Elektorowicz 2022). The MEBR technology is the first attempt to incorporate membrane processes (microfiltration), biological processes (biodegradation), and electrokinetic principles (mainly electrocoagulation processes) in a single reactor (Bani-Melhem and Elektorowicz 2010, Adam and Elektorowicz 2017, Adam and Elektorowicz 2017, Lagum 2021). The interaction between these three processes has permitted higher ammonia, nitrate, phosphorus, and chemical oxygen demand (COD) removal, in addition to decreasing membrane fouling and size of the treatment plant (Lagum 2019).

3. Human Health Risks

Waterborne diseases affecting human health are transmitted by contaminated drinking water. John Snow 1855 defined pathogens affecting human health; he discovered that the cholera break-out in London was spread by sewage-contaminated water (Bouzid and Global 2017). Today, the discharge of improperly treated effluent remains a prime source of many possible hazards to human health due to their exposure to pathogenic microorganisms (Chahal *et al.* 2016). Wastewater-related infections are, for example, dysentery, dysentery-like infections, diarrhea, typhoid, human enteritis, legionellosis, stomach ulcer and cancer, melioidosis, and *Leptospira interrogans* infections (Liang *et al.* 2006). Moreover, several skins and ear diseases may emerge due to the contaminated water coming into contact with damaged skin or penetration of the ear (Naidoo and Olaniran 2013). Bacteria include a wide variety of pathogenic strains found in wastewater and they are responsible for many diseases and pandemics. Particular attention is paid to the following bacteria which can be found worldwide: *Escherichia coli*, *Salmonella spp*, *Shigella spp*, *Giardia lamblia, Entamoeba histolytica*, and

Cryptosporidium spp. The infections and outbreaks related to these microorganisms occur more often in areas where no safe potable water supply, sanitation and hygiene are accessible.

The microbial pathogenic concentration in wastewater treatment plants is solely dependent on the source of influent entering the plant. It is basically relying on the physical, chemical and biological characteristics of the incoming waste. The symptoms related to these pathogens are varied but include dysentery, diarrhea, vomiting, gastroenteritis and typhoid. Wastewater contains mainly great amounts of excreted pathogens, particularly in places where intestinal parasites and diarrhoeal diseases are most prevalent (Moss 2016, Nwand1kor and Okolo 2016). The contamination of potable water by fecal coliforms is causing several disease outbreaks globally. The most widespread species of the family of fecal coliforms is *E. coli*, and this bacterium is considered a microbiological indicator of water quality. Thus, its presence also indicates the possibility of the co-existence of other pathogenic organisms (An *et al.* 2002). Furthermore*, E. coli*, *Giardia lamblia* and *Cryptosporidium parvum* have been recently documented as significant reasons for water and food-borne disease outbreaks connected to fecal water contamination. The issues with these pathogens are especially hard to control since they can persist in the environment and resist chlorine disinfection (Blumenthal *et al.* 2000), which consequently requires a longer contact time and higher dose concentration of chlorine. Nonetheless, chlorine is highly reactive with organic material in the water to most possible shape chlorinated organic compounds (i.e. chlorinated hydrocarbons) which are commonly known as global pollutants. Too, chlorination has shown to be insufficient to inactivate protozoa oocysts like *Cryptosporidium parvum* (Weber and Legge 2008).

Willis *et al.* (2013) studied the occurrence of *Cryptosporidium parvum* and *Giardia lamblia* in shellfish from several water bodies. They further suggested that monitoring and depuration standards should be improved to successfully eliminate the health hazards caused by harmful protozoan parasites. *Cryptosporidium* outbreaks have been recorded worldwide because of their contamination of drinking and recreational water. Recent studies of different populations show that the prevalence of *Cryptosporidium* infection is very high, with children being the most vulnerable to the risk (Osman *et al.* 2016). For instance, in China, more than half of the children by age five demonstrated antibodies to the protozoans *Cryptosporidium* and *Giardia* (Zu *et al.* 1994). *Cryptosporidium* spp. and *Giardia duodenalis* are major leading causes of diarrhea in children (Osman *et al.* 2016).

Similarly, pathogenic viruses have been recognized in recreational waters. For instance, a study by Griffin *et al.* (1999) shows that 95% of samples taken from a number of locations in surface waters nearby the Florida Keys contain hepatitis A viruses, enteroviruses, and norwalk viruses. In fact, viruses have shown higher resistance than bacterial indicators during traditional wastewater treatments like microfiltration and chlorination, and they are able to withstand lipid solvents (Fujioka and Yoneyama 2002). Osmosis and Nanofiltration membranes have shown an excellent capability to remove viruses. However, their application in wastewater treatment is mainly restricted by the high transmembrane pressure and frequent fouling. Electrokinetic processes might be the best option to remove viruses from effluent wastewater at a low cost.

4. Type of Pathogens Survive in Wastewater Treatment Plants, Effluent and Sludge

Wastewater, particularly domestic one, contains pathogens. Wastewater can cause disease prevalence when the disinfection processes for effluent are not applied appropriately. Therefore, the key objective of disinfection methods is to reduce pathogenic microorganisms such as fecal coliforms, streptococci, S*almonella spp*. and enteric viruses in receiving water and to eliminate potential health risks. However, increasing pressure on contemporary wastewater treatment processes has led to the discharge of inefficiently or untreated effluent, reinforcing the need for the adaptation of innovative techniques for the removal of pathogens before discharging. Pathogens can survive for a sufficient period in inadequately treated effluent. Although the numbers of pathogens are not very high, they still pose a grave threat to public health. For example, Anastasi *et al.* (2012) stated that several microorganisms could survive in treated wastewater effluents as a result of poorly disinfection processes. In addition, they demonstrated that "some *Escherichia coli* strains with uropathogenic properties survived treatment stages of sewage treatment plants (STPs), suggesting that they may be released into the environment". Another study was conducted by Czajkowska *et al.* (2008) on the survival of *Escherichia coli* O157:H7 strain on activated sludge from dairy sewage treatment plants and found that in all media non-pathogenic *E. coli* strain can survive longer than the *Enterohemorrhagic serotype*. Furthermore, in activated sludge after 21-28 days *E. coli* O157:H7 bacteria were not detected by using the direct plating method. In a similar fashion, the same bacteria were not detected after 21-25 days in activated sludge treating dairy wastewater. Other bacteria that survived in wastewater treatment plants are *Enterobacter aerogenes* (NCTC 10006), *Staphylococcus aureus* (negative), and *Pseudomonas aeruginosa* (Anastasi *et al.* 2012).

Worldwide, regulators rely on bacterial indicators like fecal coliform and total coliform bacteria to assess the presence of microorganisms and ultimately the water quality. Conversely, bacterial indicators do not reflect the threat from many significant pathogens. This includes viruses, stressed pathogenic bacteria (viable but nonculturable), and protozoa (Noble and Fuhrman 2001).

Recently, numerous research works on the survival of microorganisms in wastewater and sewage showed that viruses can survive longer than bacteria and its survival can significantly increase at lower temperatures. Fujioka and Yoneyama (2002) clarified that during conventional effluent treatment, like microfiltration and chlorination; pathogenic viruses are usually more resistant than bacterial indicators and are capable to withstand lipid solvents. Auer and Niehaus (1993) stated that enteric viruses can survive and grow longer than bacteria in the sediments and this is because enteric viruses are usually linked to fine sediment particles in a liquid medium. According to Dechesne *et al.* (2006), sediments can hold viruses tenfold more than water.

Fong *et al.* (2010) conducted a study to detect and track human adenoviruses in wastewater and combined sewer overflows influencing a Michigan River. They used the real-time polymerase chain reaction (RT–PCR) technique to quantify adenoviruses in wastewater, surface water, and combined sewer overflows due to their high prevalence, persistence, and survival in aquatic environments. They collected samples within two periods (August 2005 and August 2006) from raw sewage, primary-treated effluent, secondary-treated effluent, and chlorinated effluent from both a wastewater treatment plant and the lower Grand River in Michigan. The results showed that the concentrations of the viruses were above the real-time PCR detection limit (average, 7.8 - 103 Viruses/Liter). The researchers attributed the highest concentrations of adenovirus in this study to unsuccessful removal during the wastewater treatment process and to the high persistence and survival of these viruses in the environment.

Enteric viruses are prevalent in sewage, surface and drinking water. These viruses can not replicate in the environment, yet they can survive for many months in fresh water and for shorter periods in marine water (Mesquita and Emelko 2012). Enteric viruses have the ability to survive under a widespread scope of pH (3 to 10) and this ability can be extended for a period of time at low-temperatures. This period of time is different from one medium to others. To clarify, it has been observed that viruses remain active for more than 100 days in soil, for more than 120 days in sewage and fresh water, or more than 130 days in seawater at ideal temperatures (Fong and Lipp 2005). Such groups of viruses play a vital role in waterborne diseases and are not frequently investigated because of the detection limits of commonly used approaches (Poma *et al.* 2012, Jung *et al.* 2014). Viruses can be survived in sludge and soil for several months. In like manner, indicator bacteria survive for less than three months. *Salmonella spp.* remain alive up to a few months, however, frequently less than one month. *Protozoan cysts* can survive more than one month, yet, in many cases less than ten days (Feachem *et al.* 1983).

Wasted sludge in WWTP also can contain numbers of pathogens, and their number can vary as a consequence of the difference in the organic and inorganic content of the sludge. Besides that, the differences can occur geographically as well as over time at the same location. Bio-waste (sludge) may contain different species of pathogenic bacteria comprising *Escherichia coli*, *Salmonella typhi*, *Mycobacteria*, *Listeria*, *Campylobacter, Mycobacteria*, *Clostridia*, and *Yersinia* spp. (Sahlström 2003). The major dangerous pathogens that will exist in sludge are *Clostridium spp.* due to its spore-forming capacity. Such bacteria due to their ability to present in the form of spores can survive for many years in the environment (Sahlström 2003). Common bacteria like *Salmonella* can cause food poisoning. Fecal bacteria can be dominant in sludge, notwithstanding; sludge can contain a broad range of pathogens. A process to significantly reduce pathogens is the anaerobic digestion of sludge. Such processes eliminate fecal bacteria besides spores and *helminth ova* (Gerba and Smith 2005).

Environmental factors and soil amendments can play a role in the survival of microorganisms. Jamieson *et al.* (2004) pointed to a group of factors affecting the survival of the microbial organism and their transport in the aquatic environments which are represented in temperature, pH, light, availability of nutrients, and the predator presence (predator-prey cycles). The same is related to the survival and transport of microorganisms in soil environments which include pH, moisture content, temperature, soil type, nutrients and the existence of competing microorganisms. Rodriguez-Lazaro *et al.* (2012) found that enteric viruses can survive for a very long period of time (even years) at temperatures below 5 °C and particularly where the UV light is absent. Besides, they stated that the inactivation rates of these viruses vary significantly among viruses' types and inactivation rates are faster and more sufficient in liquid manure than in solid manure. Temporally and spatially variable factors also affect runoff. Microbial organisms that will be ready for transport will also be influenced by manure application rates and timing as well as a combination of physical, chemical and biological approaches which will also influence survival of microorganisms (Edwards *et al.* 1997). Likewise, factors including pathogens' persistence in water, contamination level, biological reservoirs (e.g., aquatic plants and sediments) and the capability of pathogens to be transported are determined the abundance and the occurrence of pathogens in water bodies. The survival of pathogens can also be affected by land use management practices and the size of the watershed (Ferguson *et al.* 2008, Harmel *et al.* 2010). Table 1 shows pathogens found in untreated municipal wastewater and excreta.

Table 1. Pathogens found in untreated municipal wastewater and excreta (Adapted from (Sagik *et al.* 1978, Edwards 1992, Council 1998, Lasobras *et al.* 1999).

Agent	**Disease**
Bacteria	
Campylobacter jejuni	Gastroenteritis, long term sequelae (*e.g.* arthritis)
Escherichia coli	Gastroenteritis
E. coli 0157:H7	Bloody diarrhoea, hemolytic uremic syndrome
Helicobacter pylori	Abdominal pain, peptic ulcers, gastric cancer
Legionella pneumophila	Legionnaire's disease
Leptospira (spp.)	Leptospirosis
Salmonella (many serotypes)	Salmonellosis, long term sequelae (*e.g.* arthritis)
Salmonella typhi	Typhoid fever
Shigella (several serotypes)	Shigellosis (dysentery), long term sequelae (*e.g.* arthritis)
Vibrio cholerae	Cholera
Yersinia enterocolitica	Yersiniosis, long term sequelae (*e.g.* arthritis)
Helminths	
Ascaris (roundworm)	Ascariasis
Ancylostoma (hookworm)	Hookworm
Clonorchis (liver fluke)	Clonorchiasis
Fasciola (liver fluke)	Fascioliasis
Fasciolopsis (liver fluke)	Fasciolopsiasis
Paragonimus (lung fluke)	Paragonimiasis
Schistosoma (blood fluke)	Schistosomiasis, Bilharzia
Trichuris (whipworm)	Trichuriasis
Taenia (Tapeworm)	Taeniasis
Protozoa	
Balantidium coli	Balantidiasis (dysentery)
Cryptosporidium parvum	Cryptosporidiosis, diarrhoea, fever
Cyclospora cayetanensis	Persistent diarrhoea
Entamoeba histolytica	Amebiasis (amebic dysentery)
Giardia lamblia	Giardiasis
Viruses	
Adenovirus (many types)	Respiratory disease, eye infections
Astrovirus (many types)	Gastroenteritis
Calicivirus (several types)	Gastroenteritis
Coronavirus	Gastroenteritis
Enteroviruses (many types)	Gastroenteritis, various
Coxsackie A	Herpangina, aseptic meningitis, respiratory illness
Coxsackie B	Fever; paralysis; respiratory, heart, and kidney disease
Echovirus	Fever, rash, respiratory and heart disease, aseptic meningitis
Norwalk virus	Gastroenteritis
Poliovirus	Paralysis, aseptic meningitis
Hepatitis A virus	Infectious hepatitis
Hepatitis E virus	Infectious hepatitis
Parvovirus (several types)	Gastroenteritis
Reovirus (several types)	Not clearly established
Rotavirus (several types)	Gastroenteritis

5. Microorganisms' Pathways in Surface and Groundwater

Similar to any kind of water pollution, microbial contamination occurs in surface and groundwater, and hence in potable water. Microbial water contamination is from two types of sources. These key sources are point-source and non-point source pollution. Industrial and domestic sewage belong to point sources where they are typically collected by a network of pipes or channels conveyed to a particular point of discharge into receiving water body, which is usually easier to identify and control. Urban and agriculture runoff is characterized by multiple discharge points and they are called non-point sources, which are generally complex and quite challenging to classify, and the control typically required a more comprehensive solution (Davis and Cornwell 2013).

Urban and agriculture runoff affects water quality, biological resources and habitat, then, eventually public health (Grigg 2016). Runoff is also a primary source of microorganisms in rivers and lakes. When runoff discharges to surface water catchments, there is a possible risk to public health linked to microbial contamination. *Escherichia coli*, *Streptococci* and *Enterococci* can reach surface water catchments as a result of surface runoff. Besides, viruses and protozoans such as *Giardia lamblia* and *Cryptosporidium* have been informed to reach surface water by runoff (Horner 1994).

Nationwide Urban Runoff Program (NURP) in the USA reported that urban runoff has high levels of fecal coliform concentrations at 17 different sites for 156 storm events. These concentrations can be expected to override EPA water quality in many surface water. This report concluded that (i) a dramatic seasonal effect on coliform levels and (ii) coliform counts in urban runoff during warmer periods of the year were found to be approximately 20 times greater than those observed during colder periods (Makepeace *et al.* 1995). A study by Clark and Pitt (2007) reported that a great percentage of fecal coliforms are found as a result of water runoff. *E. coli* and *fecal streptococci* were monitored in 95.5% of municipal storm sewer system outfalls. Another study documented that a large group of pathogens in water runoff and urban streams included *fecal streptococci*, *fecal coliform*, *Salmonella*, *Enterococci*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Field *et al.* 1993). In addition to the bacteria, enteroviruses (*Coxsackie virus*, *Echovirus*, and *Poliovirus*) were present in water runoff and urban streams.

It is generally agreed that the soil can act as a filter with the potential of self-purification to prevent the transmission of disease-carrying microorganisms from topsoil to groundwater resources during passage through the vadose zone and aquifer. Nevertheless, pathogens have commonly been found to occur in shallow and deep groundwater wells (WHO 2011). As well, waterborne outbreaks and foodborne diseases have been related to contamination of groundwater supplies by the microbial organism (Bradford *et al.* 2015). Different studies have reported that groundwater supplies are accountable for a significant fraction of the waterborne disease globally as a consequence of microbial contamination and the lack of adequate disinfection processes (Mesquita and Emelko 2012).

Microbial contamination can occur at different levels in groundwater. Such contamination by pathogens depends on the soil properties, adsorption process, and the ability of the pathogen to survive. Bacteria and viruses can be transported through soil media to reach groundwater by an infiltration process. The transport of bacteria depends on density and water velocity through the soil (Unice and Logan 2000). A review by Balke and Zhu (2008) shown that pathogens have been transferred to the subsurface water by the leak of contaminated water. These include bacteria such as *Escherichia coli*, *Vibrio cholerae*, *Streptococcus*, *Salmonellae*, and *Legionellae*, as well as pathogenic viruses like hepatitis-A and B, and poliomyelitis. The fate and transport of fecal bacteria are extremely associated with the dominant transport processes (Gao *et al.* 2011), whereas the transport of viruses is largely influenced by the attachment to mineral surfaces and inactivation (Soupir and Mostaghimi 2011).

Stevenson *et al.* (2015) investigated the fate and transport of bacteriophage in fine granular limestone aquifer material sampled from a borehole at a managed aquifer recharge site in Adelaide, South Australia. The findings recommend that a specific surrogate may not represent a particular pathogen solely based on similar size, morphology, and surface charge as it may be dependent on site-specific conditions. PRD1 bacteriophages was found to be the most suitable surrogate for adenovirus in an aquifer dominated by calcite material, yet not under high ionic strength or high pH conditions.

A technical report by the British Geological Survey (2009) shows that although protozoan and metazoan pathogens like *helminthic parasites* can probably be removed by filtration mechanisms, the *Cryptosporidium* at the oocyst phase is an exceptional. *Cryptosporidium* sized between (4-6 μm diameters) is larger than bacteria, which together with viruses may be transported with percolating effluent to groundwater through the soil and rock pores.

In the United States, a review by Macler and Merkle (2000) collected scientific facts about the occurrence of pathogens in U.S. groundwater based on 244 samples of public water-supply wells. This report displays that roughly 50% of wells are primarily considered more vulnerable to microbial contamination. Then, 40% of wells are considered less vulnerable, while they are positively tested for one or more faecal indicators such as total coliform bacteria, *Enterococci*, *E. coli*, viruses infecting coliform bacteria, and human viruses (e.g. enterovirus, rotavirus, and adenovirus).

Numerous research works have demonstrated that the inadequate application of manures can lead to groundwater contamination when pathogenic bacteria leach downward through the soil profile (Hooda *et al.* 2000, Unc and Goss 2004, Singh *et al.* 2018, Thorup-Kristensen *et al.* 2020). For instance, *E.coli* can migrate more than 800 meters through the soil profile and survive up to 4 months in aquifers (Cheng *et al.* 2022). Consequently, the transport of pathogenic through the soil must be identified as potential contamination of water resources. Several studies suggest designing a carbon-rich pours material for reducing the transport of *E. coli* 0157: H7 through the sandy soil (Alegbeleye and Sant'Ana 2020). By having adequate knowledge of how the bacteria can move in the soil, we can design more efficient agricultural practices with limited environmental harm. Several researchers have the idea that the soil can act as a filter with the potential for self-purification that blocks the transmission of disease-carrying microorganisms from topsoil to groundwater wells. Table 2 shows the survival times of excreted pathogens in freshwater and sewage at 20-30°C.

Table 2. Survival times of excreted pathogens in freshwater and sewage at 20-30°C (Adapted from (Feachem *et al.* 1983))

	Pathogen	Survival time (days)
1.	Viruses[a]	
	Enteroviruses[b]	<120 but usually <50
2.	Bacteria	
	Faecal coliform[a]	<60 but usually <30
	Salmonella spp.[a]	<60 but usually <30
	Shigella spp.[a]	<30 but usually <10
	Vibrio cholera[c]	<30 but usually <10
3.	Protozoa	
	Entamoeba histolytica cysts	<30 but usually <15
4.	Helminths	
	Ascaris lumbriocoides eggs	Many months

a. In seawater, viral survival is less, and bacterial survival is very much less than in freshwater

b. Includes polio-, echo-, and coxsackieviruses.
c. *V. cholera* survival in aqueous environments is still uncertain.

6. Conventional and Advanced Methods for Pathogen Removal from Wastewater

The pathogen removal from wastewater effluent is a first step to protect human health, water resources and environmental milieus. There are distinctive approaches used to remove or inactivate microorganisms "*disinfection*." Of these, chlorination, ozonation, ultraviolet light, membrane filtration, photo-Fenton, and electrokinetic processes such as electro-disinfection, electro-chlorination, and electro-Fenton processes are considered in the following discussion.

6.1 Chlorination

Chlorination is a chemical oxidation process where chlorine is applied in different forms to destroy number of pathogens. Chlorine can be added as pure chlorine (Cl_2), chlorine dioxide (ClO_2), or chlorine compounds such as sodium hypochlorite (NaOCl) and calcium hypochlorite [$Ca(OCl)_2$]. When chlorine reacts with water or wastewater, a mixture of hypochlorous acid (HOCl) and hypocaloric acid (HCl) is shaped as follows:

$CL_2\,(g) + H_2O \leftrightarrow HOCl + H^+ + Cl^-$ (1)

Chlorine disinfection is a very complicated series of events. Several factors determine the performance of chemicals used as a disinfectant agent. Of these, the contact time (CT), concentration or dose of disinfectant used (mg/L), mixing efficiency, temperature, relative humidity, pH level, water hardness, combined or free residual chlorination, and the existence of interfering substances that may decrease the effectiveness of the disinfectant are the most important factors (Rutala and Weber 2008, Chemat and Khan 2011). The reaction of chlorine is pH dependent; thus by reducing the pH of the water, the hypochlorous acid will dominate. HOCl acid is a weak acid, and nearly no dissociation will take place at pH below 6. At pH between 6.5 and 8.5, both hypochlorous acid and hypochlorite (-OCl) can be present in the solution, which are the significant disinfecting agents in chlorinated water.

$HOCl \leftrightarrow H^+ + OCl^-$ (2)

$Pk = 7.537 \text{ at } 25\ °C$

Chlorine is a universal disinfecting agent used, reacting with any organic substances present in water and wastewater. It is a strong oxidant and very toxic. Chlorine gas is the most economical form of chlorine normally used in larger treatment plants, which makes it an attractive option irrespective its health consequences (Chahal *et al.* 2016). When chlorine gas is added to water, it changes rapidly into hypochlorous acid and hypochlorite ions and hence their harmful effect on human health is reduced. Chlorine dioxide is another powerful oxidant that can oxidize iron and manganese as well as eliminate any color substances in the effluent (Ye *et al.* 2013). Chlorine dioxide is of cost-effective technique and has the advantage of not forming any chlorinated DBPs (Simoes and Simões 2013, Ling and Husain 2014). When chlorine dioxide comes into contact with water, it forms chlorite and chlorate as end-products. Such compounds are linked to human health risks (Cornwell and Davis 2012). Sodium hypochlorite also recognized as household bleach is made up of sodium salts and chlorine-containing compounds. It is the easiest form of chlorine to handle, and the required amount for treatment is much less than other types of chlorine. Calcium hypochlorite is an inorganic compound consisting of lime and calcium chloride. It is available as chlorine powder, tablets, and granules (Basu *et al.* 2007, Bester 2015).

The reaction of chlorine with ammonia to yield a range of mono-chloramine and dichloramines are of great importance in the chlorination processes. These produced compounds serve as less efficient disinfectants (Naidoo and Olaniran 2013). The formation of such complexes is dependent on temperature, time, pH, initial Cl_2: NH_3 ratio, and numerous other factors. The reaction between chlorine and ammonia is given as follows (Cornwell and Davis 2012):

$NH_3 + HOCl \leftrightarrow NH_2Cl + H_2O$ (Monochloramine) (3)
$NH_2Cl + HOCl \leftrightarrow NHCl_2 + H2O$ (Dichloramines) (4)
$NHCl_2 + HOCl \leftrightarrow NCl_3 + H_2O$ (Trichloramines or nitrogen trichloride) (5)

Nonetheless, one of the main drawbacks associated with chlorination processes is the formation of end-products like trichloromethanes (THMs) and other chloramines, some of which are very toxic to aquatic life (Hrudey 2009, Xie 2016). Such compounds create harmful effects on the receiving water bodies and may impose dechlorination before discharging. All types of chlorine are also highly corrosive and toxic, and hence its transporting and handling large amounts of such materials pose a health risk (Water 1999).

6.2 Ozonation

Ozonation is a chemical treatment process where ozone gas is used to destroy bacteria and other pathogens within water and wastewater. It is commonly agreed that the bacteria destruction through ozonation happens directly as a result of cell wall breakdown "*cell lysis*". Ozone (O_3) is an unstable gas consisting of three oxygen atoms, generated by subjecting oxygen molecules to a high voltage electrical field (Summerfelt 2003). When ozone reacts with water or wastewater, a complex chain of reaction kinetics results in the production of hydroxyl radicals (·OH). Hydroxyl radical is typically known as the most reactive aqueous and a strong oxidizing agent that may interact with various organic impurities (Dohare and Sisodia 2014, Hasan *et al.* 2014, Senghor *et al.* 2015). The hydroxyl radical is more potent than ozone itself. Due to its instability nature, ozone gas must often be produced at the point of use *"onsite."*

$O_2 + \text{Energy} \rightarrow O + O$ (6)
$O_2 + O \rightarrow O_3$ (7)

Ozone is a powerful oxidant alternative to chlorine, and it does not form any chlorinated DBPs or leave any residual behind. Ozone is a short-lived gas "*typically less than 30 minutes*", and its lifetime depends on water quality parameters such as pH, dissolved organic carbon (DOC) concentration, and others factors (Hansen *et al.* 2016). It is usually more potent than hypochlorous acid, reacting efficiently with a range of natural substances existent in the wastewater. Ozonation has been revealed to be more successful than chlorination in destroying viruses and cysts (Cornwell and Davis 2012). A widespread use of ozone is for the control of taste and the elimination of any odors as well as color-producing agents (Burton *et al.* 2013). Previous studies have shown the ability of ozone to oxidize pollutants, eliminate some natural organic matter (NOM) and color, control odor and taste as well as remove pathogens (Chen and Wang 2014). Yet, one of the main drawbacks associated with ozone application is the high cost of treatment (Water 1999), making its application for wastewater is not economical.

6.3 Ultraviolet light

Ultraviolet (UV) light is a means of disinfection that has become increasingly used due to its high effectiveness to inactivate or kill numerous species of microorganisms-causing disease. It provides rapid, more efficient inactivation of pathogens through a physical process. Ultraviolet radiation for water and wastewater disinfection provides a set of unique advantages over conventional chemical treatment (Würtele *et al.* 2011). UV treatment as an example can eliminate the need to handle, transport and store toxic chemicals. It also does an excellent job and has a better removal efficiency of microbial pathogens involving those accountable for cholera, hepatitis and other bacteria, typhoid, polio, viral and parasitic diseases. It has revealed a great ability to disinfect *Cryptosporidium parvum* (Wang *et al.* 2011). Furthermore, UV disinfection in combination with hydrogen peroxide, a process so-called UV-oxidation, can destroy chemical contaminants such as pharmaceuticals compounds, industrial solvents, and pesticides. Such process usually uses high dosages (600–2000 mJ/cm^2) of UV light in conjunction with H_2O_2 (2–15 mg/L) to produce hydroxyl radicals (Bounty *et al.* 2012). UV process has an advantage of reducing the disinfection by-products (DBPs) formation and hence, diminished human hazards and reduced environmental toxicity (Song *et al.* 2016).

The ultraviolet light disinfection takes place by rendering pathogenic microorganisms to unable them of reproducing and infecting. This losing of replication capability is performed through the formation of thymine dimers. The creation of such compounds disrupts and causes damage to genetic molecules (i.e. nucleic acids: DNA or RNA) in cells. It also prevents replication, survival, and the ability to infect. When genetic material absorbs UV light, it will be damaged and will not be able to duplicate. As a result, if a microbe cannot replicate, no infection can arise. Nevertheless, if the lower dosage of UV light is used, microorganisms have a tendency to repair the photochemical damage through different mechanisms as photorepair (photoreactivation), dark repair (nucleotide excision repair) or recombination repair (Naidoo and Olaniran 2013, Bohrerova *et al.* 2014). Many studies have documented that some microorganisms, in particular *E. coli*, have a potential capability to healing the UV disinfection damage (Bohrerova *et al.* 2014).

However, previous research works have revealed that a dose higher than 20 mJ/cm^2 will prevent any possibility for photoreactivation. Thus, an optimal wavelength is a key factor for pathogen inactivation, and the efficiency may differ from one microorganism to another (Vilhunen *et al.* 2009). Furthermore, other factors have to be taken into account for the effectiveness of the processes such as effluent quality, type of wastewater being treated, UV light intensity, and exposure or contact time. The inactivation of microorganism via UV light is associated with applied UV dosage. The average of UV dosage can be calculated as follows (Cornwell and Davis 2012):

$$D=It \quad (8)$$

Where,
D = UV dosage
I = average intensity, mW/cm^2
t = average exposure time, s

6.4 Membrane filtration

A membrane process can be defined as a thin film that is able to separate materials as function of their characteristics when a driving force is supplied across the membrane surface area. It is a selective barrier that does not allow certain materials to pass through, while it allows some particles of smaller size to get through (Davis and Cornwell 2013, Paulen and Fikar 2014). Membrane processes have been increasingly implemented for physical removal of microorganisms such as algae, bacteria, and viruses. For instance, Hai *et al.* (2014) reported that under steady state operation membrane bioreactors (MBR) have the ability to remove a number of viruses and phages. Shang *et al.* (2005) stated an excellent removal for *E. coli* and fecal coliforms using MBR system. The membrane process is divided into four main categories based on pore and particle size. Those include microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). Among of these, microfiltration (MF) and ultrafiltration (UF) membranes (pore size ranging from 0.05 to 0.4 μm) have been extensively employed for various effluent wastewater treatment applications (Le-Clech *et al.* 2006, Ramesh *et al.* 2006). However, membrane fouling has been considered a critical issue and a main obstacle facing the extensive application of membrane process for treating of effluent wastewater.

6.5 Photo-Fenton

The photo-Fenton is an advanced oxidation process (AOP) that utilizes the hydroxyl radical to disinfect water and wastewater. The produced hydroxyl radical by Fenton reaction is a very robust oxidant agent that allows for the removal of a wide array of microorganisms involving those with antimicrobial resistance (Anjali and Shanthakumar 2019). The process depends on the optimization of several parameters such as pH, temperature, hydrogen peroxide and iron concentrations as well as the intensity and wavelength of light irradiation, which are essential to accomplish an effective photo-Fenton process (Guimaraes *et al.* 2019, O'Dowd and Pillai 2020). The photo-Fenton process combines Fe^{2+}, H_2O_2 and UV irradiation. The process is basically an enhancement or improvement of the traditional Fenton reaction to increase the hydroxyl radical production and to rapidly reduce Fe^{3+} back to Fe^{2+} (Yamal-Turbay *et al.* 2013, Giannakis *et al.* 2017, Abeledo-Lameiro *et al.* 2019). The benefits of the photo-Fenton AOP in comparison with the conventional Fenton process is that the operating costs are substantially lower due to the absence of the filter press(es) and a much higher degradation rate of the photo-Fenton oxidation. Moreover, unlike a Fenton process, the photo-Fenton process can be easily automated, thereby allowing to a wide installation of a Fenton plant.

6.6 Electro-disinfection

Several terms have been used to refer to such a treating process or water produced by this process. To name but a few, electrochemical disinfection, electro-disinfection, electrolytic disinfection, anodic oxidation, electrochemically activated water, and functional water are being used to describe this type of water disinfection method. In general, electro-disinfection is a robust technology where microorganisms are inactivated or killed using an electric current applied between at least two inserted electrodes in the aqueous solution being treated (Kraft 2008). This inactivation of microorganisms takes place either directly or through the formation of very active oxides species, for example, hydroxyl radicals and other ions ($OH^{\cdot}$, $HO_2^{\cdot}$, O, Cl_2, OCl^-, and among others).

The electrochemical disinfection process can be attained with either direct current (DC) or alternating current (AC), with the frequency in a range of 0.5 ~ 800 Hz and an operating time of 5 ~ 60 minutes. A number of electrode materials have been in use including stainless steel, graphite fibers, carbon cloth, titanium, platinum, and silver (Jeong *et al.* 2009). Sometimes salt additives such as sodium chloride (NaCl) and sodium bromide (NaBr) are added to improve the performance of the process.

In electro-disinfection, electrodes are submerged either directly into the volume of mixture to be treated or into a bypass pipe. An external DC power source is connected to electrodes, leading to the electrolysis of the water. Oxygen is formed at the anode, while the key product formed at the cathode area is hydrogen (Ghernaout and Ghernaout 2010). According to such reactions, the pH around the cathode area increases and the solution becomes alkali, whereas the pH around the anode area decreases (Balasubramanian and Srinivasakannan 2010).

At the anode:

$2H_2O \rightarrow O_2 + 4H + 4e^-$ (9)

At the cathode:

$2H_2O + 2e^- \rightarrow H_2 + 2OH^-$ (10)

However, the production of hydrogen is usually undesirable and it must be removed from the water stream. Currently, an electro-disinfection process that entirely prevents hydrogen formation has been established. Atmospheric oxygen is reduced to hydroxyl ions at a gas diffusion cathode.

$O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$ (11)

Another mechanism for electro-disinfection is the formation of the disinfectant hypochlorous acid and hypochlorite ions. In the electro-disinfection process, also called electro-chlorination process, chlorine is generated electrochemically from chloride ions on the anode surface by two-electron transfer. Afterwards, the chlorine exists as hypochlorous acid and/or hypochlorite according to pH equilibrium (Choi *et al.* 2013).

$2Cl^- \rightarrow Cl_2 + 2e^-$ (12)
$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$ (13)
$HOCl \leftrightarrow H^+ + OCl^-$ (14)

The aforementioned reaction mechanism can be summarized as follows. First, chloride ions are oxidized to form chlorine dissolved in the water. Second, chlorine hydrolysis in water and hypochlorous acid (HClO) is shaped. Then, HClO and the hypochlorite anion (–OCl) form a pH-dependent equilibrium (Kraft 2008, Ghernaout and Ghernaout 2010). Yet, one of the main disadvantages of electrochemical technologies is the prohibitive costs, which relies on the energy consumption (operating), and the need of frequent electrode replacement as well as the high cost of the material being used as electrodes (Llanos *et al.* 2014).

7. Regulatory Standards for Wastewater Effluent Quality

The goal of wastewater treatment plants is to generate appropriate effluent for both agricultural and aquacultural reuse in addition to yield an effluent that can be discharged securely into lands, surface and coastal waters, crop irrigation, and groundwater recharge. Effluent quality standards would be necessary to reduce and eliminate negative impacts on human health and biological resources. It has been defined as a restriction on quantities of effluent discharges, rates of discharge, and concentrations of wastewater effluent material, and that is typically related to government and environmental agencies in a country and is based on the criteria of surface water quality, hydrodynamic conditions and characteristics of the receiving environments. These agencies constitute and define the principal legislation which is set to meet statutory laws.

The quality of effluent can be achieved by the measurement and monitoring of several parameters including dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total phosphorus (TP), total nitrogen (TN), total organic carbon (TOC), and total suspended solid (TSS), while most countries do not espouse effluent standards for all these parameters (von Sperling and de Lemos Chernicharo 2002). To our days wastewater has posed a significant threat to water catchment and agricultural soil and thus the quality of the effluents must be controlled, particularly with respect to BOD, TOC, TSS, and COD. Nutrients including phosphate, nitrate, and ammonium must be guided. Toxins such as heavy metals, phenols, solvents, and chlorinated compounds must also be tracked. The criteria of these parameters for effluent quality are different in terms of various uses of treated wastewater such as agricultural irrigation, aquifer recharge, industrial processes, and potable uses… etc. (Bakopoulou *et al.* 2011). These regulations and norms are extended to include microbial effluent quality. Such these guidelines were constituted by WHO (1989), which emphasized that a series of wastewater stabilization ponds be required to meet microbial standards of effluent quality. However, it is not in all cases standards can be applied. In some cases, these regulations and norms have to be modified. For example, Abu-Rizaiza (1999) refeed to the guidelines of the effluent of Saudi Arabia are rigid, and they impose excessive limitations on wastewater re-use and disposal. He concluded that: (i) the restricting effluent standards have prevented valuable water reuse in Saudi Arabia, and (ii) the idealistically high requirements could also discourage the development of wastewater treatment facilities. From another perspective, Blumenthal *et al.* (2000) proposed recommendations for revising WHO guidelines with respect to the microbiological quality of treated wastewater. They indicated that the norms of nematode eggs for both restricted and unrestricted irrigation is acceptable unless if conditions favor the survival of nematode eggs and where there are directly exposed to children under the age of 19. In such cases, the standards should be strength, and the criterion of nematode eggs should be reduced from < 1 egg/L to 0.1 egg/L.

8. Conclusions

Pathogens are one of the key risks affecting water resources and public health. Wastewater treatment plants are mainly aimed to reduce dangers associated with public health and the natural environment. Even though technics advance in wastewater treatment, pathogens still pose the main risk to public health worldwide, and as a result, the quality of the effluents must be controlled. This review illustrated that source of waters can be subjected to contamination to various degrees with pathogens. Owning of their presence and perseverance in water is attributed to a group of factors such as survival and transport. Infiltration depends on the type of water and aquifer characteristics such as properties of soil and adsorption to the soul components. The same is related to mineral suspended material in surface water. In the case of surface water, runoff is a major source of microorganisms in rivers and lakes. Furthermore, studies illustrated that pathogens can survive for various periods in wastewater treatment plants, effluent and sludge. This is referred to some factors such as a type of microorganisms and environmental factors. In summary, understanding the adverse environmental influences posed by untreated properly or insufficiently effluent can lead to achieve high efficiency in treating wastewater processes. Thus, understanding of survival and transport of microorganisms can result in accomplishing satisfactory effluent quality for wastewater treatment, and consequently prevent conveying of those contaminants to the natural environment. Persistence and survival, and then transport of pathogens into water resources are important issue that needs continuous attention.

9. Conflict of interests

The others declare that there is no conflict of interests regarding the publication of this article.

10. Acknowledgments

The authors express their gratitude to the Isra University (Amman, Jordan) for their support in carrying out this study. The authors also would like to thank Mr. Billa Cyprian for his helpful comments.

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