

Removal of Organisms in Domestic Wastewater by Different Treatment Processes for Reuse Purpose

Delia Teresa Sponza

Dokuz Eylül University, Engineering Faculty, Environmental Engineering Department, Buca, İzmir Turkey.

***Corresponding Author:** Delia Teresa Sponza, Dokuz Eylül University, Engineering Faculty, Environmental Engineering Department, Buca, İzmir Turkey.

Received Date: May 25, 2023; Accepted Date: June 08, 2023; Published Date: June 15, 2023.

Citation: Delia T. Sponza, (2023), Removal of Organisms in Domestic Wastewater by Different Treatment Processes for Reuse Purpose, *International Journal of Clinical and Medical Case Reports*, 2(3); **Doi:**10.31579/2834-8664/025

Copyright: © 2023, Delia Teresa Sponza. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract:

Due to limited water sources, the industrial and municipal wastewaters should be reused and can be used again after suitable treatment processes. In order to use effectively the reused waters for example for irrigation or for cleaning or for cooling water and washing water the microorganisms present in the water should be eliminated. Conventional treatment processes like GAC and activated sludge can not be treated effectively the pathogenic viruses, bacteria, protozoa and worms of which are released from the human activities. 87%-89% yields was detected in this process. The MBR reactor yields for these organisms were 90-92% while with UF and RO 97-98% and 99, 100% removal efficiencies were detected, respectively. MS2 phage, E.coli, Vibrio cholera, Cryptosporidium, Giardia, Entamoeba, round worm (Ascaris lumbricoides), hook worm (Ancylostoma duodenale) and the whip worm (Trichuris trichura) were isolated and investigated their numbers in the influent and effluent of the four treatment process to determine their log₁₀ removals. The log₁₀ removals in conventional activated sludge process varied between 1.00 and 2.01 depending to the SRT ages. As the sludge ages were increased from 10 days to 20 and 30 days the log₁₀ removal of organisms increased from 0.98 to 1.1 log₁₀, from 1.30 and 1.70 Log₁₀ and from 1.73 to 2.01, respectively. In the MBR, as the VSS was increased from 10.000 mg/l to 20.000 and to 30.000 mg/l the log₁₀ removals of organisms increased from 1.2- 1.3 to 1.6 and 1.8 and to 2.02 and to 2.32. In the UF process as the pressure was increased from 10 bar to 15 and to 30 bar the log₁₀ removals of organisms increased from 1.8-1.9 to 2.18-2.30 to 2.63 and 2.93. In the RO as the pressure was increased from 15 bar 20 30 and 40 bar the log₁₀ removals of bacteria increased from around 2.26 to 2.87 and to 4.39. The conventional parameters also such as COD, DOC, TSS, TN and TP were removed with yields as high as 99%. The results showed that the RO effluent at a pressure of 40 bar can be reused effectively. As the reverse osmosis (RO) process is often included in the treatment train to produce high quality reuse water from treated effluent for potable purposes because of its high removal efficiency of many dissolved inorganic and organic contaminants, and importantly, it also provides an excellent barrier for pathogens. In order to ensure the continued protection of public health from pathogen contamination, monitoring RO process integrity is necessary.

Keywords: domestic wastewater; reuse; log₁₀ removal; ms2 phage; e.coli; vibrio cholera; cryptosporidium, giardia; entamoeba

Introduction

The excessive global food demand and enough sources of water necessitates the utilization of treated and untreated wastewater in agriculture. The wastewater is a valuable source and increases the range of crops when can be irrigated particularly in arid and semi-arid lands (1,2). The wastewater allowed farming to be done in the dry season when farmers could sell their produce at three to six times the monsoon season prices (3,4). Wastewater reliability also allows for multiple cultivation cycles and flexibility of crops planted (5,6). The increased productivity and related income/food supply gains allow farmers a more reliable livelihood with indirect benefits of using the income for education and improving health

conditions. This is also the group potentially at risk as the possible adverse health effects to farmers and consumers are well established (7). As part of the urban food-production systems, urban livestock contributes to cities' food security by providing meat and dairy products (8-12). In semi-arid countries, livestock production relies mainly on natural pasture, which is often limited or decreasing due to low precipitation. Reusing wastewater or faecal sludge for fodder production appears an important and comparatively low-risk avenue which can contribute to enhancing the resilience to climate changes and food insecurity especially of small and middle-sized cities in developing countries.

Diseases originated from the reuse of cleaned wastewater in irrigation

The most common diseases associated with wastewater and excreta are the diarrheic ones. Examples include several kinds of helminthiasis that are caused by intestinal infestation of parasitic worms. Helminthiasis are common where poverty and poor sanitary conditions prevail; under these conditions they can affect up to 90 per cent of the population (13). Ascariasis (produced by *Ascaris* worms) is the most common one and is endemic in Africa, Latin America, and the Far East. It is estimated that 133 million people suffer from high-intensity ascariasis infections, which often lead to severe consequences, such as cognitive impairment, severe dysentery or anaemia. Even though helminthiasis have a low mortality rate (for ascariasis nearly 10,000 persons per year), most of the people affected are children under 15 years old with problems of faltering growth and/or impaired fitness. Approximately 1.5 million of these children never attain expected growth, even if treated (14-18). Another common helminthiasis is Schistosomiasis that affects approximately 246 million people worldwide (19). It causes tens of thousands of deaths every year, mainly in sub-Saharan Africa. It is strongly related to unsanitary excreta disposal and the absence of nearby sources of safe water. Another important disease is cholera, caused by bacteria named *Vibrio cholerae*. These bacteria cause not only epidemics but are responsible for several pandemics. Cholera is strongly related to the use of polluted water for irrigation or to unsafe disposal of sludge and excreta. Major risks occur where there are large concentrations of people and hygiene is poor.

Other diarrheic diseases related to unsafe agricultural practices are salmonellosis, typhoid, shigellosis, gastric ulcers (caused by *Helicobacter pylori*), giardiasis and amoebiasis (20-22). In addition, skin diseases associated with contact with untreated water have been reported. Nail problems (koilonychia) characterized by spoon-formed nails have also been reported and are associated with the anaemia produced by hookworm infections which cause iron deficiency (23). However, it must be kept in mind that in developing countries with various disease exposure pathways, the comparative risk contribution from wastewater irrigation and contaminated crops has never been comprehensively studied.

Wastewater and excreta policies to control the unplanned reuse of wastewater where it is an ongoing practice are not only hard to implement but are even difficult to develop (24-26) because governments are faced with the trade-off between public health protection and the ethical question of whether to prevent wastewater farmers from cultivating with the only source of water that is accessible to them (27). The WHO, to assist in this decision-making process, has in recent years been giving consideration both to the limitations faced by developing countries in providing sufficient wastewater treatment to meet water-quality standards and the increasingly important livelihood dimension of wastewater use. This is reflected in the 2020 Guidelines. If a government concludes that the practice must be stopped, then it has to put in place a complex process for control, with few successful examples in practice. In almost all countries legislation exists, dating back several years or decades and referring directly or indirectly to the use of polluted water or wastewater for irrigation, which is always forbidden. Many countries have irrigation water-quality guidelines, but they do not always consider microbiological standards, and where wastewater use is permitted, the legislation requires that certain quality conditions are met. Such conditions usually follow the previous WHO Guidelines which recommended water-quality thresholds. Such regulations are not followed in practice for the many reasons mentioned above. A further factor is that wastewater irrigation usually takes place outside the officially recognized formal irrigation sector. As a result, most governments ignore the situation or have no other means than to adopt a laissez-faire attitude (28). Joint efforts by WHO, FAO and United Nations Environment

Programme (UNEP) to respond to this global situation, and to encourage resource recovery, resulted in an enforceable and achievable regulatory framework to support worldwide the reuse of wastewater, greywater and excreta in agriculture and aquaculture (29). These new Guidelines build on previous ones but are in their 2020 version much more supportive of the difficult sanitation conditions in most developing countries and have suggested a multiple barrier approach for the long-term achievement of a universal health-based target. Furthermore, WHO suggests local adaptation of the Guidelines with incremental achievements towards this target. This flexibility means that authorities require support to understand and apply the new approach. The previous WHO Guidelines (30) are often considered more straightforward, especially for countries that already have comprehensive wastewater collection and treatment in place. The resulting bias towards countries at the lower part of the sanitation Ladder caused discomfort among those countries further up which have few problems in enforcing and monitoring crop or water-quality thresholds. These countries prefer to use, for example, standards similar to the California Title 22 (31). Such fixed standards are indeed most useful where they can actually be met by treatment, and wastewater use is a planned and controlled activity.

However, they are difficult to apply where treatment is rudimentary or lacking and when thousands of farmers already use polluted water sources because they have no alternative. Here, different strategies for health-risk reduction are needed. Similar regulations based on local needs and capabilities had been developed before the 2006 WHO Guidelines were released, e.g. in Australia (AATSE, 2019) and in Mexico in 1996 (32). The advantage of the WHO Guidelines is that all the developing countries that have ignored previous guidelines, because the water-quality thresholds were too high, are now challenged to control the health risks as far as possible, rather than continuing to disregard the problem. The same applies to excreta management which the WHO (2020) is also addressing. The agricultural use of treated, partially treated or untreated wastewater² or surface water contaminated with wastewater is common. An estimated 20 million Hectares worldwide are irrigated with wastewater, more of it with untreated than Treated wastewater (33). This misbalance in favour of untreated wastewater will continue to increase as long as the pollution of streams, by effluents from growing urban populations is not matched by treatment facilities. The increasing global scarcity of good-quality water will turn wastewater irrigation from an undesirable phenomenon into a necessity wherever agricultural water demand is not met by supply. This is not only the case in drier regions, but anywhere where farmers seek land and water to address market demand. Common examples are urban and peri-urban areas in most developing countries where clean water sources are hardly sufficient even to meet domestic demand. The use of untreated wastewater, or polluted water in general, poses risks to human health since it may contain excreta-related pathogens (viruses, bacteria, protozoan and multicellular parasites), skin irritants and toxic chemicals like heavy metals, pesticides and pesticide residues. When wastewater is used in agriculture, pathogens and certain chemicals are the primary hazards to human health by exposure through different routes. These exposure routes are mainly contact with wastewater (farmers, field workers and nearby communities) and consumption of wastewater-grown produce (consumers). In addition, contamination may be due to poor post-harvest handling that can also lead to cross-contamination of farm produce.

Treatments for health hazards during wastewater irrigation

The causative agents of excreta-associated infections are released from infected persons (or animals in some cases) in their excreta. They include pathogenic viruses, bacteria, protozoa and helminths of which are released from the bodies of infected persons (or animals in some cases) in their excreta (faeces or urine). The pathogens

eventually reach other people and enter either via the mouth (the faecal oral pathway, e.g. when contaminated crops are eaten) or via the skin (contact with infective larvae, e.g. hookworm infection and schistosomiasis).

Consumption of irrigated produce In relation to consumption-associated health risks, the primary concern is about vegetables eaten uncooked e.g. in raw salad dishes (34). Several studies including a prospective cohort study (35), an analytical descriptive study (22) and several descriptive studies including one done in Jerusalem (24) have shown higher *Ascaris* infections for both adults and children consuming uncooked vegetables irrigated with wastewater. Studies on the impact related to diarrhoeal diseases from consumption of contaminated vegetables have been published and reviewed extensively (21). The *Escherichia coli* strain enterotoxigenic *E. coli* (ETEC) is often Associated with diarrhoea (travellers' diarrhoea) in developing countries (23). In addition, viral enteritis (especially norovirus and rotavirus) and hepatitis A are the most commonly reported viral infections from vegetable consumption (24). Several diarrhoeal outbreaks have been associated with wastewater-irrigated vegetables (25, 36). However, in developing countries it is often a challenge to attribute Diarrhoeal outbreaks to specific exposure routes due to other contributing factors including poor hygiene, sanitation and reduced access to safe drinking water.

Health problems with wastewater use in agriculture

Not every hazard will end up causing illness and different hazards and Exposure pathways will result in different disease burdens. The relative importance of health hazards in causing illness depends on a number of factors. The ability of infectious agents to cause disease relates to their persistence in the environment, minimum infective dose, ability to induce human immunity, virulence and latency periods (37). Thus, pathogens with long persistence in the environment and low minimal infective doses that elicit little or no human immunity and having long latency periods (for example helminths) have a higher probability of causing infections than others. According to this, helminth infections, where endemic, pose the greatest risks associated with wastewater irrigation.

Risks from most chemicals are thought to be low, except in localized areas with large Industrial wastewater generation. Diseases associated with exposure to chemicals (aside from acute symptoms such as skin rashes, etc.), such as cancer, are harder to attribute to wastewater use in agriculture. This is because workers may be exposed to complex mixtures of chemicals in the wastewater and long latency periods before the disease symptoms appear, making it difficult to attribute the disease to any one specific exposure route or causal factor. The diseases of most relevance differ from area to area depending on the local status of sanitation and hygiene and the level to which wastewater is treated prior to use in agriculture. Most of these excreta related illnesses occur in children living in poor countries. The disease burden is measured in disability-adjusted life years (DALYs),³ which is increasingly becoming an essential unit in comparing disease outcomes from different exposures. More details on the use of DALYs are given in the following chapters. Overall, the WHO estimates that diarrhoea alone is responsible for nearly 3 per cent of all deaths and 3.9 per cent of DALYs worldwide (6-9). Diarrhoea is indeed a disease which can be largely attributed to environmental factors, such as unsafe drinking water, poor hygiene and sanitation, and the consumption of pathogen-contaminated crops. The question of how much of the disease burden can be attributed to poor sanitation, unsafe drinking water, poor hygiene and, in particular, to the consumption of wastewater-irrigated vegetables remains a challenging one.

There are not many comparative studies and those that exist only look at either Waterborne or foodborne pathways. Wastewater-irrigated food links both categories, but more importantly, many

factors are interwoven and not mutually exclusive. The large number of confounding factors makes any specific attribution to wastewater use difficult. One way to address the challenge is via microbiological risk assessment considering location-specific exposures.

Waterborne Pathogens

The majority of pathogens in wastewater are enteric, that is they affect the digestive system, and present a serious health risk if ingested (8-12). The adverse health effects of ingestion of pathogens are serious, and especially in the case of children under five, may be fatal if appropriate medical treatment is not administered in a timely manner. Protozoa are single-cell organisms that are important to public health because they cause life threatening diseases including giardiasis, cryptosporidiosis, dysentery and amoebic meningoencephalitis (26-28). Protozoan parasites are numerous in wastewater, including *Cryptosporidium*, *Giardia*, *Entamoeba* and *Microsporidia*. *Cryptosporidium* is highly resistant to chlorine-based disinfectants, and has been implicated in a number of gastroenteritis outbreaks around the world. Protozoa are able to survive outside their host under adverse conditions as cysts or oocysts that range in size from 3 to 14 µm in diameter (20-24). Helminths are larger multicellular organisms, which when mature can generally be seen with the naked eye. Helminth parasites commonly detected in wastewaters include the round worm (*Ascaris lumbricoides*), the hook worm (*Ancylostoma duodenale*) and the whip worm (*Trichuris trichura*). The most common microbial pathogens found in wastewater are bacteria (21-23).

These bacteria can be considered in two broad categories: enteropathogenic bacteria and opportunistic bacteria. Gastrointestinal diseases are one of the most common bacterial diseases contracted through wastewater (6-8). These include diarrhea (e.g., cholera caused by *Vibrio cholera* and salmonellosis caused by a number of *Salmonella* species) and dysentery (caused by various *Shigella* and *Salmonella* species). Other common Water diseases include typhoid and paratyphoid fever (caused by *Salmonella* species) (23-27). In addition to the established pathogens, a number of opportunistic pathogens (microorganisms causing infections and disease under optimal conditions, commonly in the very young, elderly and immune-compromised), including *Pseudomonas* and *Streptococcus*, can be found in wastewaters. Bacteria range from 0.6 to 1.0 µm in diameter and 2-3 µm in length (9). Viruses are considered as one of the most infectious pathogens common to wastewater due to their greater resistance to treatment and a smaller dose required to cause infection (19). More than 100 different viruses can be found in human feces (24). Enteroviruses, the most commonly detected viruses in wastewater, can cause paralysis, meningitis, respiratory disease, encephalitis and congenital heart anomalies, along with a range of other conditions with varying severity (29-30). Other human viruses in wastewater include coxsackie A and B, reovirus, norovirus, rotavirus, hepatitis A and E, adenovirus, echovirus and poliovirus, which can potentially cause upper respiratory and gastrointestinal illness (7, 37). Gastroenteritis is the most common wastewater related illness and can be caused by bacteria, virus or protozoa (36-38). The leading viruses responsible for gastroenteritis are rotavirus, calicivirus, enteric adenovirus and astrovirus (22). The size of different viruses ranges within a few tens of nm. For example, nominal size of hepatitis A, hepatitis E, calicivirus and astrovirus has been reported to be around 30 nm, while the nominal size of rotavirus and enteric adenovirus can be around 70 nm (26-28).

Indicator Organisms The wide variety of pathogens, including bacteria, viruses and protozoa present in most wastewater makes it impractical to test for each pathogen individually. Therefore suitable markers indicating microbial contamination are used. The indicator organisms themselves may not be pathogens. One widely used marker is the detection of coliform bacteria, either as total

coliforms or fecal coliforms. Coliforms are common inhabitants of ambient water and may be injured by environmental stresses (e.g., lack of nutrients) and water treatment (e.g., chlorine disinfection) in a manner similar to many pathogens. Fecal coliform has been shown to correlate strongly with the presence of fresh fecal matter (16-23,39). Possible indicators for protozoa suggested in the literature include aerobic spores, anaerobic spores and particle profiling. Similarly, particle profiling has been reported as a useful indicator for the removal of helminths from wastewater, with a high correlation observed between numbers of helminth ova and the volume of particles of 20–80 μm (28). Challenge testing of wastewater treatment processes for virus removal has been generally performed with model viruses having inactivation and adsorption behaviors similar to the native viruses under given conditions. Bacteriophages are viruses that infect specific bacteria and are widely considered to be process indicators for enteric virus removal or inactivation (26). A coliphage is a type of bacteriophage that infects *Escherichia coli* (a fecal coliform). Coliphages those attack *E. coli* through the “pilli” are referred to as “F-specific phage” or “Male-specific phage”, while those attacking through the cell wall are referred to as “Somatic phage”. MS2 coliphage (an F-specific phage) appears to be the most common virus. These characteristics are similar to some pathogenic human viruses found in water and wastewater, such as hepatitis A virus and poliovirus, and thus make MS2 a good indicator and surrogate for virus studies with membrane systems(29-34). T4 coliphage (a somatic coliphage) has also been used in bench-scale MBR studies since it is similar to adenoviruses, reoviruses, rotaviruses, and coronaviruses (32, 40-43).

In this study, the Log removal values (LRV) of some viruses, bacteria, protozoa and helminths in the effluents of conventional treatment processes with GAC and activated sludge, MBR reactor, UF and RO membrane processes were used to evaluate the process yields which the treated water whether reused for cleaning activities and irrigation purpose. The effects of SRT for activated sludge and, VSS concentrations in MBR treatment processes, and pressure increase on the yields of organisms, dissolved organic carbon (DOC) yields were investigated in UF and RO processes.

Materials and Methods

Reactor configurations

In this study 4 reactors namely conventional activated sludge process, MBR, UF and RO were used to detect the organism in the treated effluents which is going to be used as irrigation purpose. The MBR reactors surface was loaded with 45,000 mg/l heterogenic bacteria while UF membranes (FB02-FC-FUS1582: Daicel Membrane Systems Japan) have pore size 10 nm consisting of polymers including proprietary non-ionic polymers, polytetrafluoroethylene

(PTFE), polypropylene (PP), polysulphone (PSA spiral-wound RO reactor (SV021GV, Tokyo, Japan) with a membrane area of 0.13 m² and a molecular weight cut-off was 150,000 Da. RO is a hollow fiber reactor with a membrane area of 0.6 m² and a molecular weight cut-off of 190,000 Da.

Used organisms

MS2 phage, *E. coli*, *Vibrio cholera*, *Cryptosporidium*, *Giardia*, *Entamoeba*, round worm (*Ascaris lumbricoides*), hook worm (*Ancylostoma duodenale*) and the whip worm (*Trichuris trichura*) were isolated and investigated their numbers in the influent and effluent of the four treatment process to determine their log removals.

Log Removal of organisms

Pathogen removal is expressed in terms of log removal value (LRV), which is defined as follows:

$$\text{Log Removal} = \text{Log}_{10} (A) - \text{Log}_{10} (B);$$

Here, A is the number of organism before treatment while B is the number of microorganism after treatment.

If the log removal is equal to one then there is a 90% reduction in microorganisms. If the log reduction is two, then there is a 99% reduction, if three, then there is a 99.9% reduction and so on.

Regulations and guidelines for water recycling specify a target LRV that reduces the risk associated with exposure to the pathogen to a tolerable level. For example, the specified inactivation or removal efficiencies for various pathogens defined in Turkey. Environmental Protection Agency In Turkey mentioned limits to LRV limits which they were corresponded with 99% removal for *Cryptosporidium parvum*, 99.9% removal for *Giardia lamblia*, and 99.99% removals for viruses and bacteria (3-12).

Isolation of bacteria and measurement of all conventional pollutant parameters

All the organism isolations and pollutant analyses were performed according to Standard Methods(2022)(39,40).

Results and Discussion

Log 10 removal of organisms in conventional Activated sludge process

The effects of SRT on the yields of the organisms were tabulated in Table 1. As the SRT was increased from 10 days to 20 and 30 days the log 10 removals of all organisms increased from 1.00- 1.30 to 1.2-1.7 and to 1.7-2.01.

Organism names	Initial concentrations before treatment	SRT = 10 days Log removal (log 10 %)	SRT = 20 days Log removal (log 10 %)	SRT = 30 days Log removal (log 10 %)
MS2 phage (phage)	89 MPN/ml	1	1,7	2,01
<i>E. coli</i> (bacteria)	45x 10 ⁷ cfu/ml	1,1	1,3	1,9
<i>Vibrio cholera</i> (bacteria)	12x 10 ⁵ cfu/ml	1,2	1,3	1,8
<i>Cryptosporidium</i> (protozoan)	67 oocyst/ 100 ml	1	1,2	1,7
<i>Giardia</i> (protozoan)	67 oocyst/ 100 ml	1	1,3	1,65
<i>Entamoeba</i> (protozoan)	67 oocyst/ 100 ml	1	1,1	1,73
Covid virüs	34 MPN/ ml	1	1,2	1,45
round worm (<i>Ascaris lumbricoides</i>)	23 number/ gr	1	1	1,30
hook worm (<i>Ancylostoma duodenale</i>)	20 number/ gr	1	1	1,30
whip worm (<i>Trichuris trichura</i>)	13 number/ gr	1,1	1	1,28

Table 1: Variation of all organism log 10 removal percentageous versus SRT**Log 10removal of organisms in MBR**

the effects of VSS on the yields of the organisms were tabulated in table 2. As the VSS elevated from 10 days to 20 and 30 days the log 10 removals of all organisms increased from 1.00- 1.40 to 1.6-1.8 and to 2.11-2.32

Organism names	İnitial concentrations before treatment	VSS= 10.000 mg/l Log removal (log 10 %)	VSS= 20.000 mg/l Log removal (log 10 %)	VSS= 30.000 mg/l Log removal (log 10 %)
MS2 phage (phage)	89 MPN/ml	1	1,8	2,32
E.coli (bakteria)	45x 10 ⁷ cfu/ml	1,3	1,8	2,02
Vibrio cholera (bacteria)	12x 10 ⁵ cfu/ml	1,4	1,8	2,08
Cryptosporidium (protozoan)	67 oocyst/ 100 ml	1,2	1,6	2,11
Giardia(protozoan)	67 oocyst/ 100 ml	1,3	1,7	1,99
Entamoeba(protozoan)	67 oocyst/ 100 ml	1,3	1,8	1,99
Covid virüs	34 MPN/ ml	1,4	1,7	1,99
round worm (Ascaris lumbricoides)	23 number/ gr	1,4	1,65	2,03
hook worm (Ancylostoma duodenale)	20 number/ gr	1,3	1,6	2,30
whip worm (Trichuris trichura)	13 number/ gr	1,42	1,5	2,29

Table 2: Removals of all organisms in MBR versus VSS**Log removal of organisms in UF**

The effects of pressure n the yields of the organisms were tabulated in Table 3. As the pressure was increased from 10 bar to 20 and 30 bar the log 10 removals of all organisms increased from 1.80- 1.90 to 2.18-2.30 and to 2.78-2.93

Organism names	İnitial concentrations before treatment	Pressure = 10 bar, Log removal (log 10 %)	Pressure= 15 bar, Log removal (log 10 %)	Pressure= 30 bar, Log removal (log 10 %)
MS2 phage (phage)	89 MPN/ml	1,9	2,19	2,82
E.coli (bakteria)	45x 10 ⁷ cfu/ml	1,8	2,18	2,93
Vibrio cholera (bacteria)	12x 10 ⁵ cfu/ml	1,9	2,30	2,93
Cryptosporidium (protozoan)	67 oocyst/ 100 ml	1,8	2,28	2,78
Giardia(protozoan)	67 oocyst/ 100 ml	1,8	2,29	2,89
Entamoeba(protozoan)	67 oocyst/ 100 ml	1,9	2,23	2,63
Covid virüs	34 MPN/ ml	1,8	1,9	2,03
round worm (Ascaris lumbricoides)	23 number/ gr	1,4	1,89	2,47
hook worm (Ancylostoma duodenale)	20 number/ gr	1,3	1,99	2,40
whip worm (Trichuris trichura)	13 number/ gr	1,42	1,96	2,49

Table 3: Variations of log10 removals of all organisms versus pressure in UF**Log removal of organisms in RO**

The effects of pressure n the yields of the organisms were tabulated in Table 5. As the pressure was increased from 15 bar to 25 and 40 bar the log 10 removals of all organisms increased from 1.90- 2.38 to 2.68-2.89 and to 4.01-4.21

Organism names	İnitial concentrations before treatment	Pressure= 15 bar, Log removal (log 10 %)	Pressure = 25 bar, Log removal (log 10 %)	Pressure = 40 bar, Log removal (log 10 %)
MS2 phage (phage)	89 MPN/ml	2,26	2,89	4,03
E.coli (bakteria)	45x 10 ⁷ cfu/ml	2,38	2,68	4,01
Vibrio cholera (bacteria)	12x 10 ⁵ cfu/ml	2,01	2,87	4,21

Cryptosporidium (protozoan)	67 oocyst/ 100 ml	2,14	2,68	4,39
Giardia(protozoan)	67 oocyst/ 100 ml	2,16	2,79	4,89
Entamoeba(protozoan)	67 oocyst/ 100 ml	2,11	2,73	4,69
Covid virüs	34 MPN/ ml	2,19	2,23	4,21
round worm (Ascaris lumbricoides)	23 number/ gr	2,29	2,27	4,38
hook worm (Ancylostoma duodenale)	20 number/ gr	2,22	2,67	4,36
whip worm (Trichuris trichura)	13 number/ gr	2,18	2,69	4,43

Table 4: Variations of log10 removals of all organisms versus pressure in RO

Removals of all conventional pollutant parameters

Tables 5, 6, 7 and 8 summarize all the conventional pollutant parameters in all studied reactor types namely activated sludge, MBR, UF and RO. In activated sludge at the highest SRT the maximum yields was detected (88-89%). In MBR at the highest

VSS concentration of 30.000 mg/l the maximum pollutant yields was detected (89-91%). In UF at the highest pressure of 30 bar the maximum pollutant yields were obtained (94-96%) while in RO the maximum pollutant yields were detected as 99-99,99% at 40 bar pressure.

Pollutant parameters	Initial concentrations before treatment (mg/l)	SRT = 10 days, Removal efficiency (%)	SRT = 20 days, Removal efficiency (%)	SRT = 20 days, Removal efficiency (%)
COD	980	69	80	88
DOC	450	70	81	86
TSS	960	71	80	89
TN	56	68	82	87
TP	32	72	80	88

Table 5. Removals of conventional pollutant parameters in conventional activated sludge

Pollutant parameters	Initial concentrations before treatment (mg/l)	VSS= 10.000 mg/l, Removal efficiency (%)	VSS= 20.000 mg/l, Removal efficiency (%)	VSS=30.000 mg/l, Removal efficiency (%)
COD	980	73	86	89
DOC	450	74	87	90
TSS	960	74	86	91
TN	56	73	85	90
TP	32	74	83	89

Table 6. Removals of conventional pollutant parameters in MBR

Pollutant parameters	Initial concentrations before treatment (mg/l)	Pressure = 10 bar, Removal efficiency (%)	Pressure= 15 bar, Removal efficiency (%)	Pressure=30 bar , Removal efficiency (%)
COD	980	78	92	94
DOC	450	79	90	96
TSS	960	79	91	95
TN	56	79	90	95
TP	32	79	89	95

Table 7. Removals of conventional pollutant parameters in UF

Pollutant parameters	Initial concentrations before treatment (mg/l)	Pressure = 10 bar, Removal efficiency (%)	Pressure= 15 bar, Removal efficiency (%)	Pressure=30 bar , Removal efficiency (%)
COD	980	92	94	99,90
DOC	450	90	96	99,99
TSS	960	91	95	99,90
TN	56	90	95	99,89
TP	32	89	95	99,90

Table 8. Removals of conventional pollutant parameters in RO

Conclusions

Due to the nominal pore size of the UF and RO membranes used and the size of the coliform bacteria, membrane is considered the dominant mechanism for also the removal of coliforms and

pollutants by membranes. The pore size of common MF and UF membranes promises the removal of all bacteria from wastewater and no tertiary disinfection is required to adhere to the regulatory limit. Conversely, due to the much smaller size of viruses, there is much greater concern surrounding their removal by RO. Only the effluent (permeate) of the RO process can be reused for irrigation and clean water use.

International guidelines must therefore be practical and offer feasible risk-management solutions that will maximize health protection and facilitate the beneficial use of scarce resources. LRV correlated positively and significantly between all microorganism indicators and dissolved organic carbon (DOC), becoming a suitable monitoring technique for biological removal. LRV correlated use predominant removal mechanism. Strong correlations between LRV for protozoa, virus and bacteria indicators were established at optimum pressure,

TMP and MLSS, higher pH and temperature and longer solids times. The implications of this study provide important guidance for the validation of conventional activated sludge, MBR, UF and RO. Only RO produce safe and consistent recycled water. Strong correlations between LRV for protozoa, virus and bacteria indicators were established at optimum pressures, VSS and SRTs.

Information on the removal performances of RO would be helpful for estimating the health risks caused by organisms in the permeate, but unfortunately the information available is still insufficient. Information on the origins of organisms detected in the RO permeate is also important when considering the measures to be taken, which can change depending on the origin of the organism.

References

- Adham, S., Gagliardo, P., Smith, D., Ross, D., Gramith, K. & Trussell, R. Monitoring the integrity of reverse osmosis membranes. *Desalination* 119 (1), 143–150.
- Adham, S. S., Trussell, R. S., Gagliardo, P. F. & Trussell, R. R. Rejection of MS-2 virus by RO membranes. *Journal of American Water Works Association* 90 (9), 130–135. <https://doi.org/10.1016/j.seppur.2013.06.015>
- Ammor, M. S. Recent Advances in the Use of Intrinsic Fluorescence for Bacterial Identification and Characterization. *J. Fluoresc.* **2007**, 17 (5), 455–459.
- Ase, T. & Ohkouchi, Y. Time-dependent changes in bacterial count in permeate of spiral-wound RO modules and evaluation of factors related to their changes. *Journal of Antibacterial and Antifungal Agents* 48 (3), 101–110.
- Bellona, C., Drewes, J. E., Xu, P. & Amy, G. Factors affecting the rejection of organic solutes during NF/RO treatment – a literature review. *Water Research* 38 (12), 2795–2809.
- Buysschaert, B., Vermijs, L., Naka, A., Boon, N. & De Gussem, B. Online flow cytometric monitoring of microbial water quality in a full-scale water treatment plant. *Npj Clean Water* 1.
- Chen, W., Westerhoff, P., Leenheer, J. A., Booksh, K. Fluorescence excitation–emission matrix regional integration to quantify spectra for dissolved organic matter. *Environ. Sci. Technol.* **2003**, 37 (24), 5701–5710.
- DeCarolis, J., Adham, S., Kumar, M., Pearce, B. & Wasserman, L. Integrity and performance evaluation of new generation desalination membranes during municipal wastewater reclamation. *Proceedings of the Water Environment Federation* 3518–3529.
- Favero, M. S., Petersen, N. J., Carson, L. A., Bond, W. W. & Hindman, S. H. Gram-negative water bacteria in hemodialysis systems. *Health Laboratory Science* 12 (4), 321–334.
- Fujioka, T. & Boivin, S. Assessing the passage of particles through polyamide reverse osmosis membranes. *Separation and Purification Technology* 226, 8–12.
- Fujioka, T. & Boivin, S. Assessing bacterial infiltration through reverse osmosis membrane. *Environmental Technology & Innovation* 19, 100818. <https://doi.org/10.1016/j.eti.2020.100818>.
- Fujioka, T., Hoang, A. T., Aizawa, H., Ashiba, H., Fujimaki, M. & Leddy, M. Real-time online monitoring for assessing removal of bacteria by reverse osmosis. *Environmental Science & Technology Letters* 5 (6), 389–393.
- Fujioka, T.; Kodamatani, H.; Aizawa, H.; Gray, S.; Ishida, K. P.; Nghiem, L. D. Role of membrane fouling substances on the rejection of N-nitrosamines by reverse osmosis. *Water Res.* **2017**, 118, 187–195.
- Fujioka, T.; Oshima, N.; Suzuki, R.; Khan, S. J.; Roux, A.; Poussade, Y.; Drewes, J. E.; Nghiem, L. D. Rejection of small and uncharged chemicals of emerging concern by reverse osmosis membranes: The role of free volume space within the active skin layer. *Sep. Purif. Technol.* **2013**, 116, 426–432, DOI: 10.1016/j.seppur.2013.06.015
- Harris-Lovett, S. R.; Binz, C.; Sedlak, D. L.; Kiparsky, M.; Truffer, B. Beyond User Acceptance: A Legitimacy Framework for Potable Water Reuse in California. *Environ. Sci. Technol.* **2015**, 49 (13), 7552–7561.
- Henderson, L. W. & Beans, E. Successful production of sterile pyrogen-free electrolyte solution by ultrafiltration. *Kidney International* 14 (5), 522–525.
- Henderson, R. K.; Baker, A.; Murphy, K. R.; Hambly, A.; Stuetz, R. M.; Khan, S. J. Fluorescence as a potential monitoring tool for recycled water systems: A review. *Water Res.* **2009**, 43 (4), 863–881.
- Huang, X.; Zhao, Z.; Hernandez, D.; Jiang, S. Near Real-Time Flow Cytometry Monitoring of Bacterial and Viral Removal Efficiencies during Water Reclamation Processes. *Water* **2016**, 8 (10), 464.
- Kitis, M., Lozier, J. C., Kim, J. H., Mi, B. & Mariñas, B. J. Evaluation of biologic and non-biologic methods for assessing virus removal by and integrity of high pressure membrane systems. *Water Supply* 3 (5–6), 81–92. <https://doi.org/10.2166/ws.2003.0153>.
- Kumar, M., Adham, S. & DeCarolis, J. Reverse osmosis integrity monitoring. *Desalination* 214 (1), 138–149. <https://doi.org/10.1016/j.desal.2006.10.021>.
- Ledebo, I. On-line preparation of solutions for dialysis: practical aspects versus safety and regulations. *Journal of the American Society of Nephrology: JASN* 13 (Suppl. 1), S78–S83.
- Liu, T.; Chen, Z.-l.; Yu, W.-z.; You, S.-j. Characterization of organic membrane foulants in a submerged membrane bioreactor with pre-ozonation using three-dimensional excitation–emission matrix fluorescence spectroscopy. *Water Res.* **2011**, 45 (5), 2111–2121.
- Liu, G.; Lut, M. C.; Verberk, J. Q. J. C.; Van Dijk, J. C. A comparison of additional treatment processes to limit particle accumulation and microbial growth during drinking water distribution. *Water Res.* **2013**, 47 (8), 2719–2728.
- Lopez-Roldan, R.; Tusell, P.; Cortina, J. L.; Courtois, S.; Cortina, J. L. On-line bacteriological detection in water. *TrAC, Trends Anal. Chem.* **2013**, 44, 46–57.
- Lozier, J., Kitis, M., Colvin, C., Kim, J. & Mi, B. Microbial Removal And Integrity Monitoring Of High-Pressure Membranes. IWA Publishing, London.
- März, F., Scheer, R. & Graf, E. Microbiological aspects in the production of water for injection by reverse osmosis. *International Journal of Pharmaceutics* 58 (2), 155–164.
- Miller, S. E., Rodriguez, R. A. & Nelson, K. L. Removal and growth of microorganisms across treatment and simulated distribution at a pilot-scale direct potable reuse

- facility. *Environmental Science: Water Research & Technology*.
27. Miller, S. E.; Nelson, K. L.; Rodriguez, R. A. Microbiological Stability in Direct Potable Reuse Systems: Insights from Pilot-Scale Research Using Flow Cytometry and High-Throughput Sequencing. *Proceedings of the Water Environment Federation* **2017**, 2017 (14), 1016– 1023,
 28. Miles, S. L.; Sinclair, R. G.; Riley, M. R.; Pepper, I. L. Evaluation of Select Sensors for Real-Time Monitoring of *Escherichia coli* in Water Distribution Systems. *Appl. Environ. Microbiol.* **2011**, 77 (8), 2813– 2816,
 29. Mosher, J. J.; Vartanian, G. M.; Tchobanoglous, G. *Potable reuse research compilation: Synthesis of findings*; National Water Research Institute and Water Environment & Reuse Foundation: Fountain Valley, CA, 2016.
 30. Nam, S.-N.; Amy, G. Differentiation of wastewater effluent organic matter (EfOM) from natural organic matter (NOM) using multiple analytical techniques. *Water Sci. Technol.* **2008**, 57 (7), 1009– 1015,
 31. Ohkouchi, Y., Yata, Y., Bun, R. & Itoh, S. Chlorine requirement for biologically stable drinking water after nanofiltration. *Water Science & Technology: Water Supply* 14, 405.
 32. Park, S.; Hu, J. Y. Assessment of the extent of bacterial growth in reverse osmosis system for improving drinking water quality. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* **2010**, 45 (8), 968– 977,
 33. Pepper, I. L.; Snyder, S. A. *Monitoring for reliability and process control of potable reuse applications*; Water Environment & Reuse Foundation and IWA Publishing: Alexandria, VA, 2016.
 34. Pype, M.-L.; Lawrence, M. G.; Keller, J.; Gernjak, W. Reverse osmosis integrity monitoring in water reuse: The challenge to verify virus removal – A review. *Water Res.* **2016**, 98, 384– 395,
 35. Prest, E. I.; Hammes, F.; Köttsch, S.; van Loosdrecht, M. C. M.; Vrouwenvelder, J. S. Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method. *Water Res.* **2013**, 47 (19), 7131– 7142,
 36. Sorber, C. A., Malina, J. F. & Sagik, B. P. Virus rejection by the reverse osmosis-ultrafiltration processes. *Water Research* 6 (11), 1377–1388..
 37. *Standard Methods for Waster and Wastewater*, Newyork, USA (2022)
 38. Sherchan, S. P.; Gerba, C. P.; Pepper, I. L. Evaluation of Real-Time Water Quality Sensors for the Detection of Intentional Bacterial Spore Contamination of Potable Water. *J. Biosens. Bioelectron.* **2013**, 4 (4), 1– 5,
 39. Tchobanoglous, G.; Cotruvo, J.; Crook, J.; McDonald, E.; Olivieri, A.; Salveson, A.; Trussell, R. S. *Framework for direct potable reuse*; WateReuse Association, American Water Works Association, Water Environment Federation, National Water Research Institute: Alexandria, VA, 2015.
 40. Thomas, J. M. & Ashbolt, N. J. Do free-living amoebae in treated drinking water systems present an emerging health risk? *Environmental Science & Technology* 45 (3), 860–869.
 41. Van Nevel, S.; Koetzs, S.; Proctor, C. R.; Besmer, M. D.; Prest, E. I.; Vrouwenvelder, J. S.; Knezev, A.; Boon, N.; Hammes, F. Flow cytometric bacterial cell counts challenge conventional heterotrophic plate counts for routine microbiological drinking water monitoring. *Water Res.* **2017**, 113, 191– 206,

Ready to submit your research? Choose ClinicSearch and benefit from:

- fast, convenient online submission
- rigorous peer review by experienced research in your field
- rapid publication on acceptance
- authors retain copyrights
- unique DOI for all articles
- immediate, unrestricted online access

At ClinicSearch, research is always in progress.

Learn more <http://clinicsearchonline.org/journals/international-journal-of-clinical-and-medical-case-reports>



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.