


## Review

# Removal of Pathogens in Onsite Wastewater Treatment Systems: A Review of Design Considerations and Influencing Factors

Mian Wang <sup>1,2</sup> , Julia Zhu <sup>3</sup> and Xinwei Mao <sup>1,2,\*</sup><sup>1</sup> Department of Civil Engineering, College of Engineering and Applied Sciences, Stony Brook University, Stony Brook, NY 11794, USA; mian.wang@stonybrook.edu<sup>2</sup> New York State Center for Clean Water Technology, Stony Brook, NY 11794, USA<sup>3</sup> Ward Melville High School, Three Village Central School District, East Setauket, NY 11733, USA; zhujulia3@gmail.com

\* Correspondence: xinwei.mao@stonybrook.edu

**Abstract:** Conventional onsite wastewater treatment systems (OWTSs) could potentially contribute to the transmission of infectious diseases caused by waterborne pathogenic microorganisms and become an important human health concern, especially in the areas where OWTSs are used as the major wastewater treatment units. Although previous studies suggested the OWTSs could reduce chemical pollutants as well as effectively reducing microbial contaminants from onsite wastewater, the microbiological quality of effluents and the factors potentially affecting the removal are still understudied. Therefore, the design and optimization of pathogen removal performance necessitate a better mechanistic understanding of the hydrological, geochemical, and biological processes controlling the water quality in OWTSs. To fill the knowledge gaps, the sources of pathogens and common pathogenic indicators, along with their major removal mechanisms in OWTSs were discussed. This review evaluated the effectiveness of pathogen removal in state-of-art OWTSs and investigated the contributing factors for efficient pathogen removal (e.g., system configurations, filter materials, environmental and operational conditions), with the aim to guide the future design for optimized treatment performance.

**Keywords:** onsite wastewater; pathogen removal; filtration-based OWTS; water quality



**Citation:** Wang, M.; Zhu, J.; Mao, X. Removal of Pathogens in Onsite Wastewater Treatment Systems: A Review of Design Considerations and Influencing Factors. *Water* **2021**, *13*, 1190. <https://doi.org/10.3390/w13091190>

Academic Editor: Cristina Sousa  
Coutinho Calheiros

Received: 17 March 2021

Accepted: 21 April 2021

Published: 25 April 2021

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## 1. Introduction

More than 20% of the population in the United States rely on onsite wastewater treatment systems (OWTSs) to treat domestic wastewater [1]. Domestic wastewater contains nutrients, organic matter, suspended solids, and pathogens, which can cause a number of diseases through the oral consumption route, if not properly treated [1,2]. A conventional OWTS consists of a septic tank and a leaching field or a leaching pool, providing only primary treatment of wastewater, during which particles settle to the bottom of septic tanks, and are partially degraded. Septic tank effluent (STE) is further dispersed in a leaching field (i.e., a gravel or sand filter) or leaching pools from where it leaks into the surrounding soil and the aquifer. OWTSs have been designed for the removal of solids, organic compounds (i.e., biological oxygen demand (BOD)), and nutrients while the efficiency of pathogen removal has not been systematically evaluated [1,3,4]. Problems with the disposal of poorly treated wastewaters from OWTSs are aggravated in coastal areas and where groundwaters are shallow [5,6]. To protect water resources and human health, new treatment technologies have been developed to meet certain standards of water quality over recent years [1].

Microbial contaminant removal from onsite wastewater is important from a human health perspective, especially in the areas where OWTSs are used as the major wastewater treatment units [7]. Sanitation controls mostly involve the treatment of nutrients and organic matters, while the pathogens (bacteria and virus) removal efficiency of OWTSs is not the main focus and has been scarcely documented [8]. In addition, inadequately treated

onsite wastewater could contribute to the potential transmission of infectious diseases caused by waterborne pathogenic microorganisms. Therefore, there is a widespread need for OWTs with an optimized design that can reduce pathogen risks while being simple and affordable to build, maintain, and operate.

OWTs hold the promise to eliminate pathogens and other contaminants while offering the benefits of low cost and wide application [3,9,10]. However, few case studies or reviews have focused on the microbiological quality of effluents, and how operation conditions and design factors may affect the removal of pathogens (e.g., system configuration, filtration media, and seasonal impact, etc.). In addition, most research of advanced OWTs has not been well translated into the design and operation of OWTs for pathogen removal, due to the intertwined effects from different removal mechanisms. To optimize the system design and achieve the full potential of the OWTs for efficient pathogen removal along with nutrients and organic contaminant removal, a mechanistic understanding of the hydrological, geochemical, and biological processes controlling water quality in OWTs is needed. To fill the knowledge gap, this review evaluates the effectiveness of bacterial and viral pathogens removal by current OWTs and investigates the contributing factors that could potentially affect pathogens removal efficiency. This knowledge is beneficial to guide the design and operation of OWTs to minimize the risk of pathogenic contamination of groundwater and drinking water. Specifically, this review not only investigates the impact of common factors such as hydraulic retention time, pH and temperature, but also includes a thorough discussion regarding the OWTs configuration, filter materials, and operational conditions. Therefore, this review covers a comprehensive range of studies and provides a thorough discussion to guide the future designs of OWTs for a higher pathogen removal efficiency.

## 2. Materials and Methods

A systematic literature search of the peer-reviewed publications in Web of Science was conducted to evaluate the pathogen removal performance in OWTs. The search included the keywords (pathogen\* OR bacteria OR virus\*) AND (onsite OR on-site OR septic OR decentralized OR “sand filter\*” OR denitrifying OR filter\* OR bioreactors\* OR “constructed wetland\*” OR wetland\* OR lagoon\*). The literature search was limited to peer-reviewed publications written in English between 1990 to 2021. A total of 43,426 results can be searched from the database using the above keywords, and after full-text review, 78 references passed our criteria. Study inclusion criteria including the scope and data availability were applied to each publication. First, to be considered within scope, the article needed to have the following information: information on wastewater types, treatment configurations, types of indicator bacteria or virus, and operating seasons. Second, occurrence data for pathogens including bacteria, virus, protozoa, or helminth in wastewater influent and effluent were required for articles to pass the scope. Publications reporting only presence/absence data were excluded. In some studies, individual data points only available in figures were digitized by WebPlotDigitizer, version 4.4 (<https://automeris.io/WebPlotDigitizer/>, accessed on November 2020).

For each publication, the  $\log_{10}$  reduction and removal efficiency of different indicators were calculated using Equations (1)–(3) when the studies expressed the concentrations of pathogens using logarithmic notation.

$$\text{Log}_{10} \text{ reduction} = \log_{10} \left( \frac{C_{\text{in}}}{C_{\text{out}}} \right) \quad (1)$$

$$\text{Removal efficiency (\%)} = \left[ \left( \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{in}}} \right) \times 100 \right] \quad (2)$$

$$\text{Removal efficiency (\%)} = \left[ 1 - 10^{(\log_{10} C_{\text{out}} - \log_{10} C_{\text{in}})} \times 100 \right] \quad (3)$$

where  $C_{\text{in}}$  and  $C_{\text{out}}$  represent the influent and effluent pathogen concentration, respectively.

### 3. Sources of Pathogens and Fecal Indicators in OWTs

Domestic wastewater contains a variety of pathogens, including bacteria (e.g., coliforms, *Escherichia coli* (*E. coli*), *Salmonella*, streptococci, *Vibrio cholerae*, enterococci), viruses (e.g., enteric virus, norovirus, adenovirus, and rotavirus), protozoa (e.g., *Giardia*, *Cryptosporidium*), and helminths [11]. These pathogens are generally derived from the intestinal tract in human feces and are associated with waterborne diseases such as diarrhea, cholera, and dysentery [1,12].

It is cost-prohibitive and technically complicated to monitor all potential pathogenic microorganisms in the influents and effluents of OWTs on a regular basis. Therefore, microbial indicators are used for analytical feasibility and convenience to evaluate the levels of pathogens and indicate the extent of fecal contamination in the wastewater [13]. Coliform bacteria, including total coliforms (TCs) and fecal coliforms (FCs), are the most commonly measured pathogen indicators. Coliform bacteria are nonpathogenic but their detections in the water are generally accepted as the reliable indicators of fecal contamination [14]. *Escherichia coli*, a member of the FCs, has been widely used as a pathogen indicator. However, it is more sensitive to disinfection than other pathogens (e.g., viruses and protozoa) [15]. As a result, enterococci and fecal streptococci (FS) are also used as indicators due to their higher resistance to environmental stresses (e.g., temperature changes) and general longer survival times [15,16]. Their presence in wastewater may also signal the presence of human enteric pathogens such as hepatitis A virus [17]. These bacterial indicators have long been utilized as microbial measures of water quality, largely because they are easy to detect and enumerate in water. Different types of enteric viruses excreted in human feces have been detected in domestic wastewater [18,19]. Previous studies have demonstrated that viruses such as enteric adenovirus, rotavirus, human calicivirus, hepatitis A and E, and astrovirus in domestic wastewater are the leading causes of gastrointestinal and respiratory illness [20–22]. Although the relationship between enteric viruses and infectious diseases has been discovered for a long time, no regulation has been implemented to monitor the concentrations of viral pathogens in the wastewater treatment effluent prior to discharging into water bodies [23]. Other indicators such as F-specific RNA and somatic bacteriophages have been chosen as surrogate viruses as their size and morphology are similar to human enteric viruses [24,25]. F-specific RNA bacteriophages can infect bacteria through the F-pili, while somatic bacteriophages infect bacteria via the cell wall. Both bacteriophages are frequently found in human and animal feces, and are non-invasive to humans [15,26]. Therefore, they are widely accepted as indicators for human enteric viruses and are commonly used in studies on the removal of viruses in OWTs [25,27–29]. *Clostridium perfringens*, whose spores are used as the proxy for protozoan (oo)cysts, are also typical pathogen indicators in wastewater quality measurements [30]. Compared to protozoan (oo)cysts (generally around 5 µm in diameter), *C. perfringens* are more resistant to predation and inactivation, but less retained by filtration due to their smaller sizes ( $\leq 1$  µm in diameter) [31–33]. *Cryptosporidium* is also one of the most commonly detected protozoan parasites in domestic wastewater and has been implicated in numerous gastroenteritis outbreaks worldwide [34]. Additionally, helminth eggs represent the infective stage of helminths, which are excreted in feces and are prevalent in domestic wastewater especially in developing countries [35]. *Ascaris lumbricoides* is the most frequently tested helminth indicator for water quality as *Ascariasis* is one of the most common worms associated with excreta in low-income countries and the eggs of *Ascariasis* are more resistant than other helminth eggs (e.g., *Trichuris* and *Toxocara*) [36–38]. Table 1 provides a list of quantitative measures of the most commonly detected pathogen indicators' concentrations in domestic wastewater.

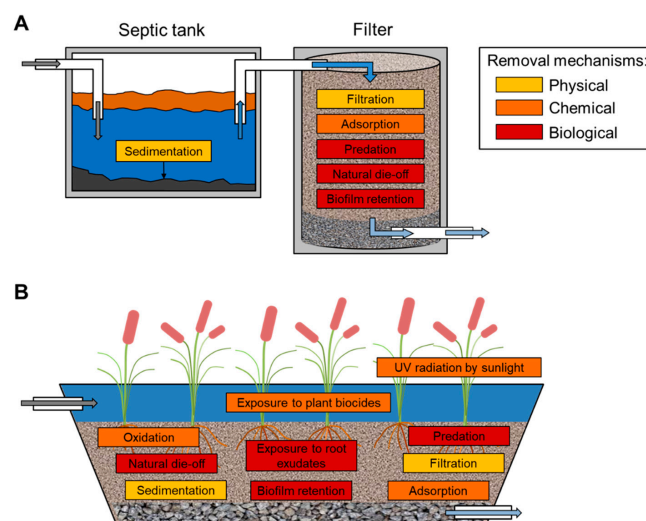
**Table 1.** The concentrations of indicator pathogens in domestic wastewaters.

Pathogenic Microorganisms	Concentration in Domestic Wastewater	Removal Efficiency by OWTs (%)	Reference
<b>Bacterial pathogen</b>			
Total coliform	4.4–8.6 log <sub>10</sub> CFU/100 mL <sup>a</sup>	84.15–99.99	[25,39–46]
Fecal coliform	4.1–7.9 log <sub>10</sub> CFU/100 mL	96.02–99.99	[10,24,25,30,40,45–48]
Fecal streptococci	3.1–6.1 log <sub>10</sub> CFU/100 mL	99.29–99.93	[41,44,47,49]
<i>Escherichia coli</i>	5.8–7.8 log <sub>10</sub> CFU/100 mL	73.91–99.99	[4,10,39,41–44,50–52]
<i>Salmonella</i>	4.7 log <sub>10</sub> CFU/100 mL	68.38–99.99	[4,48,53]
<i>Shigella</i>	1.0–3.8 log <sub>10</sub> CFU/100 mL	96.33–99.72	[4,54]
<i>Clostridium perfringens</i>	0–6.0 log <sub>10</sub> CFU/100 mL	93.69–99.96	[30,41,55]
<i>Pseudomonas aeruginosa</i>	3.8–5.0 log <sub>10</sub> CFU/100 mL	96.92–99.21	[39,55]
Enterococci	3.3–6.6 log <sub>10</sub> CFU/100 mL	80.05–99.99	[10,43,51,56]
<b>Viral pathogen</b>			
F-specific bacteriophage	2.8–6.3 log <sub>10</sub> PFU/100 mL <sup>b</sup>	36.90–99.99	[24,25,40,51,56,57]
Rotavirus	2.9–8.1 log <sub>10</sub> copies/mL	97.28–99.99	[51,54,58]
Norovirus genogroup I and II	3.1–8.9 log <sub>10</sub> copies/mL	73.80–99.99	[4,51,55,58–61]
Adenovirus	5.0–7.3 log <sub>10</sub> copies/mL	77.83–99.99	[51,58,60–62]
Enterovirus	7.6–7.8 log <sub>10</sub> copies/mL	98.73–99.99	[51,62]
<b>Protozoa</b>			
<i>Giardia</i>	0.6–4.9 log <sub>10</sub> cysts/L	99.91	[4,24,25,40,41]
<i>Cryptosporidium</i>	0–140 oocysts/L	99.87	[24,25,40,41]
<b>Helminths</b>	9.6–244 eggs/L	53.70–99.98	[41,44,47,63]

<sup>a</sup> The concentrations of bacteria are expressed using logarithmic notation, where the values shown in the table are the base 10 logarithm colony-forming unit (CFU) per 100 mL of samples. <sup>b</sup> PFU: plaque-forming unit.

#### 4. Fate and Removal Mechanisms of Pathogens in OWTs

The removal of pathogenic microorganisms in OWTs is accomplished through a combination of physical (e.g., sedimentation and filtration), chemical (e.g., adsorption to substrates or particles, UV radiation by sunlight, and exposure to root exudates), and biological (e.g., predation, natural die-off, and retention in biofilm) mechanisms [9,64–67]. Conventional OWTs (e.g., septic tank with cesspools/leaching fields), filtration-based OWTs, and constructed wetlands (CWs) are the most frequently used onsite wastewater treatment systems [1]. Filtration-based OWTs such as sand filters and peat filters provide a high level of treatment for chemical and microbial pollutants by filtering the STE through filters packed with different media [65]. CWs utilize a variety of processes including contaminant removal by vegetation, soil filtration, and biodegradation by associated microbial assemblages on the media surface to assist in treating onsite wastewater [3,68]. Wastewater flows from the septic tank and then enters the wetland cell where the wastewater can be treated by microbes, vegetations, and other media that remove pathogens and nutrients [9]. An example of different pathogen removal mechanisms involved in a filtration-based OWT and a CW is shown in Figure 1. The most significant removal mechanisms of pathogens in OWTs may vary depending on numerous factors such as the configuration of the treatment units, filtration materials, hydraulic loadings, and seasonal variations [65,67,69]. This section is dedicated to reviewing the major pathogen removal mechanisms that occurred in OWTs.



**Figure 1.** The removal mechanisms of pathogens in OWTs. (A): Major pathogen removal mechanisms involved in a conventional septic tank and sand filtration system. (B): Major pathogen removal mechanisms involved in a constructed wetland.

#### 4.1. Physical Removal Mechanism

##### 4.1.1. Sedimentation

Sedimentation has been demonstrated as an effective removal mechanism, which is controlled by the settling velocity of pathogens [24,70,71]. Therefore, the pathogen reduction in septic tanks was most effective for helminth eggs (e.g.,  $87\text{--}158.2\ \mu\text{m s}^{-1}$  for *Ascaris suum*, *Trichuris suis*, and *Oesophagostomum* spp.), followed by protozoa (e.g.,  $0.27\text{--}1.4\ \mu\text{m s}^{-1}$  for *Giardia* and *Cryptosporidium*), bacteria (e.g.,  $0.14\text{--}0.5\ \mu\text{m s}^{-1}$  for FC, *E. coli*, enterococci, and *C. perfringens*), and least for viruses (e.g.,  $0.0001\ \mu\text{m s}^{-1}$  for F-specific and somatic bacteriophages) [72–74]. Pathogens with low settling velocities can only be effectively eliminated by sedimentation when they were aggregated or associated with larger particles [25]. When assessing the influence of suspended particles on settling out of FC, fresh peat was added to wetland water and led to a higher removal rate of FC compared to plain wetland water [75]. On the other hand, the adsorption of pathogens onto settleable solids is not always conducive to pathogen reduction. Boutilier et al. found that sedimentation was not observed to contribute to *E. coli* removal despite the fact that approximately 50% of *E. coli* in the STE were associated with particles  $> 5\ \mu\text{m}$ . Only the bacteria associated with larger particles ( $>80\ \mu\text{m}$ ) could be removed by sedimentation, indicating that the size and density of the particles which the bacteria associated with could affect the pathogen sedimentation performance in water columns [70]. In septic tanks, 99.9% removal of helminth ova was observed through sedimentation, while a limited removal of fecal bacteria (37.4 to 63.5%) was observed [46,76,77]. In another study, the moderate removal of FC (52.1 to 56.6%), *Giardia* (35.1%), and *Cryptosporidium* (45%) from domestic wastewater was observed in settling tanks prior to the wetland cells [25]. Since limited pathogen removal occurs in the septic tank, especially for viruses due to their low settling velocities, the STE needs further treatment where pathogens could be eliminated by other mechanisms [65,67].

##### 4.1.2. Filtration

Pathogens that survive and remain infective in the STE can be further reduced with mechanical filtration before discharging to groundwater or surface water. Filtration refers to the physical blocking of pathogen movement through smaller pores in the filter media. The effectiveness of filtration mainly depends on the characteristics of the pathogen and filtration media, including the type, texture, and size [55,78]. Previous studies have reported efficient bacteria ( $4.85\text{--}6.8\ \log_{10}\ \text{CFU}/100\ \text{mL}$ ) and protozoa ( $2\ \log_{10}\ \text{CFU}/100\ \text{mL}$ )



removal through the filtration process [79,80]. Filtration can be considered as a significant removal mechanism when the ratio of the diameter of the bio-colloid ( $d_p$ ) to the diameter of median grain size ( $d_{50}$ ) was greater than 0.005 [81]. Filtration can be more effective during unsaturated flow than during saturated flow through the same filter media since most of the transport of bacteria would occur in the smaller pores. In addition, clogging of the filtration systems could restrict pore sizes and hence increase the pathogen filtration efficiency [65,69]. The removal of FC was more efficient in a heavily clogged sand filter (SF) compared with a less clogged SF [82]. On the other hand, the presence of macropores or channeling in the unsaturated flow filter media would decrease the removal rates by filtration and results in a more rapid movement of bacteria [65]. Although bacteria, protozoa, and helminth eggs can be eliminated by filtration, viruses are not effectively removed by filtration as their sizes (20–300 nm) are much smaller than the pores sizes (0.4–400  $\mu\text{m}$ ) of most filtration media [2,26].

#### 4.2. Chemical Removal Mechanism

##### 4.2.1. Adsorption

When the pathogen size is much smaller than the media pores, mechanical filtration becomes ineffective, and adsorption becomes the dominant mechanism for pathogen removal [83–85]. Adsorption of pathogens on the filtration matrix occurs during the filtration process and can be influenced by the interaction with plant roots, filter media type, and the associated biofilm on the media surface [70]. The interactions between bio-colloids (e.g., bacteria and viruses) and solid surfaces have been comprehensively explained by the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory [86,87]. In the DLVO theory, the attractive/repulsive interaction potential is calculated based on both van der Waals force and electrostatic force, which vary with distance (short distance as the primary minimum:  $\leq 1$  nm or long distance as the secondary minimum: 5–10 nm). Moreover, the adsorption of bio-colloids to the porous media is a two-step process [65]. The first step is reversible adsorption, which is a weak interaction between bacteria and the solid surface [88]. Reversible adsorption could be a temporary process at the secondary minimum distance, where attached bacteria can detach from the solid surface and return to the water. The second step is irreversible adsorption to the surface of media at the primary minimum distance. Despite the fact that the DLVO theory has been applied successfully to explain the bacterial and viral adhesion to the surface for some strains, non-DLVO forces from hydrophobic and steric interactions were also proposed to affect the partitioning of bacteria and viruses onto a solid surface [89–91]. Overall, the theories provide a general understanding of the relative contributions of the physical (e.g., media types, organic matter content, and temperature), chemical (e.g., pH and ionic strength), and microbiological (e.g., cell types and cell surface characteristics) factors to the adsorption process [70,92–94].

Adsorption of viruses to porous media is related to the characteristics of proteins in the virus outer capsid, and the electrostatic repulsive forces between viruses and media can be reduced by low pH, high ionic strength, and the presence of divalent cations in the systems, hence improving the removal efficiency [90]. As most protein coats of viruses and porous media are both negatively charged at natural pH, the increase in pH would result in less adsorption of virus to filter media due to the enhanced electrostatic repulsion between them [95]. Similarly, increasing the ionic strength would decrease the energy barriers, extending the secondary minimum distance and promoting bacterial cells and virus attachment to the solid surface, thus enhancing the adsorption [91]. An increased level of divalent cations was reported to be more effective than monovalent cations in increasing virus adsorption to filtration media in a soil column [96]. It has been shown that soil with high contents of iron (Fe) and aluminum (Al) oxides and low pH showed a greater reduction in MS2 bacteriophages, mainly due to irreversible adsorption. The soil particle surface charge became more positive by coating the  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$ , so the adsorption of bacteriophages increased [93]. The amendment of clay to soil filters could also increase the

adsorption of bacteria as a result of increased surface areas due to small particle sizes along with large cation exchange capacity [83,97].

In addition, viral and bacterial adsorption to soils is strongly affected by the type and strain of virus and bacteria as their surface characteristics vary significantly [98]. For example, two fecal indicator bacteria (*E. faecalis* and *E. coli*) with distinct surface characteristics resulted in different transport and retention behaviors in sand media: the adsorption between the Gram-positive *E. faecalis* and sand was dominated by the combination of DLVO and hydrophobic or polymer bridging, while the combination of DLVO and steric interaction governed the adsorption of Gram-negative *E. coli* to sand [91]. As a result, *E. faecalis* were irreversibly attached to sand in primary minimum at increased salt conditions, whereas *E. coli* were reversibly attached to sand in secondary minimum at both freshwater and seawater conditions [91].

#### 4.2.2. UV Radiation by Sunlight

UV radiation by sunlight is an effective mechanism for pathogen removal mainly in open-water treatment wetlands (e.g., free water surface flow CWs and waste stabilization ponds) [99,100]. The effect of sunlight UV radiation on pathogen removal heavily depends on the amount of sunlight the systems have access to. The open-water treatment systems were reported to achieve increased inactivation of pathogens due to more sunlight exposure than vegetated wetlands [67,101]. UV-B ( $\lambda = 280\text{--}320\text{ nm}$ ), UV-A ( $\lambda = 320\text{--}400\text{ nm}$ ), and visible light ( $\lambda = 400\text{--}700\text{ nm}$ ) of the sunlight spectrum are responsible for pathogen inactivation in open-water wetlands [102–104]. All three regions of sunlight contributed approximately equally in enterococci and F-specific bacteriophage inactivation, while UV-B dominated the inactivation of *E. coli*, poliovirus, adenovirus, PRD1, and MS2 bacteriophages [99,104–106]. There are three sunlight inactivation mechanisms proposed for bacteria and viruses: the direct, indirect endogenous mechanisms, as well as exogenous mechanisms [99,107]. Through the direct endogenous mechanism, sunlight causes direct damage to nucleic acids or proteins that absorb the photons, while the indirect endogenous mechanism occurs when photons are absorbed by endogenous photosensitizers (e.g., NADH/NADPH, flavins, and porphyrins) to produce reactive intermediates (e.g., singlet oxygen) that can indirectly damage bacteria and viruses [108,109]. In exogenous mechanisms, indirect damage may occur when photons are absorbed by photosensitizers in the water (e.g., natural organic matter) to form exogenous reactive intermediates (e.g., superoxide, hydroxyl radical, and triplet dissolved organic matter) [110,111]. The contribution of each mechanism to sunlight inactivation varies with different organisms' survivability, water depth, and water quality (e.g., turbidity, dissolved oxygen (DO), pH) [3,9,99]. The effect of UV radiation by sunlight is negligible in subsurface flow CWs due to the limited pathogen exposure to sunlight [3,112]. Furthermore, most filtration-based OWTs are constructed underground, therefore sunlight radiation has little impact on pathogen removal.

### 4.3. Biological Removal Mechanism

#### 4.3.1. Natural Die-Off

Natural die-off is another important mechanism in the removal of bacterial pathogens from domestic wastewater, controlled by predation, starvation, and exposure to unfavorable factors [64,70,113]. The unfavorable conditions for bacterial survival include high temperature, low moisture content, low pH, and low organic matter content [65,114]. Previous studies have reported that the survival times of pathogenic bacteria in soil were reduced by increasing soil temperatures from 5 °C to 30 °C [14]. In addition, it has been found that the die-off rates of pathogenic bacteria increased as the organic matter decreased, presumably due to competition for nutrients [115]. The low pH (below 5.5) in some OWTs such as sand (pH 3.9–6.9) and peat (pH 3.7–4.7) filters also have an adverse effect on bacterial survival [48,116]. The survival of *E. coli* has been found to be more improved in neutral-to-alkaline soils (pH 6.8–8.3) than in acidic soils (pH 5.5–7.2) [117]. In another study, the enhanced survival of *E. coli* and *Enterococcus faecalis* was observed in limestone

soil (pH 5.8–7.8), compared to peat (pH 2.9–4.5) [118]. Moreover, dissolved oxygen increase has been linked with bacterial die-off in OWTs [119,120]. For example, the die-off rates of fecal bacteria increased from 17–95% to 53–99% when the wastewater lagoon was aerated [119]. In CWs, natural die-off rates of bacteriophages were higher in the water column ( $0.198\text{--}0.397 \log_{10} \text{d}^{-1}$ ) than in the sediments ( $0.054\text{--}0.107 \log_{10} \text{d}^{-1}$ ) due to the protective effect of sediments on bacteriophages [24]. In addition, another study suggested that natural die-off of *E. coli* was the major removal mechanism after wastewater has undergone pretreatment such as a septic tank [70]. Likewise, helminth eggs can remain viable for months in soil, freshwater, and sewage, and for years in feces and sludge [19,37,121]. For example, *Ascaris* eggs can remain viable for 14 months in sludge while the inactivation rate ranged from  $0.0007$  to  $0.001 \log_{10} \text{d}^{-1}$  [36]. It was hard to inactivate the helminth eggs unless the temperature was over  $40^\circ\text{C}$  and the moisture content was below 5% [122]. Therefore, helminth eggs are more likely to be eliminated by filtration from soils and by sedimentations in septic tanks as discussed above [123].

#### 4.3.2. Predation

Predation has been found to be an important factor in attenuating pathogenic bacteria by other bacteria, bacteriophages, protozoa, and nematodes [3,64,124,125]. Predation rates depend on the characteristics of the prey (e.g., concentration and species), the predator (e.g., morphology and feeding behavior), and physicochemical factors (e.g., temperature and hydrodynamics in the microenvironment) [113,126,127]. Protozoa predation has been reported in various OWTs, such as subsurface flow CWs and SFs, and it was suggested to be the main mechanism for bacteria elimination in CWs [64,113,128]. *Shigella flexneri*, *E. coli*, and *Salmonella typhi* were efficiently removed (98 to 99.9%) by free-living ciliate protozoans, and the grazing rates ( $10^3$  to  $10^5$  bacteria/ciliate/h) increased with prey densities ( $10^6$  to  $10^8$  bacteria/mL) [124]. In another lab-scale CW study, the predation of bacteria by protozoa was found to be the dominant mechanism for *E. coli* removal while the roles of adsorption and natural die-off in the elimination processes were insignificant [64]. On the other hand, researchers found predation was the most likely removal mechanism for *Cryptosporidium parvum* in slow SFs, while it was less relevant to *C. perfringens* removal [129]. Moreover, grazing rates in actual OWTs have not been studied, and more research is needed to investigate the predation activity of pathogens by different types of predators in OWTs.

### 5. Factors Affecting the Removal of Bacterial and Viral Pathogens in OWTs

The efficiency of pathogenic microorganism removal in OWTs depends on many variables including system configuration, filter material, operational conditions (such as hydraulic retention time, flow pattern, recirculation, and aeration), and other environmental factors (such as temperature and pH). Other influencing factors such as vegetation on the removal of pathogens in OWTs were investigated and well explained by other reviews [3,9,65]. Therefore, in this review, we focus on the factors related to the design and operation of OWTs. Based on the literature review, OWTs can achieve high pathogen reduction rates up to 99.99% (Table 2). However, in some OWTs, the level of pathogens in the effluent does not meet the regulatory standards for wastewater reuse in agriculture [130].



**Table 2.** Performance of pathogen removal in various OWTsS.

[illegible]

Table 2. Cont.

Treatment Type	Total Coliform			Fecal Coliform			<i>Escherichia coli</i>			In <sup>b</sup>	Helminth		Bacteriophage <sup>c</sup>			Virus <sup>e</sup>		Reference
	In <sup>a</sup>	Out <sup>a</sup>	RD <sup>a</sup>	In	Out	RD	In	Out	RD		Out <sup>b</sup>	RD <sup>b</sup>	In <sup>d</sup>	Out <sup>d</sup>	RD <sup>d</sup>	In <sup>f</sup>	Out <sup>f</sup>	
SF + HF CW + VF CW	5.16	2.13	3.03	4.30	1.04	3.26	-	-	-	-	-	-	-	-	-	-	-	[137]
HF CW + HF CW	7.08–	4.70–	1.56–	-	-	-	5.64–	2.68–	1.36–	27	5.25	21.75	-	-	2.50–3	-	-	[44,156, 157]
HF CW + HF CW + VF CW	7.77	6.22	2.38	-	-	-	6.81	5.45	2.96	-	-	-	-	-	-	-	-	[156]
HF CW + VF CW	-	-	-	-	-	-	6.54	4.08	2.46	-	-	-	-	-	-	-	-	[46,146]
HF CW + pond	6.40–	3.65–	2.19–	7.63	3.48	4.15	6.20	3	3.20	-	-	-	-	-	-	-	-	[146,147]
HF CW + pond	7.98	4.20	4.33	-	-	-	5.40–	2.40–	0.82–	-	-	-	2.90	1	1.90	-	-	[146,147]
HF CW + SF	6.440	5.58	3.00	-	-	-	6.20	3.62	3.20	-	-	-	-	-	-	-	-	[62]
HF CW + SF	5.83	3.94	1.89	-	-	-	6.11	3.93	2.19	-	-	-	-	-	-	-	-	[62]
FWS CW+HF CW + SF	6.39	2.93	3.46	-	-	-	6.68	3.19	3.49	-	-	-	-	-	-	8.81–	5.46–	[62]
VF CW + HF CW	6.40–	2.15–	1.55–	6.73–	2.16–	3.88–	6.18–	2.32–	3.51–	-	-	-	-	-	-	10.31	6.30	[44,146, 157,158]
VF CW + HF CW	6.95	4.85	4.8	6.83	2.85	4.67	6.81	3.30	3.88	27	1.90	25.10	4.89	2.07	2.83	-	-	[44,132, 157]
VF CW + VF CW	6.00–	3.98	2.00–	-	-	-	6.00–	2.50–	2.00–	27	2.40	24.60	4.41	3	1.41	-	-	[44,132, 157]
VF CW + VF CW + bioreactors	7.77	-	3.80	-	-	-	7.10	4.70	4.50	-	-	-	-	-	-	-	-	[156]
VF CW + VF CW + bioreactors	-	-	-	-	-	-	6.54	2.34–	3.46–	-	-	-	-	-	-	-	-	[156]
Bioreactor + VF CW + bioreactors	-	-	-	-	-	-	3.08	4.08	4.20	-	-	-	-	-	-	-	-	[156]
Pond + FSF CW + SSF CW	6.75	4.40	2.35	-	-	-	6.54	4.08	2.46	-	-	-	-	-	-	-	-	[156]
<b>OWTSs with disinfection units</b>																		
SF + UV unit	6.35	3.23	3.12	6.35	3.23	3.12	9.56	<1	9.56	4.86	2.62	2.24	-	-	-	-	-	[41]
SF + UV unit	-	-	-	8.23	4.97	3.26	6	0.91–	4.47–	-	-	-	-	-	-	-	-	[159,160]
CWs/pond + UV unit	4.85–	1	4.4–5.1	6.23–	0–	-	3.88–	0.041–	1.78–	-	-	-	2.90	<1	1.20	-	-	[143,147, 161,162]
CWs/pond + UV unit	5.4	-	-	9.11	4.81	-	7.07	4.54	5.90	-	-	-	-	-	-	-	-	[162]
Activated sludge system + Filter+ VF CW + chlorination	4.82	ND	4.90	-	-	-	3.74	-	>3.20	-	-	-	-	-	-	-	-	[162]
Fine screen + A <sup>2</sup> O-MBR + chlorination	-	-	-	6.72	0	6.72	5.41	0	5.41	-	-	-	-	-	-	4.96–	0	[54]
ST + peat filter + chlorination	-	-	-	-	-	-	5.40	0	5.40	-	-	-	4.61	2.360	2.25	5.62	4.96–	[138]

<sup>a</sup> In: influent concentrations of pathogens; Out: effluent concentrations of pathogens; RD: reduction rate of pathogens; the units of total coliform, fecal coliform, and *E. coli* concentrations are log<sub>10</sub> CFU/100 mL.

<sup>b</sup> The unit of helminth concentrations is egg/L. <sup>c</sup> Detection of F-specific, somatic, and phiX174 bacteriophages. <sup>d</sup> The unit of bacteriophage concentrations is log<sub>10</sub> PFU/100 mL. <sup>e</sup> Detection of adenovirus, echovirus, rotavirus, norovirus, poliovirus, Aichi virus 1, and pepper mild mottle virus. <sup>f</sup> The unit of virus concentrations is log<sub>10</sub> copies/100 mL. <sup>g</sup> No data reported in the studies. <sup>h</sup> ND presents not detected in the studies. ST: septic tank; FSF CW: free water surface flow constructed wetland; HSSF CW: horizontal subsurface flow constructed wetland; VSSF CW: vertical subsurface flow constructed wetland; MBR: membrane bioreactor; A<sup>2</sup>O: anaerobic/anoxic/oxic; RBC: rotating biological contactor; SF: sand filter; VF CW: vertical flow constructed wetland; HF CW: horizontal flow constructed wetland; SSF CW: subsurface flow constructed wetland.

### 5.1. Effect of Configurations

#### 5.1.1. System Configuration

Conventional OWTs generally consist of a septic tank and a subsurface infiltration system, commonly known as the drain field. Septic tanks usually consist of a one- or two-chamber system that provides quiescent and anaerobic conditions to facilitate the removal of pathogens from domestic wastewater. The quality of STE depends on the characteristics of the raw domestic wastewater (e.g., size of the suspended particles, concentration and chemical composition of the influent) that enters the septic tank, but the reductions in bacteria, viruses are limited (approximately 1–2 log<sub>10</sub>) in the septic tanks [135,163,164].

Besides the traditional septic system, surface flow CWs, subsurface horizontal flow CWs, and vertical flow CWs have been widely employed in many countries and can achieve 0.4–4.48 log<sub>10</sub> removal of pathogens (bacteria, viruses, protozoa, and helminths) treating domestic wastewater [3,9]. Furthermore, the filtration-based treatment system filled with sand, peat, carbonaceous or other media is another technological solution for onsite wastewater treatment [28,136,165]. The installation of an SF in drain fields greatly increased the removal of FC (99.8%) compared with the soil-only filter systems (91%) [133]. When the pathogen removal performance of TC, *E. coli*, clostridia, *Bacteroides* spp. and enterococci was compared among different OWTs (a horizontal flow CW, a vertical flow CW, an SF, and a biofilter consisted of specific light weight aggregates (LWA)), the mean pathogen reductions ranged from 1.41 to 4.12 log<sub>10</sub> units in different systems, with the best overall performance (3.04 log<sub>10</sub>) observed in the biofilter due to the long hydraulic retention time (HRT) (>20 days) resulted from its configuration [43].

More recently, hybrid OWTs which comprise a CW or an infiltration percolation system with a subsequent filter or CW have been found to achieve higher pathogen removal efficiency compared to single-stage systems [41,44,137,156,166], because the hybrid system synergistically integrated the benefits of both types of CWs which created optimal hydraulic conditions and longer HRT [9]. For example, the highest removal rates of TC, FC, and FS from 97.7 to 99.4% were observed in hybrid systems, followed by subsurface flow CWs and free water surface CWs [167]. Enhanced pathogen reductions were also reported in multi-stage sand filters (3–5 log<sub>10</sub>) compared to single vertical-flow sand filters (1.5–2.5 log<sub>10</sub>) [157]. Furthermore, the hybrid OWTs (CWs and a denitrifying bioreactor) could not only improve *E. coli* reduction (3.5–4.7 log<sub>10</sub>) but also reduced energy inputs and land area requirement compared with a horizontal subsurface flow CW system [156]. Most of the hybrid OWTs were designed to operate in series with recirculation to obtain maximum removal performance [43,137,156]. Few studies evaluated the system configuration impact on viral pathogen removal efficiencies. A study showed the rotating biological contactor (RBC) achieved a higher removal efficiency of viral (MS2, phiX174, and PR772 bacteriophages) pathogen (>3.63 log<sub>10</sub>) in comparison to the conventional septic tank system (>3.35 log<sub>10</sub>) [168].

In addition, activated sludge-based OWTs have also been evaluated for pathogen removal efficiencies. For example, an anaerobic/anoxic/oxic (A<sup>2</sup>O) biological treatment unit with a membrane bioreactor (A<sup>2</sup>O-MBR) has been used to treat onsite wastewater [54]. The 0.2–0.4 log<sub>10</sub> removal of bacterial and viral pathogens was achieved by fine screening in the preliminary treatment of wastewater, and 1.3–1.7 log<sub>10</sub> removal of pathogens in the A<sup>2</sup>O biological treatment unit, followed by 0.7–4.7 log<sub>10</sub> removal in the MBR [54]. Although other filtration-based OWTs such as passive nitrogen removal systems seem to have the potential for pathogen reduction due to the physical, chemical, and biological processes within the systems, the capability of them to remove pathogens has not been systematically evaluated [169].

Studies have revealed that the flow direction in a treatment system is an important factor affecting the pathogen removal performance. A better removal rate of total coliforms was observed in the vertical flow wetland (92.3–93.1%) than in the horizontal flow wetland (96.8–97.0%) [170]. Analysis of the vertical flow CWs compared to horizontal flow CWs showed a higher DO concentration (2.35 mg/L vs. 0.21 mg/L), which was the main factor

for the better pathogen removal efficiency. Many studies suggested that such results were due to the unfavorable environment for bacteria survival in aerobic conditions and the distribution of microbial communities varied in response to the horizontal or vertical flow direction [64,71,167]. Another study also observed more effective eliminations of *E. coli* ( $0.65 \log_{10}$ ), clostridia ( $0.42 \log_{10}$ ), and *Bacteroides* spp. ( $0.44 \log_{10}$ ) in vertical flow CWs than horizontal flow CWs [43]. The poorer removal performance in the horizontal flow CW was probably due to clogging problems after over 20 years of operation [43]. When comparing different system configuration impacts on *E. coli* areal load removal rate (i.e., the geometric mean of pathogen removal rate), it was found that the aerated vertical/horizontal flow wetlands with recirculation had the highest reduction rates ( $9.9\text{--}10.5 \log_{10}$  MPN/m<sup>2</sup> d), followed by unsaturated vertical flow wetlands ( $9.7\text{--}9.8 \log_{10}$  MPN/m<sup>2</sup> d) and passive horizontal flow systems ( $9.1\text{--}9.4 \log_{10}$  MPN/m<sup>2</sup> d), indicating that the configuration and flow direction had significant impacts on the removal performance of pathogens [120]. Although the pathogen removal rate was often recognized as the overall performance of the system, it requires caution to fairly assess the overall capability when comparing the effluent concentrations or removal rates under vastly different design and operational conditions.

### 5.1.2. Disinfection Treatment Unit

Although substantial pathogen reduction can be achieved in conventional OWTs, effluent concentrations of pathogens are generally high, especially above legislation limits for wastewater reclamation and reuse [138,171–173]. Therefore, a disinfection step is imperative to remove pathogens for minimizing health risks that are associated with pathogens in reclaimed water. UV radiation, ozone, or chlorination are commonly adapted for enhanced pathogen inactivation after conventional OWTs [54,143,159,161,174–176]. The effluent concentrations of *E. coli* and FC can be reduced to extremely low levels ( $<10$  CFU/100 mL) by using the combination of OWTs and UV radiation systems [143,161]. Likewise, the concentrations of TC and *E. coli* were considerably reduced in the effluent to meet the regulation for agricultural reuse by 5 mg O<sub>3</sub>/L with a contact time of 5 min [174]. Post-treatment by chlorine with a dose of 2.5 mg/L was also proved to be effective for eliminating all pathogens including FC, *E. coli*, *Salmonella*, *Shigella*, enterovirus, rotavirus, and norovirus from MBR effluent for water reuse [54]. Therefore, the combination of the OWT and a disinfection treatment unit could significantly improve the water quality by reducing the pathogen concentration, while minimizing the operational cost.

## 5.2. Effect of Filter Materials

### 5.2.1. Media Grain Size

Media grain size and the filter material type are two main factors in the elimination of pathogens in OWTs. The media grain size determines the effectiveness of mechanical filtration while the intrinsic properties of the materials can be utilized to enhance pathogen removal performance. Smaller media grain sizes are beneficial for pathogen immobilization by filtration and can also provide larger surface areas for adsorption [65,83,177]. Many studies have demonstrated the positive influence of fine grain sizes on the removal of pathogenic bacteria (Table 3). Silt, clay, and fine sand have pore sizes within the range of most protozoa (10–100 µm in diameter) and bacterial cells (0.2–5 µm in diameter), therefore improving the pathogenic bacteria and protozoa removal [26]. For example, the removal of *C. parvum* and *Giardia lamblia* were investigated in CWs with different filtration media: one set of CWs were filled with washed sand (grain size of 0–2 mm) and the other set of CWs were filled with a mixture of expanded clay (grain size of 2–4 mm) and sand (grain size of 0–2 mm) [79]. The results showed that washed sand in the filter bed was the most effective filter material to achieve 2  $\log_{10}$  reductions of protozoa, indicating small media size played an important role in eliminating protozoa by mechanical filtration. In another study, 18 times more *C. parvum* oocysts were detected in the effluent from the coarse sand column than from the fine sand column [178]. A modeling study revealed that the removal rates of bacteria and viruses increased by 0.16–0.3  $\log_{10}$  and

0–0.1 log<sub>10</sub>, respectively, when comparing a sand filter with fine size ( $d_{10}$  = 0.17 mm) and one with coarse size ( $d_{10}$  = 0.52 mm). The increase in pathogen removal efficiency was independent of the HRT changes [177]. These results are in agreement with a previous study in which a significantly greater removal of *E. coli* was observed in the vertical-flow wetlands with fine sand media (3.2 log<sub>10</sub>) than that with the coarse gravel media (1.9 log<sub>10</sub>) under the same operational condition treating wastewater [156]. Another study reported a substantial removal of *E. coli* and F-specific bacteriophage (5 log<sub>10</sub> and 3 log<sub>10</sub> reductions, respectively) in fine soil matrix (1–4 mm), while no removal of those pathogens was observed in coarse soil matrix (4–11 mm) [179]. Likewise, Ausland et al. observed that the reduction in FC in the media with fine media size (unsorted sand) was higher (>3 log<sub>10</sub>) than in the media with coarser size (LWA 0–4 mm and LWA 2–4 mm) at the same hydraulic loading (80 mm d<sup>−1</sup>) [180]. However, when hydraulic loading decreased to 40 mm d<sup>−1</sup>, no considerable difference of pathogen removal was found between different media grain sizes, indicating the contribution of grain sizes becomes marginal when HRT is sufficient [180].

**Table 3.** The removal performance of pathogens in OWTs with various grain sizes of the filter media.

Filter Material	Size <sup>a</sup> (mm)	Pathogen Reduction (log <sub>10</sub> CFU/100 mL or log <sub>10</sub> PFU/100 mL)							Reference
		Total Coliform	Fecal Coliform	<i>Escherichia coli</i>	Fecal Streptococci <sup>b</sup>	F-Specific Bacteriophage	Somatic Bacteriophage	<i>Cryptosporidium parvum</i>	
Gravel	$d_{60}$ = 3.5; Cu = 1.7	2.0–2.4	- <sup>c</sup>	2.2–2.4	1.3–1.7	-	-	-	[55]
	$d_{60}$ = 10; Cu = 1.6	1.2–2.7	-	1.5–2.6	1.3–2.2	-	-	-	
	5–13 ( $d_{60}$ = 9.5)	-	0.2–2.8	-	-	-	0.9–1.8	-	[181]
	5–25 ( $d_{60}$ = 17)	-	0.1–2.7	-	-	-	0.5–1.7	-	
Sand	$d_{10}$ = 5; $d_{60}$ = 12	0 <sup>d</sup>	-	0–0.5	-	-	-	-	[151]
	$d_{10}$ = 0.24; $d_{50}$ = 0.65; Cu = 3.13	-	5.6–6.2	-	4.9	-	-	-	[180]
	$d_{10}$ = 0.13; $d_{50}$ = 0.84; Cu = 9.85	-	6.2–6.3	-	4.9	-	-	-	[151]
	$d_{10}$ = 0.34; $d_{60}$ = 0.9	1.5–2.9	-	1.5–3.3	-	-	-	-	
Mixture of river sand and gravels	2–13 ( $d_{60}$ = 9.5)	-	0.7–3.4	-	-	-	0.9–2.6	-	[181]
Pebbles	5–20 ( $d_{60}$ = 12.7)	-	0.1–2.7	-	-	-	0.5–1.2	-	[180]
Light weight aggregates (LWA)	0–3 ( $d_{10}$ = 0.08; $d_{50}$ = 0.7; Cu = 15)	-	5.6–6.2	-	4.9	-	-	-	
	0–4 ( $d_{10}$ = 0.8; $d_{50}$ = 1.9; Cu = 2.68)	-	3.4	-	4.7	-	-	-	
	2–4 ( $d_{10}$ = 2.05; $d_{50}$ = 2.8; Cu = 1.46)	-	2.9	-	4.5	-	-	-	
Biochar	$d_{10}$ = 1.4; Cu = 2.2	-	-	3.3–6.5	3.0–6.4	2.7–4.3	-	3.5–3.9	[56]
	$d_{10}$ = 2.8; Cu = 2.2	-	-	2.6–3.8	2.5–3.6	2.2–3.0	-	2.9–3.1	
	$d_{10}$ = 5.0; Cu = 2.2	-	-	2.2–2.5	2.3–2.4	2.2	-	2.3–2.6	

<sup>a</sup>  $d_{50}$  and  $d_{60}$  represent the grain diameters where 50% and 60% weight of the mass is of smaller size. Cu is the uniformity coefficient and is calculated as the ratio between  $d_{60}$  and  $d_{10}$ . <sup>b</sup> Fecal streptococci = fecal enterococci. <sup>c</sup> No data reported in the studies. <sup>d</sup> The mean removal log<sub>10</sub> was zero due to the same average concentrations of influent and effluent measured in the study.

Although the fine grain size has been reported to be a key design factor in removing pathogenic microorganisms, a filter media with too fine grain sizes may lead to rapid clogging [182]. Therefore, selecting a proper media size to maximize the removal performance and minimize the risk of clogging is an important filter design parameter.  $d_{10}$  (i.e., the effective media size, that 10% of the media by weight is smaller than this particular size),  $d_{60}$  (60% of the media by weight is smaller than this particular size), and the uniformity coefficient should be considered when selecting the filter media to avoid clogging. The uniformity coefficient represents the uniformity of the media size distribution in the filter media and is calculated as the ratio of  $d_{60}$  to  $d_{10}$ . Based on previous studies, a selected range of  $d_{10}$  (0.3 to 2.0 mm),  $d_{60}$  (0.5 to 8.0 mm), and the uniformity coefficient (<4) for filter media in OWTs were recommended to achieve a high HRT without clogging [53,183].

### 5.2.2. Type of Filter Media

A variety of filtration media have been used in OWTs, including sand, peat, wood byproducts (e.g., woodchip, sawdust, and bark), zeolite, biochar, oyster shells, coconut



shells, glass bead, geotextile, earthworms, and commercially available filtration media [27,51,134,136,160,184–187]. The selection of filter material largely depends on the pollutants of concern in the domestic wastewater, the operational strategies, and the configurations of treatment systems. Pundsack et al. compared the pathogen removal efficiencies of subsurface-flow CWs filled with a variety of media (i.e., gravel, sand, and peat). The highest reductions in *Salmonella* were found in the intermittent peat filters (99.99% removal), followed by sand filters (95.69–99.93% removal) and gravel CWs (94.94–95.82% removal) [48]. The high reduction rates were strongly related to the media size and the type. Smaller media size in the sand and peat filters enhanced the filtration efficiency of finer particles and improved the possibility of pathogen adsorption to media surface and the biofilms in the systems [48]. In addition, peat functioned as an effective sorbent in wastewater treatment, offering higher removal through the adsorption process [188]. A previous study evaluated the log reductions in FC by sand, crushed glass, peat, and geotextile as filter media for onsite wastewater treatment, and the peat filter showed the best FC removal (3.9 log<sub>10</sub>) compared with other filter materials (1.3–3.1 log<sub>10</sub>) in the recirculating biofilters [160].

Porous media such as woodchip and coconut husk, commonly found in denitrifying bioreactors as the carbon source, are also promising materials for pathogen removal. The wood byproduct materials have been selected mainly due to their readily availability, low cost, high permeability, and high C:N ratio [189]. Previous studies have evaluated the ability of wood-based bioreactors to reduce pathogens in wastewater [27,136,156,189]. Among those studies, 0.2–2.9 log<sub>10</sub> reductions in *E. coli* and 3.9 log<sub>10</sub> reductions in F-specific bacteriophages were reported by the denitrification bioreactors. Specifically, no detectable *E. coli* (<10 CFU/100 mL) in effluent has been achieved in the full-scale OWTSSs using wood byproduct media [189]. In addition, coconut husk, employed as an effective alternative carbon-rich media in the denitrifying bioreactors, attenuated pathogens as a low-cost natural material. In a case study, the removal performance of TC, *E. coli*, and F-specific bacteriophage were evaluated by woodchip and coconut husk bioreactors with gravel filters as the control, treating primarily treated municipal wastewater [27]. The removal rates of pathogens by woodchip and coconut husk bioreactors were comparable (1.3–1.6 log<sub>10</sub>) to those gravel-based bioreactors (1.5–1.6 log<sub>10</sub>) under the same operational conditions, suggesting that denitrifying bioreactors filled with carbonaceous media could be considered as an effective option for pathogen removal from domestic wastewater [27]. Enhanced overall reduction rates of pathogens were observed in newly constructed (fresh) woodchip and coconut husk bioreactors (1.72 log<sub>10</sub>) compared to the 8-year old (mature) bioreactors (1.42 log<sub>10</sub>), suggesting a negative effect of media age on the attenuation of microbial contaminants in carbonaceous bioreactors [27].

In developing countries, earthworms have been used in vermifilters to treat domestic wastewater as a low-cost and bio-safe material [134,190,191]. Vermifilters are engineered natural systems involving the joint action of earthworms and microorganisms, in which microorganisms biodegrade the waste materials while earthworms ingest organic matter, bacteria, protozoa, nematodes, algae, and fungi and secrete mucus to increase the available surface area for microbial action [192–194]. Considerable reductions in pathogens (TC: 3.91 log<sub>10</sub>, FC: 2.82–6.4 log<sub>10</sub>, FS: 2.65 log<sub>10</sub>, *E. coli*: 2.51 log<sub>10</sub>, *Salmonella*: 2.2–8.6 log<sub>10</sub>, enteric virus: 4.6 log<sub>10</sub>, helminth eggs: 1.9 log<sub>10</sub>) have been observed in these earthworms amended vermifilters treating domestic wastewater [134,195].

Zeolite has also been used as the filter media to enhance pathogen removal efficiency due to its ability to adsorb positively charged ions [76,196]. A zeolite tank was used as a polishing unit after the conventional CW to further remove nutrients from domestic wastewater. The zeolite tank was also able to offer additional removal of total coliforms (0.78 log<sub>10</sub>; relative removal rate: 83.7%) besides nitrogen, phosphorus, and organic matter [76]. In recent years, biochar as an environmentally friendly sorbent has gained attention for contaminant removal in wastewaters [197]. It was considered as a suitable medium to improve pathogen removal due to its small pore size and large surface area [94,166,198].

In a case study, the removal performance of bacterial (*E. coli* and *Enterococcus* spp.), viral (phiX174 and MS2 bacteriophages), and protozoan (*C. parvum*) pathogens were studied in biochar filters treating wastewater for irrigation [56]. The average reduction in bacteria (0.2 to 4.5 log<sub>10</sub>), viruses (0.2 to 2.3 log<sub>10</sub>), and protozoa (0.3 to 1.9 log<sub>10</sub>) by biochar filters were comparable to that of sand filters [56]. Another study also demonstrated that sand filters amended with 5% (by weight) biochar retained up to 1000 times more *E. coli* than plain sand filters, indicating that the pathogen removal efficiency could be improved by the amendment of biochar to the filter [198]. Other materials such as recycled tires and mulch have been applied for onsite wastewater treatment [196,199]. However, most of the studies only focused on the removal of nutrient and organic pollutants.

### 5.3. Effect of Environmental Conditions

#### 5.3.1. Seasonal Changes

Temperature, which fluctuates with different seasons, is commonly considered as the dominant factor for the survival of viruses in the environment [30,116,200,201]. Higher temperatures can damage viral DNA or RNA, increase predation through enhanced microbial metabolism, and also increase the natural die-off rates of bacteria [28,202,203]. A significant die-off of *Pseudomonas* sp. was observed at 25 °C [204], while the best survival of enteric bacteria was at 5 °C [117]. A few case studies have reported higher removal rates of pathogens in warm seasons compared to those in cold seasons [28,43,48,52,55,60,134,157]. On the other hand, several studies suggested seasonal differences did not have a significant impact on the removal of pathogens in OWTs [57,136,137,180,205]. The discrepancy was likely due to the considerable differences in the system configuration and filter media.

The removal efficiencies of FC, F-specific and somatic bacteriophages were compared in an SF and a CW at all seasons. Both the SF and the CW performed better in summer with 1.4–3.9 log<sub>10</sub> removal of all pathogens. While in winter, 0.8–2.7 log<sub>10</sub> removal of all pathogens was observed [28]. In another study, the removal rate of *Salmonella choleraesuis* increased by 1.1–6.2 log<sub>10</sub> during summer when a variety of filters (peat filters, SFs and CWs) were tested [48]. These results were similar to previous reports that the removal of bacterial indicators (e.g., *Salmonella* and FC) was higher in summer than in winter in CWs [55,157]. Furthermore, the seasonal impact on pathogen removal rates was also observed in vermifilters. The removal efficiencies of TC, FC, FS, and *E. coli* in winter were in the range of 33.93–52.60%, while in summer the removal efficiencies increased to 98.20–99.88% [134].

On the other hand, only a minor or no difference in the removal of TC, FC, fecal indicator bacteria, *E. coli*, F-specific, and somatic bacteriophages were observed between winter and summer seasons in a few case studies using CWs, peat filters, and denitrifying bioreactors to treat onsite wastewater [28,136,137,150,205]. For example, a greater reduction in *E. coli* (0.6 log<sub>10</sub>) was achieved in woodchip bioreactors operating at 22 °C compared to 10 °C [52]. However, in another study, no impact of seasonal changes on pathogen removal (e.g., *E. coli*, TC, and F-specific bacteriophage) was found in denitrification bioreactors with woodchip and coconut husk media [27].

Meanwhile, the filter media type also plays an important role in the pathogen removal efficiency at different seasons. The SF filled with gravels and the SF filled with sand showed a reduced removal performance of bacteriophages, enteric viruses, and other microbes during winter, while the SF filled with a mixture of gravel, sand, and biotite showed an increase in the removal of enterococci and heterotrophic bacteria during winter [60]. It was speculated that this increase was due to the decreased biofilm formation in the winter, leading to lower shedding of these pathogens. Furthermore, one study found that the seasonal changes were greater close to the surface than for the rest of the intermittent sand filters, thereby establishing no significant correlation between FC and FS removal rates and filter temperatures [180]. Overall, these studies demonstrated that seasonal variations in removal rates varied significantly based on the configuration and filter media of OWTs.

### 5.3.2. pH

The wastewater pH may affect the extent of bacterial and viral adsorption to colloidal particles and filter materials [92,206]. The optimum pH for coliform bacteria survival was in the range of 5.5 to 7.5, and the chance of survival would rapidly decline outside this pH range [207]. Therefore, the low pH in some OWTs can result in faster natural die-off rates of bacteria, thus increasing the bacteria removal efficacy [48]. A previous study showed that increased pH (from 5 to 7) posed a negative impact on the removal of F-specific bacteriophages (87% decrease) in the silica-bead columns [208]. Similarly, a decrease in the removal of F-specific bacteriophages ( $0.6 \log_{10}$ ) was observed in a quartz granular media column after the pH increased from 3.5 to 5.0 [90]. The plausible explanation is that viruses such as F-specific bacteriophages and enteric viruses tend to have a positive surface charge at pH below 5, increasing their affinity to negatively charged soil thus increasing the viral removal performance [28,92,209]. These results were consistent with the previous studies, where higher reductions in F-specific bacteriophages, FC, and *S. choleraesuis* were observed in peat filters (pH  $4.7 \pm 0.6$ ) compared to the SFs (pH  $6.7 \pm 0.2$ ) and CWs (pH  $7.1 \pm 0.1$ ) [28,48]. On the other hand, a higher pH outside the bacteria survival range also leads to enhanced pathogen inactivation. A compact filter system amended with positively charged iron and aluminum oxides showed a complete removal of *E. coli* and somatic bacteriophages when treating domestic wastewater [154]. This performance may be explained by an inactivation effect resulting from the high initial pH (12–13) of the filter media. A follow-up study of the same system after three years of operation still reported a complete reduction with pH at 9–10 [155]. At higher pH values, the negatively charged virus particles were easily attracted by the positively charged filters, and were more easily removed by the system.

### 5.3.3. Water Composition

The quality of wastewater such as turbidity and organic and nutrient content has been found to be an effective factor in the removal of pathogens in OWTs [177,210–212]. A relatively large negative effect of influent turbidity on MS2 bacteriophage removal (one additional NTU of influent turbidity decreased  $0.017\text{--}0.019 \log_{10}$  reduction), and a smaller positive effect of influent turbidity on FC removal (one additional NTU of influent turbidity increased  $0.0035 \log_{10}$  reduction) in an intermittent slow SF [177]. The plausible explanation was that the particles in the influents competed with the MS2 bacteriophages for adsorption sites while the particles associated with the bacterial pathogens for sedimentation [70,177]. Moreover, the influent particles provided protection to coliforms, which were resistant to high initial chlorine concentrations of  $80 \text{ mg L}^{-1}$ , decreasing the disinfection performance [213].

Organic and nutrient contents of the wastewater also play important roles in pathogen reductions in OWTs [211,212]. Dissolved organic carbon (DOC) in the wastewater may decrease bacterial adsorption by compositing with bacteria for adsorption sites on the grain surface [214]. Increased DOC in silica-bead columns may enhance the hydrophobic interactions between the hydrophobic virus (MS2 bacteriophages) and grain surfaces while inhibiting the adsorption of the hydrophilic virus (phiX174 bacteriophages) by competing for binding sites; therefore, the removal efficiency of the hydrophobic virus was improved [92,95]. A positive correlation between bacteria indicators' (*E. coli* and enterococci) levels and total nitrogen concentrations in CWs has been detected by previous studies, showing that the survival of bacteria could be enhanced due to the presence of available nitrogen [212].

### 5.3.4. Sunlight Intensity

Sunlight inactivation of bacteria and viruses can be affected by various factors such as water depth, vegetation density, and water quality [100,105,106,215]. High sunlight intensity enhances the effect of sunlight radiation, thus promoting pathogen removal. However, the effect of sunlight has a very limited penetration depth. Sunlight intensity and

the inactivation rates of bacteriophages reduced rapidly within the first few centimeters of the open-water systems such as free water surface flow CWs and waste stabilization ponds [100,216]. The density of vegetation in CWs also significantly impedes sunlight penetration into the wastewater which lowers the fecal bacteria removal [217].

The effect of sunlight radiation to remove pathogens also depends on the quality of wastewater including DO and pH [112,215,218]. Previous studies have reported a strong influence of DO on the sunlight inactivation of FC, *E. faecalis* in waste stabilization ponds [106,219]. In a previous study, the sunlight inactivation rates of enterococci, *E. coli*, and F-specific bacteriophage increased from ~80% to ~99% with increased DO (4.8% to 148% of saturation) in waste stabilization ponds [99]. In the presence of oxygen, sunlight radiation can produce exogenous reactive oxygen species which can damage the DNA, RNA, or proteins of bacteria and viruses [106]. In addition, the sunlight inactivation of *E. coli*, FC, and mesophilic *Aeromonas* increased with pH increased from 7 to 10 [99,112,215,219]. A study also found a strong synergy between increased DO and pH on sunlight inactivation of *E. coli*, and the highest inactivation rate was observed at high DO and high pH [99].

### 5.3.5. Pathogen Species and Morphologies

The removal performance of pathogens varies among different species in the OWTs. This is attributed to two contributing factors: survivability and the morphology of pathogenic microorganisms [65,220]. Certain species such as *Salmonella* and several *Streptococcus* have longer survival times than *E. coli* in soil, whereas *Shigella* dies more quickly [221]. The longer survivability of some bacteria is attributed to their greater ability to compete for nutrients with indigenous microorganisms, enhanced adsorption to the soil surface, or higher resistance to physical, chemical, or biological stressors in the environment [65]. It has been reported that *Salmonella* can survive under unfavorable conditions by entering a viable but nonculturable state [221]. In addition, the estimated inactivation rates of various pathogens (e.g., *C. perfringens* and *C. parvum*) in a slow SF were drastically different ( $0.005 \log_{10} \text{ d}^{-1}$  and  $0.014\text{--}0.019 \log_{10} \text{ d}^{-1}$ , respectively) [129].

On the other hand, the morphology of pathogens would affect their transport through filter media [65]. It was found that the size of bacteria significantly impacted the bacterial transport through the soil. For example, cells with smaller diameters ( $<1 \mu\text{m}$ ) transported further than cells with larger sizes ( $>1 \mu\text{m}$ ) [222]. Another study reported that the cell shape (as the ratio of cell width to cell length) influenced the transport of bacteria through porous media, and the long, rod-shaped bacterial cells showed preferential removal in porous media [223]. Surface appendages (e.g., flagella and pili) produced by pathogens have been found to facilitate adsorption to the filter media [65]. For example, the hydrophobic pili can result in enhanced adhesive properties of the bacteria to filter media [224].

## 5.4. Effect of Operational Conditions

### 5.4.1. Hydraulic Loading Rate

Hydraulic loading plays an important role in the pathogen removal rates in OWTs, since longer HRTs increase the contact time between pathogens and the filtration media. The HRT changes depend on the properties of filter material (e.g., texture and porosity), loading rates, water depth, and the vegetation on top of the OWTs [57,137]. Higher pathogen removal rates, as a result of decreased loading rates, have been observed in different OWTs such as SFs, denitrification bioreactors, biochar filters, and CWs [11,56,57,137,157,180,225,226]. Several studies showed a positive relationship between microbial reduction and HRTs in CWs [181,212,227,228]. A previous study also observed slightly higher removal percentages of *Salmonella* (4% higher) and *E. coli* (5% higher) at 24 h HRT compared to 12 h HRT under  $21.5^\circ\text{C}$  in woodchip bioreactors [52]. In addition, the removal performance of FC and SC increased as the HRT increased until saturation values were reached at approximately three days of HRT [181]. This result might be related to the assumption that first-order microbial decay was valid only at short HRT [205]. Similar results were reported that the removal rates of TC, FC, *E. coli*, *Salmonella*, and *Shigella*



were higher during the initial three days of treatment. After day 3, the removal efficiency appeared to be negligible [141]. In addition, a lower hydraulic loading rate allows for the development of thicker biofilm and could create a closer and longer contact between the porous media and pathogens, thus enhancing the mechanical filtration and adsorption for pathogen removal [65,226]. Filter depth change also affected the HRT in CWs and sand filters [57]. Higher removal rates of FC, *E. coli*, somatic and F-specific bacteriophages were achieved in the filters with a depth of 65 cm than those with 25 cm, due to the increased 3–5 h HRT [57]. Although an average wastewater loading is specified in most OWTs with a septic tank as the pretreatment unit for solids separation and flow stabilization, in some cases where a pretreatment unit is lacking, the wastewater loading variation throughout the year may lead to fluctuating pathogen removing performance. If the OWTs rely heavily on the predation mechanism, the performance deterioration can be significant, especially when a high loading follows a long pause of wastewater.

On the other hand, high hydraulic loading rates increased larger pores and decreased the exchange between mobile and less mobile water in the systems [225]. Previous studies reported that the increase in hydraulic loading rates would considerably decrease the reduction in FC, *E. coli*, and somatic bacteriophage, but there was a minimum impact on the removal of F-specific bacteriophages ( $p > 0.05$ ) in vertical flow CWs and soil-based filters at 200–800 mm d<sup>−1</sup> loading rates [57,180,225].

#### 5.4.2. Loading Regime and Distribution Method

Loading regime in terms of the influent dosing frequency and the volume of each dosing is considered as an important operation condition that impacts the pathogen removal in OWTs [57,120,151,229–231]. A previous study observed enhanced FC reduction rates ( $\sim 1.9 \log_{10}$  to  $\sim 4.3 \log_{10}$ ) in sand columns with increased daily hydraulic loading frequency (fractionation of the total hydraulic loading was from 2 to 12) [230]. When the daily hydraulic loading (800 mm d<sup>−1</sup>) was divided into 32 pulses rather than 16 pulses, a significant improvement of FC, *E. coli*, and somatic bacteriophage removal was obtained in vertical flow CWs and intermittent sand filters [57]. However, the removal of MS2 bacteriophage in the same study was not significantly affected by the loading regime change [57]. Similarly, several studies found no effect on the *E. coli*, MS2, and PRD1 bacteriophages reductions at different loading frequencies (12 and 24 pulses per day) in sand-based vertical flow filters [120,231]. From a hydraulic point of view, longer intervals between single loadings would lead to better drainage of the system and lower residual water content, resulting in better oxygen renewal and pollutant removal [198,232]. A case study has demonstrated in intermittent slow sand filters, increased HRT (experimental average: 16 h) with an overnight pause between feedings was beneficial for the removal of bacteria by 0.29 log<sub>10</sub> and viruses by 0.67–0.77 log<sub>10</sub> compared to a short HRT (average: 5 h) with continuous flow [177]. Pause time may also help to avoid viral shedding by increasing the residence time for adsorption and provide sufficient time to clear pore spaces with contaminants before the next dosing [177]. Nevertheless, the hydraulic effect of longer intervals became marginal on pathogen removal when the volume of each dosing was smaller, due to the resulted longer hydraulic contact time with the media and biomass in the systems [229,232]. Furthermore, the effect of various distribution methods on the removal of FC was significant in buried infiltration systems treating STE, and a higher removal of FC was observed in the uniformly loaded filters than in the point loaded filters [180]. On the other hand, excessive loading frequency could decrease the hydraulic conductivity, diminish the oxygen supply, and damage the infiltration surface, thereby inducing short-circuiting in the treatment systems [57,230].

Short-circuiting caused by preferential flow paths may reduce the pathogen removal efficiency since the actual residence time of pathogens remaining in the OWTs could be less than expected [142,233]. For example, the hydraulic short-circuiting in CWs was caused primarily by uneven plant distribution, channelized flow, and malfunctions (e.g., the clogging and freezing of inlet pipes) [157,234]. Results from another study indicated



that low numbers of helminth eggs in the influent may be more difficult to be eliminated by waste stabilization ponds due to the extent of short-circuiting compared to influent with higher helminth egg concentration [63]. Besides short-circuiting, many studies have indicated that preferential flow, flow circulation, and dead zones are major limitations to reduce hydraulic efficiency, thus decreasing the pathogen removal in conventional soil-based treatment systems [142,235]. The hydraulic efficiency represents the ability of a system to distribute the inflow uniformly across its volume, maximizing the contact time between pollutants and the media. OWTs with high hydraulic efficiency are expected to achieve a better treatment efficiency [236,237]. For example, the mean reduction rates of TC, FC, and FS increased from 1.25, 1.26, and 1.15  $\log_{10}$  in standard multi-soil-layering (S-MSL) filters to 2.36, 2.38, and 2.11  $\log_{10}$  in modified multi-soil-layering (M-MSL) filters [142]. The hydraulic profile analysis showed a smaller HRT (15.79 h vs. 22.07 h), a lower effective volume ratio (0.26 vs. 0.84), and a higher dead zone rate (38.25% vs. 8.19%) in the S-MSL filters, which were the main factors for its poor pathogen removal performance [142]. To obtain maximum pathogen removal efficiency, the hydraulic efficiency can be improved by allowing the flow to be uniformly distributed through the media and avoiding short-circuiting and dead zones.

#### 5.4.3. Degree of Water Saturation

The substrate saturation level has also been found to play an important role in determining the removal efficiency of pathogens in OWTs due to its effect on pathogen survivability [28]. The virus inactivation mainly occurs at the air–water interface in unsaturated porous media where viruses are vulnerable to environmental stressors [96]. In a case study, the inactivation rate of poliovirus type 1 was demonstrated to fluctuate in soil with a water content in the range of 5–25% [238]. Although the resistance to moisture change varies among different pathogen species, the general survival time is longer in moist soil than in dry soil [93,220]. Pathogenic microorganisms can be eliminated from unsaturated soil not only by inactivation and adsorption to soil but also by filtration. Under unsaturated conditions, pathogens move through micropores via matrix flow and filtration can be more effective compared to the saturated conditions in the same media [65,239]. Under saturated conditions, pathogens transport mainly through macropores, which may reduce the effectiveness of filtration [65]. Therefore, OWTs that operate under unsaturated conditions are more likely to achieve better pathogen removal efficiency.

Previous studies showed higher removal rates of different types of viruses (e.g., poliovirus type 1, MS2, and PRD1 bacteriophages) under unsaturated flow than saturated flow in OWTs [96,240]. Similarly, the lower removal of viruses in saturated CWs was observed when compared to unsaturated peat and sand filters [28,48]. Field and laboratory studies have reported that pathogens can be significantly reduced through 0.35 to 0.9 m depth of unsaturated subsoil achieving 99–99.9% removal of MS2 and PRD1 bacteriophages and a near-complete removal of *E. coli* [168,241,242]. In addition, saturated conditions are more likely to cause freezing problems (e.g., inlet or outlet pipes) in cold seasons which can also reduce the removal of pathogens [116,243]. Therefore, a constant unsaturated flow pattern may be beneficial for bacterial pathogen removal since this regime resulted in considerably less bacterial transport compared to saturated and variable unsaturated flow patterns. It could be more effective to remove pathogens by converting variable unsaturated flow to constant unsaturated flow in OWTs by dosing wastewater at a steady rate [244].

#### 5.4.4. Recirculation and Aeration

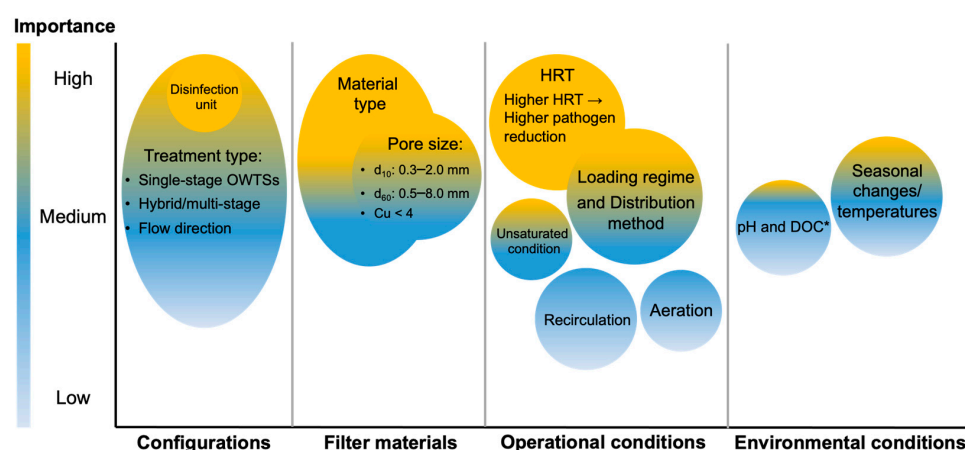
Recirculation has been applied in various OWTs to promote nitrification and denitrification processes for enhanced nitrogen removal [161,185,245]. However, it did not appear to influence the pathogen removal performance, as reported by most studies [131,137]. For example, the mean removal rates of TC and FC were at the same levels (<2% differences) with and without recirculation when different CW configurations were tested [137]. Sim-

ilarly, no impact on *E. coli* removal was observed when preliminary treated wastewater was recirculated in the vertical sand filter [157]. A possible reason is that pathogens differ in sizes, survivability, and surface morphologies. There may be a stable removal rate for chemical contaminants, while the remaining pathogens with smaller sizes and longer die-off periods were unlikely to be removed even with recirculation. On the other hand, a case study found recirculation was beneficial for *E. coli* removal. When a recirculating vertical flow CW was used to treat artificial domestic wastewater, the effluent concentration of *E. coli* increased by 2.4 to 5.5 log<sub>10</sub> in the absence of recirculation during a 72-h treatment [245]. Possible reasons for the higher reduction rates might be: (1) recirculation increased aeration and consequently promoted faster biodegradation; (2) recirculation created an almost completely mixed reactor to dilute influent domestic wastewater with the treated residual wastewater from the previous batch, thus easing the hydraulic loading to systems and enhancing the pathogen reduction [245].

Aerated systems also showed improved pathogen removal performance due to the enhanced biological removal process [39,119]. One study found that the vertical flow CW achieved better TC, *E. coli*, enterococci, clostridia, and heterotrophs removal (approximately 1 to 3 log<sub>10</sub> higher) than the horizontal flow CW under the same conditions, indicating that aerobic unsaturated conditions benefited the pathogen reduction in greywater treatment [39]. Moreover, a comparison of CWs with various designs revealed a significantly improved *E. coli* removal performance in aerated systems, indicating that the bacterial pathogen removal may be related to the dissolved oxygen concentration in the water [120].

## 6. Future Perspectives

The various OWTS designs, water sources, and operating conditions in different studies make it difficult to reach a conclusive understanding of the impact of each contributing factor on pathogen removal efficiency. Therefore, a systematic level comprehensive investigation on the factors that affect pathogen removal performance is needed to develop the design and operating guidelines for OWTSs to help prevent pathogen transport from septic tanks into natural environments. It is also worth noting that there are factors outside the user's control such as climate and the formation of preferential flow pathways in natural media; thus, the need for more controllable and predictable treatment systems should be considered. Here, in Figure 2, we provided a qualitative summary of the contributing factors to the pathogen removal performance based on previous studies. However, these factors can be significantly intervened, and lead to inconsistent conclusions in the previous literature. There is also no clear distinction between physicochemical mechanisms and biological mechanisms because they are usually coupled with microbial transport [84,246]. Limited work has been reported to better understand the effects of design and operation on overall system performance. A quantitative approach is critical and urgent to investigate the effect of each factor, in order to identify the potential bottleneck of OWTS systems, optimize the design based on the local environment and water source, and therefore further enhance the removal performance. Besides conventional OWTSs, newly developed innovative and alternative (I/A) systems with different configurations, filter material, and operational conditions need further investigations on their performance and dominant mechanisms of pathogen removal. Although indicator pathogens such as TC, FC, *E. coli*, and F-specific bacteriophage have been widely used to represent the water quality, they are not able to provide information about the existence of a specific pathogen, such as SARS-CoV-2 and its fate within treatment systems. Therefore, it is necessary to explore a wider spectrum of pathogen indicators to further assess and interpret the pathogen removal in OWTSs. OWTSs integrated with chemical or physical disinfection methods such as chlorination and UV radiation could further enhance the pathogen removal efficiency, but more research is required to evaluate their performance and the potential of disinfection byproduct (DBP) formation.



**Figure 2.** A bubble graph illustrating the significance of each contributing factor to the pathogen removal rates. Different factors are categorized into four aspects. The colors represent the importance of these factors in previous studies. A larger portion of the yellow region indicates that the factor is significant for high removal rates reported by a large number of studies, while the light blue color region represents that its effect is negligible in some studies. The size stands for the design space of the contributing factor. \*Pathogen reductions based on the filter material and pathogen types.

Policies and standards related to pathogen removal are also complicated topics due to the wide range of pathogen types and their environmental impacts. The pathogen removal capabilities in both conventional OWTs and I/A systems should be taken into consideration in future standards and regulations controlling the microbial contamination in OWTs. Although the policy and standards evaluations are out of the scope of this review, they are also essential and urgent matters for the development and optimization of OWTs to enhance pathogen removal, which future works can also focus on.

## 7. Conclusions

The reduction in pathogens from domestic wastewater is a high priority for environmental and public health protection. Both filtration-based treatment systems and CWs have shown the potential to remove substantial quantities of pathogens, and the hybrid/multi-stages OWTs have been found to remove a significantly higher level of pathogens from domestic wastewater than traditional OWTs. HRT is the most significant contributing factor that can be optimized for higher pathogen removal. The grain sizes of filter media and the water distribution method are also important factors for pathogen elimination, as they are all directly related to the HRT in the systems. The fine size of filter media, uniform distribution of wastewater over the filter surface, and low hydraulic loading rate could enhance the HRT, and subsequently, increase the removal of pathogens. Furthermore, emerging materials such as zeolite and biochar can enhance the pathogen removal efficiency via a variety of mechanisms. OWTs with subsequent disinfection units could significantly improve the pathogen removal performance, which is important to eliminate the health risks associated with onsite wastewater used for agriculture applications.

**Author Contributions:** Conceptualization, M.W. and X.M.; methodology, M.W.; formal analysis, M.W. and J.Z.; investigation, M.W. and J.Z.; resources, X.M.; writing—original draft preparation, M.W. and J.Z.; writing—review and editing, M.W., J.Z. and X.M.; visualization, M.W. and J.Z.; supervision, X.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by a grant to the Center for Clean Water Technology (CCWT) from the New York State Department of Environmental Conservation [NYS-DEC01-C00366GG-3350000].

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

- USEPA. *Onsite Wastewater Treatment System Manual*; U.S. Environmental Protection Agency: Washington, DC, USA, 2002.
- Lusk, M.G.; Toor, G.S.; Yang, Y.Y.; Mechtensimer, S.; De, M.; Obreza, T.A. A review of the fate and transport of nitrogen, phosphorus, pathogens, and trace organic chemicals in septic systems. *Crit. Rev. Environ. Sci. Technol.* **2017**, *47*, 455–541. [[CrossRef](#)]
- Wu, S.; Carvalho, P.N.; Müller, J.A.; Manoj, V.R.; Dong, R. Sanitation in constructed wetlands: A review on the removal of human pathogens and fecal indicators. *Sci. Total Environ.* **2016**, *541*, 8–22. [[CrossRef](#)]
- Amin, N.; Liu, P.; Foster, T.; Rahman, M.; Miah, M.R.; Ahmed, G.B.; Kabir, M.; Raj, S.; Norman, G.; Moe, C.L.; et al. Pathogen flows from on-site sanitation systems in low-income urban neighborhoods, Dhaka: A quantitative environmental assessment. *Int. J. Hyg. Environ. Health* **2020**, *230*, 113619. [[CrossRef](#)]
- Withers, P.J.A.; Jarvie, H.P.; Stoate, C. Quantifying the impact of septic tank systems on eutrophication risk in rural headwaters. *Environ. Int.* **2011**, *37*, 644–653. [[CrossRef](#)]
- Gardner, T.; Geary, P.; Gordon, I. Ecological sustainability and on-site effluent treatment systems. *Aust. J. Environ. Manag.* **1997**, *4*, 144–156. [[CrossRef](#)]
- United Nations. *Factsheet International Year of Freshwater 2003*; UNESCO and the UN Department of Economic and Social Affairs: New York, NY, USA, 2003.
- Saeed, T.; Sun, G. A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media. *J. Environ. Manag.* **2012**, *112*, 429–448. [[CrossRef](#)]
- Shingare, R.P.; Thawale, P.R.; Raghunathan, K.; Mishra, A.; Kumar, S. Constructed wetland for wastewater reuse: Role and efficiency in removing enteric pathogens. *J. Environ. Manag.* **2019**, *246*, 444–461. [[CrossRef](#)]
- Akunna, J.C.; O’Keeffe, J.M.; Allan, R. Reviewing factors affecting the effectiveness of decentralised domestic wastewater treatment systems for phosphorus and pathogen removal. *Desalin. Water Treat.* **2017**, *91*, 40–47. [[CrossRef](#)]
- Yates, M.V. *Septic Tank Siting to Minimize the Contamination of Ground Water by Microorganisms*; US Environmental Protection Agency, Office of Ground-Water Protection, Office of Water: Washington, DC, USA, 1987.
- Yoder, J.; Roberts, V.; Craun, G.F.; Hill, V.; Hicks, L.; Alexander, N.T.; Radke, V.; Calderon, R.L.; Hlavsa, M.C.; Beach, M.J.; et al. Morbidity and Mortality Weekly Report Surveillance for Waterborne Disease and Outbreaks Associated with Recreational Water Use and Other Aquatic Facility-Associated Health Events—United States, 2005–2006 and Surveillance for Waterborne Disease and Outbreaks. *Morb. Mortal. Wkly. Rep.* **2008**, *57*, 39–63.
- Payment, P.; Locas, A. Pathogens in Water: Value and Limits of Correlation with Microbial Indicators. *Ground Water* **2011**, *49*, 4–11. [[CrossRef](#)]
- Smith, J.E. *Technology Transfer Handbook: Management of Water Treatment Plant Residuals*; U.S. Environmental Protection Agency: Washington, DC, USA, 1996.
- Payment, P.; Waite, M.; Dufour, A. Introducing parameters for the assessment of drinking water quality. *Assess. Microb. Saf. Drink. Water Improv. Approaches Methods* **2003**, 9789264099, 47–77. [[CrossRef](#)]
- Yates, M.V. Classical Indicators in the 21st Century—Far and Beyond the Coliform. *Water Environ. Res.* **2007**, *79*, 279–286. [[CrossRef](#)] [[PubMed](#)]
- Simpson, J.M.; Santo Domingo, J.W.; Reasoner, D.J. Microbial source tracking: State of the science. *Environ. Sci. Technol.* **2002**, *36*, 5279–5288. [[CrossRef](#)]
- Wong, K.; Fong, T.T.; Bibby, K.; Molina, M. Application of enteric viruses for fecal pollution source tracking in environmental waters. *Environ. Int.* **2012**, *45*, 151–164. [[CrossRef](#)] [[PubMed](#)]
- Melnick, J.L.; Gerba, C.P.; Wallis, C. Viruses in water. *Bull. World Health Organ.* **1978**, *56*, 499–508.
- Desselberger, U.; Gray, J. Viral gastroenteritis. *Medicine* **2009**, *37*, 594–598. [[CrossRef](#)] [[PubMed](#)]
- Carter, M.J. Enterically infecting viruses: Pathogenicity, transmission and significance for food and waterborne infection. *J. Appl. Microbiol.* **2005**, *98*, 1354–1380. [[CrossRef](#)]
- Okoh, A.I.; Sibanda, T.; Gusha, S.S. Inadequately treated wastewater as a source of human enteric viruses in the environment. *Int. J. Environ. Res. Public Health* **2010**, *7*, 2620–2637. [[CrossRef](#)]
- Osuolale, O.; Okoh, A. Human enteric bacteria and viruses in five wastewater treatment plants in the Eastern Cape, South Africa. *J. Infect. Public Health* **2017**, *10*, 541–547. [[CrossRef](#)]
- Karim, M.R.; Manshadi, F.D.; Karpiscak, M.M.; Gerba, C.P. The persistence and removal of enteric pathogens in constructed wetlands. *Water Res.* **2004**, *38*, 1831–1837. [[CrossRef](#)]
- Quiñónez-Díaz, M.D.J.; Karpiscak, M.M.; Ellman, E.D.; Gerba, C.P. Removal of pathogenic and indicator microorganisms by a constructed wetland receiving untreated domestic wastewater. *J. Environ. Sci. Health Part A* **2001**, *36*, 1311–1320. [[CrossRef](#)] [[PubMed](#)]
- Azadpour-keiley, A.; Faulkner, B.R.; Chen, J. *Movement and Longevity of Viruses in the Subsurface*; Environmental Protection Agency, National Risk Management Research Laboratory: Washington, DC, USA, 2003; pp. 1–25.



27. Rambags, F.; Tanner, C.C.; Stott, R.; Schipper, L.A. Bacteria and virus removal in denitrifying bioreactors: Effects of media type and age. *Ecol. Eng.* **2019**, *138*, 46–53. [\[CrossRef\]](#)
28. Olson, M.R.; Axler, R.P.; Hicks, R.E.; Henneck, J.R.; McCarthy, B.J. Seasonal virus removal by alternative onsite wastewater treatment systems. *J. Water Health* **2005**, *3*, 139–155. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Barrett, E.C.; Sobsey, M.D.; House, C.H.; White, K.D. Microbial indicator removal in onsite constructed wetlands for wastewater treatment in the southeastern U.S. *Water Sci. Technol.* **2001**, *44*, 177–182. [\[CrossRef\]](#)
30. García, M.; Soto, F.; González, J.M.; Bécares, E. A comparison of bacterial removal efficiencies in constructed wetlands and algae-based systems. *Ecol. Eng.* **2008**, *32*, 238–243. [\[CrossRef\]](#)
31. Hijnen, W.A.M.; Schijven, J.F.; Bonné, P.; Visser, A.; Medema, G.J. Elimination of viruses, bacteria and protozoan oocysts by slow sand filtration. *Water Sci. Technol.* **2004**, *50*, 147–154. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Schijven, J.F.; De Bruin, H.A.M.; Hassanizadeh, S.M.; De Roda Husman, A.M. Bacteriophages and clostridium spores as indicator organisms for removal of pathogens by passage through saturated dune sand. *Water Res.* **2003**, *37*, 2186–2194. [\[CrossRef\]](#)
33. Novak, J.S.; Juneja, V.K.; McClane, B.A. An ultrastructural comparison of spores from various strains of *Clostridium perfringens* and correlations with heat resistance parameters. *Int. J. Food Microbiol.* **2003**, *86*, 239–247. [\[CrossRef\]](#)
34. Hai, F.I.; Riley, T.; Shawkat, S.; Magram, S.F.; Yamamoto, K. Removal of pathogens by membrane bioreactors: A review of the mechanisms, influencing factors and reduction in chemical disinfectant dosing. *Water* **2014**, *6*, 3603–3630. [\[CrossRef\]](#)
35. Fuhriemann, S.; Winkler, M.S.; Pham-Duc, P.; Do-Trung, D.; Schindler, C.; Utzinger, J.; Cissé, G. Intestinal parasite infections and associated risk factors in communities exposed to wastewater in urban and peri-urban transition zones in Hanoi, Vietnam. *Parasites Vectors* **2016**, *9*, 1–14. [\[CrossRef\]](#)
36. Nelson, K.L.; Cisneros, B.J.; Tchobanoglous, G.; Darby, J.L. Sludge accumulation, characteristics, and pathogen inactivation in four primary waste stabilization ponds in central Mexico. *Water Res.* **2004**, *38*, 111–127. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Koné, D.; Cofie, O.; Zurbrugg, C.; Gallizzi, K.; Moser, D.; Drescher, S.; Strauss, M. Helminth eggs inactivation efficiency by faecal sludge dewatering and co-composting in tropical climates. *Water Res.* **2007**, *41*, 4397–4402. [\[CrossRef\]](#)
38. Sanguinetti, G.S.; Tortul, C.; García, M.C.; Ferrer, V.; Montangero, A.; Strauss, M. Investigating helminth eggs and *Salmonella* sp. in stabilization ponds treating septage. *Water Sci. Technol.* **2005**, *51*, 239–247. [\[CrossRef\]](#)
39. Winward, G.P.; Avery, L.M.; Frazer-Williams, R.; Pidou, M.; Jeffrey, P.; Stephenson, T.; Jefferson, B. A study of the microbial quality of grey water and an evaluation of treatment technologies for reuse. *Ecol. Eng.* **2008**, *32*, 187–197. [\[CrossRef\]](#)
40. Falabi, J.A.; Gerba, C.P.; Karpiscak, M.M. Giardia and Cryptosporidium removal from waste-water by a duckweed (*Lemna gibba* L.) covered pond. *Lett. Appl. Microbiol.* **2002**, *34*, 384–387. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Reinoso, R.; Torres, L.A.; Bécares, E. Efficiency of natural systems for removal of bacteria and pathogenic parasites from wastewater. *Sci. Total Environ.* **2008**, *395*, 80–86. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Horn, T.B.; Zerwes, F.V.; Kist, L.T.; Machado, Ê.L. Constructed wetland and photocatalytic ozonation for university sewage treatment. *Ecol. Eng.* **2014**, *63*, 134–141. [\[CrossRef\]](#)
43. Adrados, B.; Arias, C.A.; Pérez, L.M.; Codony, F.; Bécares, E.; Brix, H.; Morató, J. Comparison of removal efficiency of pathogenic microbes in four types of wastewater treatment systems in Denmark. *Ecol. Eng.* **2018**, *124*, 1–6. [\[CrossRef\]](#)
44. García, J.A.; Paredes, D.; Cubillos, J.A. Effect of plants and the combination of wetland treatment type systems on pathogen removal in tropical climate conditions. *Ecol. Eng.* **2013**, *58*, 57–62. [\[CrossRef\]](#)
45. Richter, A.Y.; Weaver, R.W. Treatment of domestic wastewater by subsurface flow constructed wetlands filled with gravel and tire chip media. *Environ. Technol.* **2003**, *24*, 1561–1567. [\[CrossRef\]](#)
46. Mbuligwe, S.E. Applicability of a septic tank/engineered wetland coupled system in the treatment and recycling of wastewater from a small community. *Environ. Manag.* **2005**, *35*, 99–108. [\[CrossRef\]](#)
47. Latrach, L.; Ouazzani, N.; Masunaga, T.; Hejjaj, A.; Bouhoum, K.; Mahi, M.; Mandi, L. Domestic wastewater disinfection by combined treatment using multi-soil-layering system and sand filters (MSL-SF): A laboratory pilot study. *Ecol. Eng.* **2016**, *91*, 294–301. [\[CrossRef\]](#)
48. Pundsack, J.; Axler, R.; Hicks, R.; Henneck, J.; Nordman, D.; McCarthy, B. Seasonal Pathogen Removal by Alternative On-Site Wastewater Treatment Systems. *Water Environ. Res.* **2001**, *73*, 204–212. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Abdel-Shafy, H.I.; El-Khateeb, M.A. Integration of septic tank and constructed wetland for the treatment of wastewater in Egypt. *Desalin. Water Treat.* **2013**, *51*, 3539–3546. [\[CrossRef\]](#)
50. Boutilier, L.; Jamieson, R.; Gordon, R.; Lake, C.; Hart, W. Performance of surface-flow domestic wastewater treatment wetlands. *Wetlands* **2010**, *30*, 795–804. [\[CrossRef\]](#)
51. Humphries, B.; Weaver, L.; Burberry, L.; Webber, J.; Morgan, L.; Gregor, J. Microbial pathogen removal from domestic effluent using coral sand in Kiribati. *J. Appl. Microbiol.* **2020**, *128*, 1208–1220. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Soupier, M.L.; Hoover, N.L.; Moorman, T.B.; Law, J.Y.; Bearson, B.L. Impact of temperature and hydraulic retention time on pathogen and nutrient removal in woodchip bioreactors. *Ecol. Eng.* **2018**, *112*, 153–157. [\[CrossRef\]](#)
53. Eregno, F.E.; Heistad, A. On-site treated wastewater disposal systems—The role of stratified filter medias for reducing the risk of pollution. *Environ. Int.* **2019**, *124*, 302–311. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Zhou, J.; Wang, X.C.; Ji, Z.; Xu, L.; Yu, Z. Source identification of bacterial and viral pathogens and their survival/fading in the process of wastewater treatment, reclamation, and environmental reuse. *World J. Microbiol. Biotechnol.* **2015**, *31*, 109–120. [\[CrossRef\]](#) [\[PubMed\]](#)



55. Morató, J.; Codony, F.; Sánchez, O.; Pérez, L.M.; García, J.; Mas, J. Key design factors affecting microbial community composition and pathogenic organism removal in horizontal subsurface flow constructed wetlands. *Sci. Total Environ.* **2014**, *481*, 81–89. [[CrossRef](#)] [[PubMed](#)]
56. Perez-Mercado, L.F.; Lalander, C.; Joel, A.; Ottoson, J.; Dalahmeh, S.; Vinnerås, B. Biochar filters as an on-farm treatment to reduce pathogens when irrigating with wastewater-polluted sources. *J. Environ. Manag.* **2019**, *248*, 109295. [[CrossRef](#)] [[PubMed](#)]
57. Torrens, A.; Molle, P.; Boutin, C.; Salgot, M. Removal of bacterial and viral indicator in vertical flow constructed wetlands and intermittent sand filters. *Desalination* **2009**, *246*, 169–178. [[CrossRef](#)]
58. Zhang, C.M.; Xu, L.M.; Xu, P.C.; Wang, X.C. Elimination of viruses from domestic wastewater: Requirements and technologies. *World J. Microbiol. Biotechnol.* **2016**, *32*, 1–9. [[CrossRef](#)]
59. Jahne, M.A.; Brinkman, N.E.; Keely, S.P.; Zimmerman, B.D.; Wheaton, E.A.; Garland, J.L. Droplet digital PCR quantification of norovirus and adenovirus in decentralized wastewater and graywater collections: Implications for onsite reuse. *Water Res.* **2020**, *169*, 115213. [[CrossRef](#)] [[PubMed](#)]
60. Kauppinen, A.; Martikainen, K.; Matikka, V.; Veijalainen, A.M.; Pitkänen, T.; Heinonen-Tanski, H.; Miettinen, I.T. Sand filters for removal of microbes and nutrients from wastewater during a one-year pilot study in a cold temperate climate. *J. Environ. Manag.* **2014**, *133*, 206–213. [[CrossRef](#)]
61. Rachmadi, A.T.; Kitajima, M.; Pepper, I.L.; Gerba, C.P. Enteric and indicator virus removal by surface flow wetlands. *Sci. Total Environ.* **2016**, *542*, 976–982. [[CrossRef](#)] [[PubMed](#)]
62. Kaliakatsos, A.; Kalogerakis, N.; Manios, T.; Venieri, D. Efficiency of two constructed wetland systems for wastewater treatment: Removal of bacterial indicators and enteric viruses. *J. Chem. Technol. Biotechnol.* **2019**, *94*, 2123–2130. [[CrossRef](#)]
63. Stott, R.; May, E.; Mara, D.D. Parasite removal by natural wastewater treatment systems: Performance of waste stabilisation ponds and constructed wetlands. *Water Sci. Technol.* **2003**, *48*, 97–104. [[CrossRef](#)]
64. Wand, H.; Vacca, G.; Kusch, P.; Krüger, M.; Kästner, M. Removal of bacteria by filtration in planted and non-planted sand columns. *Water Res.* **2007**, *41*, 159–167. [[CrossRef](#)] [[PubMed](#)]
65. Stevik, T.K.; Aa, K.; Ausland, G.; Hanssen, J.F. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: A review. *Water Res.* **2004**, *38*, 1355–1367. [[CrossRef](#)] [[PubMed](#)]
66. Ansari, A.A.; Gill, S.S.; Gill, R.; Lanza, G.R.; Newman, L. Phytoremediation: Management of environmental contaminants, volume 4. *Phytoremediation Manag. Environ. Contam.* **2016**, *4*, 1–409. [[CrossRef](#)]
67. Jasper, J.T.; Nguyen, M.T.; Jones, Z.L.; Ismail, N.S.; Sedlak, D.L.; Sharp, J.O.; Luthy, R.G.; Horne, A.J.; Nelson, K.L. Unit process wetlands for removal of trace organic contaminants and pathogens from municipal wastewater effluents. *Environ. Eng. Sci.* **2013**, *30*, 421–436. [[CrossRef](#)]
68. USEPA. *A Handbook of Constructed Wetlands: Guide to Creating Wetlands for; Agricultural Wastewater, Domestic Wastewater, Coal Mine Drainage, Stormwater in the Mid-Atlantic Region*; Office of Wetlands, Oceans and Watersheds: Washington, DC, USA, 2000; Volume 1.
69. Sen, T.K. Processes in Pathogenic Biocolloidal Contaminants Transport in Saturated and Unsaturated Porous Media: A Review. *Water. Air. Soil Pollut.* **2011**, *216*, 239–256. [[CrossRef](#)]
70. Boutilier, L.; Jamieson, R.; Gordon, R.; Lake, C.; Hart, W. Adsorption, sedimentation, and inactivation of *E. coli* within wastewater treatment wetlands. *Water Res.* **2009**, *43*, 4370–4380. [[CrossRef](#)]
71. Vacca, G.; Wand, H.; Nikolausz, M.; Kusch, P.; Kästner, M. Effect of plants and filter materials on bacteria removal in pilot-scale constructed wetlands. *Water Res.* **2005**, *39*, 1361–1373. [[CrossRef](#)] [[PubMed](#)]
72. Cizek, A.R.; Characklis, G.W.; Krometis, L.A.; Hayes, J.A.; Simmons, O.D.; Di Lonardo, S.; Alderisio, K.A.; Sobsey, M.D. Comparing the partitioning behavior of *Giardia* and *Cryptosporidium* with that of indicator organisms in stormwater runoff. *Water Res.* **2008**, *42*, 4421–4438. [[CrossRef](#)] [[PubMed](#)]
73. Sengupta, M.E.; Thamsborg, S.M.; Andersen, T.J.; Olsen, A.; Dalsgaard, A. Sedimentation of helminth eggs in water. *Water Res.* **2011**, *45*, 4651–4660. [[CrossRef](#)] [[PubMed](#)]
74. Dai, X.; Boll, J. Settling velocity of *Cryptosporidium parvum* and *Giardia lamblia*. *Water Res.* **2006**, *40*, 1321–1325. [[CrossRef](#)] [[PubMed](#)]
75. Kansime, F.; Van Bruggen, J.J.A. Distribution and retention of faecal coliforms in the Nakivubo wetland in Kampala, Uganda. *Water Sci. Technol.* **2001**, *44*, 199–206. [[CrossRef](#)]
76. Gikas, G.D.; Tsihrintzis, V.A. A small-size vertical flow constructed wetland for on-site treatment of household wastewater. *Ecol. Eng.* **2012**, *44*, 337–343. [[CrossRef](#)]
77. Zhang, Z.N.; Su, G.; Ge, G.L.; Zhao, G.Y.; Wu, Z.Y. Rural sanitary domestic flushing toilet system. *Water Res.* **1996**, *30*, 2826–2829. [[CrossRef](#)]
78. Alufasi, R.; Gere, J.; Chakauya, E.; Lebea, P.; Parawira, W.; Chingwaru, W. Mechanisms of pathogen removal by macrophytes in constructed wetlands. *Environ. Technol. Rev.* **2017**, *6*, 135–144. [[CrossRef](#)]
79. Redder, A.; Dürr, M.; Daeschlein, G.; Baeder-Bederski, O.; Koch, C.; Müller, R.; Exner, M.; Borneff-Lipp, M. Constructed wetlands—Are they safe in reducing protozoan parasites? *Int. J. Hyg. Environ. Health* **2010**, *213*, 72–77. [[CrossRef](#)] [[PubMed](#)]
80. Sleytr, K.; Tietz, A.; Langergraber, G.; Haberl, R. Investigation of bacterial removal during the filtration process in constructed wetlands. *Sci. Total Environ.* **2007**, *380*, 173–180. [[CrossRef](#)] [[PubMed](#)]

81. Bradford, S.A.; Simunek, J.; Bettahar, M.; Tadassa, Y.F.; Van Genuchten, M.T.; Yates, S.R. Straining of colloids at textural interfaces. *Water Resour. Res.* **2005**, *41*, 1–18. [[CrossRef](#)]
82. Kristiansen, R. Sand-Filter Trenches for Purification of Septic Tank Effluent: III. The Microflora. *J. Environ. Qual.* **1981**, *10*, 361–364. [[CrossRef](#)]
83. Tan, Y.; Bond, W.J.; Griffin, D.M. Transport of Bacteria during Unsteady Unsaturated Soil Water Flow. *Soil Sci. Soc. Am. J.* **1992**, *56*, 1331–1340. [[CrossRef](#)]
84. Tufenkji, N. Modeling microbial transport in porous media: Traditional approaches and recent developments. *Adv. Water Resour.* **2007**, *30*, 1455–1469. [[CrossRef](#)]
85. Sharma, M.M.; Chang, Y.I.; Yen, T.F. Reversible and irreversible surface charge modification of bacteria for facilitating transport through porous media. *Colloids Surf.* **1985**, *16*, 193–206. [[CrossRef](#)]
86. Derjaguin, B.; Landau, L. Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. *Prog. Surf. Sci.* **1993**, *43*, 30–59. [[CrossRef](#)]
87. Verwey, E.J.W. Theory of the stability of lyophobic colloids. *J. Phys. Colloid Chem.* **1947**, *51*, 631–636. [[CrossRef](#)] [[PubMed](#)]
88. Mozes, N.; Marchal, F.; Hermesse, M.P.; Van Haecht, J.L.; Reuliaux, L.; Leonard, A.J.; Rouxhet, P.G. Immobilization of microorganisms by adhesion: Interplay of electrostatic and nonelectrostatic interactions. *Biotechnol. Bioeng.* **1987**, *30*, 439–450. [[CrossRef](#)]
89. Poortinga, A.T.; Bos, R.; Norde, W.; Busscher, H.J. Electric double layer interactions in bacterial adhesion to surfaces. *Surf. Sci. Rep.* **2002**, *47*, 1–32. [[CrossRef](#)]
90. Penrod, S.L.; Olson, T.M.; Grant, S.B. Deposition kinetics of two viruses in packed beds of quartz granular media. *Langmuir* **1996**, *12*, 5576–5587. [[CrossRef](#)]
91. Chen, G.; Walker, S.L. Fecal indicator bacteria transport and deposition in saturated and unsaturated porous media. *Environ. Sci. Technol.* **2012**, *46*, 8782–8790. [[CrossRef](#)]
92. Schijven, J.F.; Hassanizadeh, S.M. Removal of viruses by soil passage: Overview of modeling, processes, and parameters. *Crit. Rev. Environ. Sci. Technol.* **2000**, *30*, 49–127. [[CrossRef](#)]
93. Zhao, B.; Zhang, H.; Zhang, J.; Jin, Y. Virus adsorption and inactivation in soil as influenced by autochthonous microorganisms and water content. *Soil Biol. Biochem.* **2008**, *40*, 649–659. [[CrossRef](#)]
94. Sasidharan, S.; Torkzaban, S.; Bradford, S.A.; Kookana, R.; Page, D.; Cook, P.G. Transport and retention of bacteria and viruses in biochar-amended sand. *Sci. Total Environ.* **2016**, *548–549*, 100–109. [[CrossRef](#)]
95. Bales, R.C.; Hinkle, S.R.; Kroeger, T.W.; Stocking, K.; Gerba, C.P. Bacteriophage Adsorption during Transport through Porous Media: Chemical Perturbations and Reversibility. *Environ. Sci. Technol.* **1991**, *25*, 2088–2095. [[CrossRef](#)]
96. Lance, J.C.; Gerba, C.P. Virus movement in soil during saturated and unsaturated flow. *Appl. Environ. Microbiol.* **1984**, *47*, 335–337. [[CrossRef](#)] [[PubMed](#)]
97. Huysman, F.; Verstraete, W. Water-facilitated transport of bacteria in unsaturated soil columns: Influence of cell surface hydrophobicity and soil properties. *Soil Biol. Biochem.* **1993**, *25*, 83–90. [[CrossRef](#)]
98. Goyal, S.M.; Gerba, C.P. Comparative adsorption of human enteroviruses, simian rotavirus, and selected bacteriophages to soils. *Appl. Environ. Microbiol.* **1979**, *38*, 241–247. [[CrossRef](#)]
99. Davies-Colley, R.J.; Donnison, A.M.; Speed, D.J.; Ross, C.M.; Nagels, J.W. Inactivation of faecal indicator micro-organisms in waste stabilisation ponds: Interactions of environmental factors with sunlight. *Water Res.* **1999**, *33*, 1220–1230. [[CrossRef](#)]
100. Davies-Colley, R.J.; Craggs, R.J.; Park, J.; Sukias, J.P.S.; Nagels, J.W.; Stott, R. Virus removal in a pilot-scale “advanced” pond system as indicated by somatic and F-RNA bacteriophages. *Water Sci. Technol.* **2005**, *51*, 107–110. [[CrossRef](#)] [[PubMed](#)]
101. Nguyen, M.T.; Jasper, J.T.; Boehm, A.B.; Nelson, K.L. Sunlight inactivation of fecal indicator bacteria in open-water unit process treatment wetlands: Modeling endogenous and exogenous inactivation rates. *Water Res.* **2015**, *83*, 282–292. [[CrossRef](#)] [[PubMed](#)]
102. Davies-Colley, R.J.; Donnison, A.M.; Speed, D.J. Towards a mechanistic understanding of pond disinfection. *Water Sci. Technol.* **2000**, *42*, 149–158. [[CrossRef](#)]
103. Kohn, T.; Nelson, K.L. Sunlight-mediated inactivation of MS2 coliphage via exogenous singlet oxygen produced by sensitizers in natural waters. *Environ. Sci. Technol.* **2007**, *41*, 192–197. [[CrossRef](#)] [[PubMed](#)]
104. Silverman, A.I.; Peterson, B.M.; Boehm, A.B.; McNeill, K.; Nelson, K.L. Sunlight inactivation of human viruses and bacteriophages in coastal waters containing natural photosensitizers. *Environ. Sci. Technol.* **2013**, *47*, 1870–1878. [[CrossRef](#)]
105. Muela, A.; García-Bringas, J.M.; Arana, I.; Barcina, I. The effect of simulated solar radiation on *Escherichia coli*: The relative roles of UV-B, UV-A, and photosynthetically active radiation. *Microb. Ecol.* **2000**, *39*, 65–71. [[CrossRef](#)]
106. Kadir, K.; Nelson, K.L. Sunlight mediated inactivation mechanisms of *Enterococcus faecalis* and *Escherichia coli* in clear water versus waste stabilization pond water. *Water Res.* **2014**, *50*, 307–317. [[CrossRef](#)]
107. Sinton, L.W.; Hall, C.H.; Lynch, P.A.; Davies-Colley, R.J. Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. *Appl. Environ. Microbiol.* **2002**, *68*, 1122–1131. [[CrossRef](#)] [[PubMed](#)]
108. Davies, M.J.; Truscott, R.J.W. Photo-oxidation of proteins and its role in cataractogenesis. *J. Photochem. Photobiol. B Biol.* **2001**, *63*, 114–125. [[CrossRef](#)]
109. Cunningham, M.L.; Krinsky, N.I.; Giovanazzi, S.M.; Peak, M.J. Superoxide anion is generated from cellular metabolites by solar radiation and its components. *J. Free Radic. Biol. Med.* **1985**, *1*, 381–385. [[CrossRef](#)]
110. Blough, N.V.; Zepp, R.G. Reactive Oxygen Species in Natural Waters. *Act. Oxyg. Chem.* **1995**, 280–333. [[CrossRef](#)]

111. Cooper, W.J.; Zika, R.G.; Petasne, R.G.; Fischer, A.M. Sunlight-Induced Photochemistry of Humic Substances in Natural Waters: Major Reactive Species. *Aquat. Humic Subst.* **1989**, *22*, 333–362.
112. Mayo, A.W. Kinetics of bacterial mortality in granular bed wetlands. *Phys. Chem. Earth* **2004**, *29*, 1259–1264. [[CrossRef](#)]
113. Decamp, O.; Warren, A. Bacterivory in ciliates isolated from constructed wetlands (reed beds) used for wastewater treatment. *Water Res.* **1998**, *32*, 1989–1996. [[CrossRef](#)]
114. Zaleski, K.J.; Josephson, K.L.; Gerba, C.P.; Pepper, I.A.N.L. Survival, Growth, and Regrowth of Enteric Indicator and Pathogenic Bacteria in Biosolids, Compost, Soil, and Land Applied Biosolids. *J. Residuals Sci. Technol.* **2005**, *2*, 49–63.
115. Horswell, J.; Hewitt, J.; Prosser, J.; Van Schaik, A.; Croucher, D.; MacDonald, C.; Burford, P.; Susarla, P.; Bickers, P.; Speir, T. Mobility and survival of *Salmonella* Typhimurium and human adenovirus from spiked sewage sludge applied to soil columns. *J. Appl. Microbiol.* **2010**, *108*, 104–114. [[CrossRef](#)]
116. Olson, M.R.; Axler, R.P.; Hicks, R.E. Effects of freezing and storage temperature on MS2 viability. *J. Virol. Methods* **2004**, *122*, 147–152. [[CrossRef](#)]
117. Sjogren, R.E. Prolonged survival of an environmental *Escherichia coli* in laboratory soil microcosms. *Water. Air. Soil Pollut.* **1994**, *75*, 389–403. [[CrossRef](#)]
118. Cuthbert, W.A.; Panes, J.J.; Hill, E.C. Survival of *Bacterium coli* type I and *Streptococcus faecalis* in soil. *J. Appl. Bacteriol.* **1955**, *18*, 408–414. [[CrossRef](#)]
119. Fernández, A.; Tejedor, C.; Chordi, A. Effect of different factors on the die-off of fecal bacteria in a stabilization pond purification plant. *Water Res.* **1992**, *26*, 1093–1098. [[CrossRef](#)]
120. Headley, T.; Nivala, J.; Kassa, K.; Olsson, L.; Wallace, S.; Brix, H.; van Afferden, M.; Müller, R. *Escherichia coli* removal and internal dynamics in subsurface flow ecotechnologies: Effects of design and plants. *Ecol. Eng.* **2013**, *61*, 564–574. [[CrossRef](#)]
121. Atlas, R.M. Sanitation and Disease—Health Aspects of Excreta and Waste-water Management. *JAWRA J. Am. Water Resour. Assoc.* **1984**, *20*, 803. [[CrossRef](#)]
122. Akpor, O.B.; Ogundeyi, M.D.; Olaolu, T.D.; Aderiyi, B.I. Microbial Roles and Dynamics in Wastewater Treatment Systems: An Overview. *Int. J. Pure Appl. Biosci.* **2014**, *2*, 156–168.
123. Jiménez, B.; Maya, C.; Galván, M. Helminth ova control in wastewater and sludge for advanced and conventional sanitation. *Water Sci. Technol.* **2007**, *56*, 43–51. [[CrossRef](#)]
124. Ali, T.H.; Saleh, D.S. A simplified experimental model for clearance of some pathogenic bacteria using common bacterivorous ciliated spp. in Tigris river. *Appl. Water Sci.* **2014**, *4*, 63–71. [[CrossRef](#)]
125. Shapiro, O.H.; Kushmaro, A.; Brenner, A. Bacteriophage predation regulates microbial abundance and diversity in a full-scale bioreactor treating industrial wastewater. *ISME J.* **2010**, *4*, 327–336. [[CrossRef](#)] [[PubMed](#)]
126. Alexander, M. Why microbial predators and parasites do not eliminate their prey and hosts. *Annu. Rev. Microbiol.* **1981**, *35*, 113–133. [[CrossRef](#)]
127. Pernthaler, J. Predation on prokaryotes in the water column and its ecological implications. *Nat. Rev. Microbiol.* **2005**, *3*, 537–546. [[CrossRef](#)]
128. Decamp, O.; Warren, A.; Sanchez, R. The role of ciliated protozoa in subsurface flow wetlands and their potential as bioindicators. *Water Sci. Technol.* **1999**, *40*, 91–98. [[CrossRef](#)]
129. Hijnen, W.A.M.; Dullemon, Y.J.; Schijven, J.F.; Hanzens-Brouwer, A.J.; Rosielle, M.; Medema, G. Removal and fate of *Cryptosporidium parvum*, *Clostridium perfringens* and small-sized centric diatoms (*Stephanodiscus hantzschii*) in slow sand filters. *Water Res.* **2007**, *41*, 2151–2162. [[CrossRef](#)] [[PubMed](#)]
130. USEPA. *Guidelines for Water Reuse*; U.S. Environmental Protection Agency: Washington, DC, USA, 2012.
131. Arias, C.A.; Cabello, A.; Brix, H.; Johansen, N.H. Removal of indicator bacteria from municipal wastewater in an experimental two-stage vertical flow constructed wetland system. *Water Sci. Technol.* **2003**, *48*, 35–41. [[CrossRef](#)] [[PubMed](#)]
132. Nivala, J.; Abdallat, G.; Aubron, T.; Al-Zreiqat, I.; Abbassi, B.; Wu, G.M.; van Afferden, M.; Müller, R.A. Vertical flow constructed wetlands for decentralized wastewater treatment in Jordan: Optimization of total nitrogen removal. *Sci. Total Environ.* **2019**, *671*, 495–504. [[CrossRef](#)]
133. Harrison, R.B.; Turner, N.S.; Hoyle, J.A.; Krejzl, J.; Tone, D.D.; Henry, C.L.; Isaksen, P.J.; Xue, D. Treatment of Septic Effluent for Fecal Coliform and. *Water. Air. Soil Pollut.* **2000**, *124*, 205–215. [[CrossRef](#)]
134. Arora, S.; Kazmi, A.A. The effect of seasonal temperature on pathogen removal efficacy of vermifilter for wastewater treatment. *Water Res.* **2015**, *74*, 88–99. [[CrossRef](#)] [[PubMed](#)]
135. Kadam, A.M.; Oza, G.H.; Nemade, P.D.; Shankar, H.S. Pathogen removal from municipal wastewater in Constructed Soil Filter. *Ecol. Eng.* **2008**, *33*, 37–44. [[CrossRef](#)]
136. Rambags, F.; Tanner, C.C.; Stott, R.; Schipper, L.A. Fecal Bacteria, Bacteriophage, and Nutrient Reductions in a Full-Scale Denitrifying Woodchip Bioreactor. *J. Environ. Qual.* **2016**, *45*, 847–854. [[CrossRef](#)]
137. Tunçsiper, B.; Ayaz, S.Ç.; Akça, L. Coliform bacteria removal from septic wastewater in a pilot-scale combined constructed wetland system. *Environ. Eng. Manag. J.* **2012**, *11*, 1873–1879. [[CrossRef](#)]
138. Park, E.; Mancl, K.M.; Tuovinen, O.H.; Bisesi, M.S.; Lee, J. Ensuring safe reuse of residential wastewater: Reduction of microbes and genes using peat biofilter and batch chlorination in an on-site treatment system. *J. Appl. Microbiol.* **2016**, *121*, 1777–1788. [[CrossRef](#)]

139. Friedler, E.; Kovalio, R.; Ben-Zvi, A. Comparative study of the microbial quality of greywater treated by three on-site treatment systems. *Environ. Technol.* **2006**, *27*, 653–663. [\[CrossRef\]](#)
140. de Oliveira Cruz, L.M.; Tonetti, A.L.; Gomes, B.G.L.A. Association of septic tank and sand filter for wastewater treatment: Full-scale feasibility for decentralized sanitation. *J. Water Sanit. Hyg. Dev.* **2018**, *8*, 268–277. [\[CrossRef\]](#)
141. Shingare, R.P.; Nanekar, S.V.; Thawale, P.R.; Karthik, R.; Juwarkar, A.A. Comparative study on removal of enteric pathogens from domestic wastewater using *Typha latifolia* and *Cyperus rotundus* along with different substrates. *Int. J. Phytoremediation* **2017**, *19*, 899–908. [\[CrossRef\]](#)
142. Latrach, L.; Ouazzani, N.; Hejjaj, A.; Zouhir, F.; Mahi, M.; Masunaga, T.; Mandi, L. Optimization of hydraulic efficiency and wastewater treatment performances using a new design of vertical flow Multi-Soil-Layering (MSL) technology. *Ecol. Eng.* **2018**, *117*, 140–152. [\[CrossRef\]](#)
143. Azaizeh, H.; Linden, K.G.; Barstow, C.; Kalbouneh, S.; Tellawi, A.; Albalawneh, A.; Gerchman, Y. Constructed wetlands combined with UV disinfection systems for removal of enteric pathogens and wastewater contaminants. *Water Sci. Technol.* **2013**, *67*, 651–657. [\[CrossRef\]](#) [\[PubMed\]](#)
144. Kadlec, R.H.; Cuvellier, C.; Stober, T. Performance of the Columbia, Missouri, treatment wetland. *Ecol. Eng.* **2010**, *36*, 672–684. [\[CrossRef\]](#)
145. Caselles-Osorio, A.; Villafañe, P.; Caballero, V.; Manzano, Y. Efficiency of mesocosm-scale constructed wetland systems for treatment of sanitary wastewater under tropical conditions. *Water. Air. Soil Pollut.* **2011**, *220*, 161–171. [\[CrossRef\]](#)
146. Zurita, F.; Carreón-Álvarez, A. Performance of three pilot-scale hybrid constructed wetlands for total coliforms and *Escherichia coli* removal from primary effluent—A 2-year study in a subtropical climate. *J. Water Health* **2015**, *13*, 446–458. [\[CrossRef\]](#)
147. Russo, N.; Marzo, A.; Randazzo, C.; Caggia, C.; Toscano, A.; Cirelli, G.L. Constructed wetlands combined with disinfection systems for removal of urban wastewater contaminants. *Sci. Total Environ.* **2019**, *656*, 558–566. [\[CrossRef\]](#)
148. Nokes, R.L.; Gerba, C.P.; Karpiscak, M.M. Microbial water quality improvement by small scale on-site subsurface wetland treatment. *J. Environ. Sci. Health Part A* **2003**, *38*, 1849–1855. [\[CrossRef\]](#) [\[PubMed\]](#)
149. Davison, L.; Headley, T.; Edmonds, M. On-site domestic wastewater treatment by reed bed in the moist subtropics. *Water Sci. Technol.* **2001**, *44*, 353–360. [\[CrossRef\]](#)
150. Galvão, A.; Matos, J.; Silva, M.; Ferreira, F. Constructed wetland performance and potential for microbial removal. *Desalin. Water Treat.* **2009**, *4*, 76–84. [\[CrossRef\]](#)
151. Bohórquez, E.; Paredes, D.; Arias, C.A. Vertical flow-constructed wetlands for domestic wastewater treatment under tropical conditions: Effect of different design and operational parameters. *Environ. Technol.* **2017**, *38*, 199–208. [\[CrossRef\]](#)
152. Chang, N.B.; Xuan, Z.; Daranpob, A.; Wanielista, M. A subsurface upflow wetland system for removal of nutrients and pathogens in on-site sewage treatment and disposal systems. *Environ. Eng. Sci.* **2011**, *28*, 11–24. [\[CrossRef\]](#)
153. Salter, H.E.; Boyle, L.; Ouki, S.K.; Quarmby, J.; Williams, S.C. The performance of tertiary lagoons in the United Kingdom: I. *Water Res.* **1999**, *33*, 3775–3781. [\[CrossRef\]](#)
154. Heistad, A.; Paruch, A.M.; Vråle, L.; Ádám, K.; Jenssen, P.D. A high-performance compact filter system treating domestic wastewater. *Ecol. Eng.* **2006**, *28*, 374–379. [\[CrossRef\]](#)
155. Jenssen, P.D.; Krogstad, T.; Paruch, A.M.; Mæhlum, T.; Adam, K.; Arias, C.A.; Heistad, A.; Jonsson, L.; Hellström, D.; Brix, H.; et al. Filter bed systems treating domestic wastewater in the Nordic countries—Performance and reuse of filter media. *Ecol. Eng.* **2010**, *36*, 1651–1659. [\[CrossRef\]](#)
156. Tanner, C.C.; Sukias, J.P.S.; Headley, T.R.; Yates, C.R.; Stott, R. Constructed wetlands and denitrifying bioreactors for on-site and decentralised wastewater treatment: Comparison of five alternative configurations. *Ecol. Eng.* **2012**, *42*, 112–123. [\[CrossRef\]](#)
157. Ulrich, H.; Klaus, D.; Irmgard, F.; Annette, H.; Juan, L.P.; Regine, S. Microbiological investigations for sanitary assessment of wastewater treated in constructed wetlands. *Water Res.* **2005**, *39*, 4849–4858. [\[CrossRef\]](#) [\[PubMed\]](#)
158. Elfanssi, S.; Ouazzani, N.; Latrach, L.; Hejjaj, A.; Mandi, L. Phytoremediation of domestic wastewater using a hybrid constructed wetland in mountainous rural area. *Int. J. Phytoremediation* **2018**, *20*, 75–87. [\[CrossRef\]](#) [\[PubMed\]](#)
159. Sherman-Wood, R.; Tikekar, R.V. Decontamination of irrigation water using a combined sand filtration and UV-C light treatment. *J. Food Saf.* **2019**, *40*, e12744.
160. Hu, Z.; Gagnon, G.A. Impact of filter media on the performance of full-scale recirculating biofilters for treating multi-residential wastewater. *Water Res.* **2006**, *40*, 1474–1480. [\[CrossRef\]](#)
161. Sklarz, M.Y.; Gross, A.; Yakirevich, A.; Soares, M.I.M. A recirculating vertical flow constructed wetland for the treatment of domestic wastewater. *Desalination* **2009**, *246*, 617–624. [\[CrossRef\]](#)
162. Otter, P.; Hertel, S.; Ansari, J.; Lara, E.; Cano, R.; Arias, C.; Gregersen, P.; Grischek, T.; Benz, F.; Goldmaier, A.; et al. Disinfection for decentralized wastewater reuse in rural areas through wetlands and solar driven onsite chlorination. *Sci. Total Environ.* **2020**, *721*. [\[CrossRef\]](#) [\[PubMed\]](#)
163. Gill, L.W.; O’Súilleabháin, C.; Misstear, B.D.R.; Johnston, P.J. The Treatment Performance of Different Subsoils in Ireland Receiving On-Site Wastewater Effluent. *J. Environ. Qual.* **2007**, *36*, 1843–1855. [\[CrossRef\]](#) [\[PubMed\]](#)
164. Kay, D.; Crowther, J.; Stapleton, C.M.; Wyer, M.D.; Fewtrell, L.; Edwards, A.; Francis, C.A.; McDonald, A.T.; Watkins, J.; Wilkinson, J. Faecal indicator organism concentrations in sewage and treated effluents. *Water Res.* **2008**, *42*, 442–454. [\[CrossRef\]](#)
165. White, K.D.; Byrd, L.A.; Robertson, S.C.; O’Driscoll, J.P.; King, T. Evaluation of peat biofilters for onsite sewage management. *J. Environ. Health* **1995**, *58*, 11–17.



166. Dalahmeh, S.S.; Assayed, A.; Stenström, Y. Combined vertical-horizontal flow biochar filter for onsite wastewater treatment—Removal of organic matter, nitrogen and pathogens. *Appl. Sci.* **2019**, *9*, 5386. [\[CrossRef\]](#)
167. Vymazal, J. Removal of enteric bacteria in constructed treatment wetlands with emergent macrophytes: A review. *J. Environ. Sci. Health* **2005**, *40*, 1355–1367. [\[CrossRef\]](#) [\[PubMed\]](#)
168. O’Luanaigh, N.D.; Gill, L.W.; Misstear, B.D.R.; Johnston, P.M. The attenuation of microorganisms in on-site wastewater effluent discharged into highly permeable subsoils. *J. Contam. Hydrol.* **2012**, *142–143*, 126–139. [\[CrossRef\]](#) [\[PubMed\]](#)
169. Gobler, C.J.; Waugh, S.; Asato, C.; Clyde, P.M.; Nyer, S.C.; Graffam, M.; Brownawell, B.; Venkatesan, A.K.; Goleski, J.A.; Price, R.E.; et al. Removing 80–90% of nitrogen and organic contaminants with three distinct passive, lignocellulose-based on-site septic systems receiving municipal and residential wastewater. *Ecol. Eng.* **2021**, *161*, 106157. [\[CrossRef\]](#)
170. Zurita, F.; De Anda, J.; Belmont, M.A. Treatment of domestic wastewater and production of commercial flowers in vertical and horizontal subsurface-flow constructed wetlands. *Ecol. Eng.* **2009**, *35*, 861–869. [\[CrossRef\]](#)
171. Hespanhol, I.; Prost, A.M.E. Who guidelines and national standards for reuse and water quality. *Water Res.* **1994**, *28*, 119–124. [\[CrossRef\]](#)
172. World Health Organization. *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture: Report of a WHO Scientific Group [Meeting held in Geneva from 18 to 23 November 1987]*; World Health Organization: Geneva, Switzerland, 1989.
173. Angelakis, A.; Gikas, P. Water reuse: Overview of current practices and trends in the world with emphasis on EU states. *Water Util. J.* **2014**, *8*, 67–78.
174. Miranda, N.D.; Oliveira, E.L.; Silva, G.H.R. Study of constructed wetlands effluent disinfected with ozone. *Water Sci. Technol.* **2014**, *70*, 108–113. [\[CrossRef\]](#) [\[PubMed\]](#)
175. Toscano, A.; Hellio, C.; Marzo, A.; Milani, M.; Lebre, K.; Cirelli, G.L.; Langergraber, G. Removal efficiency of a constructed wetland combined with ultrasound and UV devices for wastewater reuse in agriculture. *Environ. Technol.* **2013**, *34*, 2327–2336. [\[CrossRef\]](#)
176. Babcock, R.W.; McNair, D.A.; Edling, L.A.; Nagato, H. Evaluation of a System for Residential Treatment and Reuse of Wastewater. *J. Environ. Eng.* **2004**, *130*, 766–773. [\[CrossRef\]](#)
177. Jenkins, M.W.; Tiwari, S.K.; Darby, J. Bacterial, viral and turbidity removal by intermittent slow sand filtration for household use in developing countries: Experimental investigation and modeling. *Water Res.* **2011**, *45*, 6227–6239. [\[CrossRef\]](#)
178. Darnault, C.J.G.; Garnier, P.; Kim, Y.-J.; Oveson, K.L.; Steenhuis, T.S.; Parlange, J.-Y.; Jenkins, M.; Ghiorse, W.C.; Baveye, P. Preferential Transport of *Cryptosporidium parvum* Oocysts in Variably Saturated Subsurface Environments. *Water Environ. Res.* **2003**, *75*, 113–120. [\[CrossRef\]](#)
179. Ushijima, K.; Ito, K.; Ito, R.; Funamizu, N. Greywater treatment by slanted soil system. *Ecol. Eng.* **2013**, *50*, 62–68. [\[CrossRef\]](#)
180. Ausland, G.; Stevik, T.K.; Hanssen, J.F.; Köhler, J.C.; Jenssen, P.D. Intermittent filtration of wastewater—Removal of fecal coliforms and fecal streptococci. *Water Res.* **2002**, *36*, 3507–3516. [\[CrossRef\]](#)
181. García, J.; Vivar, J.; Aromir, M.; Mujeriego, R. Role of hydraulic retention time and granular medium in microbial removal in tertiary treatment reed beds. *Water Res.* **2003**, *37*, 2645–2653. [\[CrossRef\]](#)
182. Nam, T.K.; Timmons, M.B.; Montemagno, C.D.; Tsukuda, S.M. Biofilm characteristics as affected by sand size and location in fluidized bed vessels. *Aquac. Eng.* **2000**, *22*, 213–224. [\[CrossRef\]](#)
183. Brix, H.; Arias, C.A.; Del Bubba, M. Media selection for sustainable phosphorus removal in subsurface flow constructed wetlands. *Water Sci. Technol.* **2001**, *44*, 47–54. [\[CrossRef\]](#) [\[PubMed\]](#)
184. Chang, N.B.; Wanielista, M.; Daranpob, A.; Xuan, Z.; Hossain, F. New performance-based passive septic tank underground drainfield for nutrient and pathogen removal using sorption media. *Environ. Eng. Sci.* **2010**, *27*, 469–482. [\[CrossRef\]](#)
185. Hossain, F.; Chang, N.-B.; Wanielista, M.; Xuan, Z.; Daranpob, A. Nitrification and Denitrification in a Passive On-site Wastewater Treatment System with a Recirculation Filtration Tank. *Water Qual. Expo. Heal.* **2010**, *2*, 31–46. [\[CrossRef\]](#)
186. Nilsson, C.; Lakshmanan, R.; Renman, G.; Rajarao, G.K. Efficacy of reactive mineral-based sorbents for phosphate, bacteria, nitrogen and TOC removal—Column experiment in recirculation batch mode. *Water Res.* **2013**, *47*, 5165–5175. [\[CrossRef\]](#)
187. Nilsson, C.; Renman, G.; Johansson Westholm, L.; Renman, A.; Drizo, A. Effect of organic load on phosphorus and bacteria removal from wastewater using alkaline filter materials. *Water Res.* **2013**, *47*, 6289–6297. [\[CrossRef\]](#)
188. Bashan, Y.; Levany, H. Adsorption of the rhizosphere bacterium *Azospirillum brasilense* Cd to soil, sand and peat particles. *J. Gen. Microbiol.* **1988**, *134*, 1811–1820. [\[CrossRef\]](#)
189. Robertson, W.D.; Ford, G.I.; Lombardo, P.S. Wood-based filter for nitrate removal in septic systems. *Trans. ASAE* **2005**, *48*, 121–128. [\[CrossRef\]](#)
190. Wang, L.; Zheng, Z.; Luo, X.; Zhang, J. Performance and mechanisms of a microbial-earthworm ecofilter for removing organic matter and nitrogen from synthetic domestic wastewater. *J. Hazard. Mater.* **2011**, *195*, 245–253. [\[CrossRef\]](#)
191. Rajpal, A.; Bhargava, R.; Sasi, S.K.; Chopra, A.K. On site domestic organic waste treatment through vermiculture using indigenous earthworm species. *Waste Manag. Res.* **2012**, *30*, 266–275. [\[CrossRef\]](#)
192. Sinha, R.K.; Bharambe, G.; Chaudhari, U. Sewage treatment by vermifiltration with synchronous treatment of sludge by earthworms: A low-cost sustainable technology over conventional systems with potential for decentralization. *Environmentalist* **2008**, *28*, 409–420. [\[CrossRef\]](#)
193. Rajpal, A.; Bhargava, R.; Sasi, S.K.; Chopra, A.K.; Bajsa, O.; Nair, J.; Mathew, K.; Ho, G.E. Vermiculture as a tool for domestic wastewater management. *Water Sci. Technol.* **2012**, *48*, 266–275. [\[CrossRef\]](#)



194. Bajsa, O.; Nair, J.; Mathew, K.; Ho, G.E. Vermiculture as a tool for domestic wastewater management. *Water Sci. Technol.* **2004**, *48*, 125–132. [\[CrossRef\]](#)
195. Eastman, B.R.; Kane, P.N.; Edwards, C.A.; Trytek, L.; Gunadi, B.; Stermer, A.L.; Mobley, J.R. The effectiveness of vermiculture in human pathogen reduction for USEPA biosolids stabilization. *Compost Sci. Util.* **2001**, *9*, 38–49. [\[CrossRef\]](#)
196. Rodriguez-Gonzalez, L.; Miriyala, A.; Rice, M.; Delgado, D.; Marshall, J.; Henderson, M.; Ghebremichael, K.; Mihelcic, J.R.; Ergas, S.J. A Pilot-Scale Hybrid Adsorption-Biological Treatment System for Nitrogen Removal in Onsite Wastewater Treatment. *J. Sustain. Water Built Environ.* **2020**, *6*, 04019014. [\[CrossRef\]](#)
197. Ahmad, M.; Rajapaksha, A.U.; Lim, J.E.; Zhang, M.; Bolan, N.; Mohan, D.; Vithanage, M.; Lee, S.S.; Ok, Y.S. Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere* **2014**, *99*, 19–33. [\[CrossRef\]](#)
198. Mohanty, S.K.; Cantrell, K.B.; Nelson, K.L.; Boehm, A.B. Efficacy of biochar to remove *Escherichia coli* from stormwater under steady and intermittent flow. *Water Res.* **2014**, *61*, 288–296. [\[CrossRef\]](#) [\[PubMed\]](#)
199. Krayzelova, L.; Lynn, T.J.; Banihani, Q.; Bartacek, J.; Jenicek, P.; Ergas, S.J. A Tire-Sulfur Hybrid Adsorption Denitrification (T-SHAD) process for decentralized wastewater treatment. *Water Res.* **2014**, *61*, 191–199. [\[CrossRef\]](#)
200. Yates, M.V.; Gerba, C.P.; Kelley, L.M. Virus persistence in groundwater. *Appl. Environ. Microbiol.* **1985**, *49*, 778–781. [\[CrossRef\]](#) [\[PubMed\]](#)
201. Blanc, R.; Nasser, A. Effect of effluent quality and temperature on the persistence of virus in soil. *Water Sci. Technol.* **1996**, *33*, 237–242. [\[CrossRef\]](#)
202. Nasser, A.M.; Oman, S.D. Quantitative assessment of the inactivation of pathogenic and indicator viruses in natural water sources. *Water Res.* **1999**, *33*, 1748–1752. [\[CrossRef\]](#)
203. Haig, S.J.; Schirmer, M.; D'Amore, R.; Gibbs, J.; Davies, R.L.; Collins, G.; Quince, C. Stable-isotope probing and metagenomics reveal predation by protozoa drives *E. Coli* removal in slow sand filters. *ISME J.* **2015**, *9*, 797–808. [\[CrossRef\]](#)
204. Vandenhove, H.; Merckx, R.; Wilmots, H.; Vlassak, K. Survival of *Pseudomonas fluorescens* inocula of different physiological stages in soil. *Soil Biol. Biochem.* **1991**, *23*, 1133–1142. [\[CrossRef\]](#)
205. Kadlec, R.H.; Wallace, S. *Treatment Wetlands*; CRC Press: Boca Raton, FL, USA, 2008.
206. Klánová, K. Effect of chemical fertilizers on the transport of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella infantis* through sand columns. *Folia Microbiol. Off. J. Inst. Microbiol. Acad. Sci. Czech Repub.* **1994**, *39*, 283–286. [\[CrossRef\]](#)
207. McFeters, G.A.; Stuart, D.G. Survival of coliform bacteria in natural waters: Field and laboratory studies with membrane-filter chambers. *Appl. Microbiol.* **1972**, *24*, 805–811. [\[CrossRef\]](#) [\[PubMed\]](#)
208. Bales, R.C.; Li, S.; Maguire, K.M.; Yahya, M.T.; Gerba, C.P. MS-2 and poliovirus transport in porous media: Hydrophobic effects and chemical perturbations. *Water Resour. Res.* **1993**, *29*, 957–963. [\[CrossRef\]](#)
209. Šolić, M.; Krstulović, N. Separate and combined effects of solar radiation, temperature, salinity, and pH on the survival of faecal coliforms in seawater. *Mar. Pollut. Bull.* **1992**, *24*, 411–416. [\[CrossRef\]](#)
210. Tate, R.L. Cultural and environmental factors affecting the longevity of *Escherichia coli* in histosols. *Appl. Environ. Microbiol.* **1978**, *35*, 925–929. [\[CrossRef\]](#)
211. Gagliardi, J.V.; Karns, J.S. Leaching of *Escherichia coli* O157:H7 in diverse soils under various agricultural management practices. *Appl. Environ. Microbiol.* **2000**, *66*, 877–883. [\[CrossRef\]](#) [\[PubMed\]](#)
212. Díaz, F.J.; O'Geen, A.T.; Dahlgren, R.A. Efficacy of constructed wetlands for removal of bacterial contamination from agricultural return flows. *Agric. Water Manag.* **2010**, *97*, 1813–1821. [\[CrossRef\]](#)
213. Winward, G.P.; Avery, L.M.; Stephenson, T.; Jefferson, B. Chlorine disinfection of grey water for reuse: Effect of organics and particles. *Water Res.* **2008**, *42*, 483–491. [\[CrossRef\]](#) [\[PubMed\]](#)
214. Harvey, R.W.; George, L.H.; Smith, R.L.; LeBlanc, D.R. Transport of Microspheres and Indigenous Bacteria through a Sandy Aquifer: Results of Natural and Forced-Gradient Tracer Experiments. *Environ. Sci. Technol.* **1989**, *23*, 51–56. [\[CrossRef\]](#)
215. Benchokroun, S.; Imzilen, B.; Hassani, L. Solar inactivation of mesophilic *Aeromonas* by exogenous photooxidation in high-rate algal pond treating waste water. *J. Appl. Microbiol.* **2003**, *94*, 531–538. [\[CrossRef\]](#)
216. Nguyen, M.T.; Silverman, A.I.; Nelson, K.L. Sunlight inactivation of ms2 coliphage in the absence of photosensitizers: Modeling the endogenous inactivation rate using a photoaction spectrum. *Environ. Sci. Technol.* **2014**, *48*, 3891–3898. [\[CrossRef\]](#)
217. Martin, L. Removal of faecal bacteria and nutrients from domestic wastewater in a horizontal surface flow wetland vegetated with *Echinochloa pyramidalis*. *Afr. J. Environ. Sci. Technol.* **2012**, *6*, 337–345. [\[CrossRef\]](#)
218. Ansa, E.D.O.; Lubberding, H.J.; Ampofo, J.A.; Gijzen, H.J. The role of algae in the removal of *Escherichia coli* in a tropical eutrophic lake. *Ecol. Eng.* **2011**, *37*, 317–324. [\[CrossRef\]](#)
219. Curtis, T.P.; Mara, D.D.; Silva, S.A. Influence of pH, oxygen, and humic substances on ability of sunlight to damage fecal coliforms in waste stabilization pond water. *Appl. Environ. Microbiol.* **1992**, *58*, 1335–1343. [\[CrossRef\]](#)
220. Rudolfs, W.; Falk, L.L.; Ragotzkie, R.A. Literature review on the occurrence and survival of enteric, pathogenic, and relative organisms in soil, water, sewage, and sludges, and on vegetation: I. Bacterial and virus diseases. *Sew. Ind. Waste.* **1950**, 1261–1281. [\[CrossRef\]](#)
221. Winfield, M.D.; Groisman, E. Role of Nonhost Environments in the Lifestyles of. *Appl. Environ. Microbiol.* **2003**, *69*, 3687–3694. [\[CrossRef\]](#) [\[PubMed\]](#)
222. Gannon, J.T.; Manilal, V.B.; Alexander, M. Relationship between cell surface properties and transport of bacteria through soil. *Appl. Environ. Microbiol.* **1991**, *57*, 190–193. [\[CrossRef\]](#) [\[PubMed\]](#)

223. Weiss, T.H.; Mills, A.L.; Hornberger, G.M.; Herman, J.S. Effect of Bacterial Cell Shape on Transport of Bacteria in Porous Media. *Environ. Sci. Technol.* **1995**, *29*, 1737–1740. [[CrossRef](#)]
224. Harvey, R.W. Parameters involved in modeling movement of bacteria in groundwater. *Model. Environ. Fate Microorg.* **1991**, *89*–114.
225. Stevik, T.K.; Ausland, G.; Hanssen, J.F.; Jenssen, P.D. The influence of physical and chemical factors on the transport of *E. coli* through biological filters for wastewater purification. *Water Res.* **1999**, *33*, 3701–3706. [[CrossRef](#)]
226. Smith, M.S.; Thomas, G.W.; White, R.E.; Ritonga, D. Transport of *Escherichia coli* Through Intact and Disturbed Soil Columns. *J. Environ. Qual.* **1985**, *14*, 87–91. [[CrossRef](#)]
227. Polprasert, C.; Sawaitayothin, V. Nitrogen mass balance and microbial analysis of constructed wetlands treating municipal landfill leachate. *Water Sci. Technol.* **2006**, *54*, 147–154. [[CrossRef](#)]
228. Giacomán-Vallejos, G.; Ponce-Caballero, C.; Champagne, P. Pathogen removal from domestic and swine wastewater by experimental constructed wetlands. *Water Sci. Technol.* **2015**, *71*, 1263–1270. [[CrossRef](#)]
229. Olsson, L. Effect of Design and Dosing Regime on the Treatment Performance of Vertical Flow Constructed Wetlands. Master's Thesis, Linköping University, Linköping, Sweden, 2011.
230. Brissaud, F.; Salgot, M.; Bancolé, A.; Campos, C.; Folch, M. Residence time distribution and disinfection of secondary effluents by infiltration percolation. *Water Sci. Technol.* **1999**, *40*, 215–222. [[CrossRef](#)]
231. Van Cuyk, S.; Siegrist, R.L. Virus removal within a soil infiltration zone as affected by effluent composition, application rate, and soil type. *Water Res.* **2007**, *41*, 699–709. [[CrossRef](#)]
232. Molle, P.; Liénard, A.; Grasmick, A.; Iwema, A. Effect of reeds and feeding operations on hydraulic behaviour of vertical flow constructed wetlands under hydraulic overloads. *Water Res.* **2006**, *40*, 606–612. [[CrossRef](#)]
233. Lightbody, A.F.; Avenier, M.E.; Nepf, H.M. Observations of short-circuiting flow paths within a free-surface wetland in Augusta, Georgia, U.S.A. *Limnol. Oceanogr.* **2008**, *53*, 1040–1053. [[CrossRef](#)]
234. Kjellin, J.; Wörman, A.; Johansson, H.; Lindahl, A. Controlling factors for water residence time and flow patterns in Ekeby treatment wetland, Sweden. *Adv. Water Resour.* **2007**, *30*, 838–850. [[CrossRef](#)]
235. Bracho, N.; Lloyd, B.; Aldana, G. Optimisation of hydraulic performance to maximise faecal coliform removal in maturation ponds. *Water Res.* **2006**, *40*, 1677–1685. [[CrossRef](#)] [[PubMed](#)]
236. Persson, J.; Somes, N.L.; Wong, T.H.F. Hydraulics efficiency of constructed wetlands and ponds. *Water Sci. Technol.* **1999**, *40*, 291–300. [[CrossRef](#)]
237. Holland, J.F.; Martin, J.F.; Granata, T.; Bouchard, V.; Quigley, M.; Brown, L. Effects of wetland depth and flow rate on residence time distribution characteristics. *Ecol. Eng.* **2004**, *23*, 189–203. [[CrossRef](#)]
238. Hurst, C.J.; Gerba, C.P.; Cech, I. Effects of environmental variables and soil characteristics on virus survival in soil. *Appl. Environ. Microbiol.* **1980**, *40*, 1067–1079. [[CrossRef](#)]
239. Qin, Y.; Wen, Z.; Zhang, W.; Chai, J.; Liu, D. Different roles of silica nanoparticles played in virus transport in saturated and unsaturated porous media. *Environ. Pollut.* **2020**, *259*, 113861. [[CrossRef](#)] [[PubMed](#)]
240. Powelson, D.K.; Gerba, C.P. Virus removal from sewage effluents during saturated and unsaturated flow through soil columns. *Water Res.* **1994**, *28*, 2175–2181. [[CrossRef](#)]
241. Van Cuyk, S.; Siegrist, R.; Logan, A.; Masson, S.; Fischer, E.; Figueroa, L. Hydraulic and purification behaviors and their interactions during wastewater treatment in soil infiltration systems. *Water Res.* **2001**, *35*, 953–964. [[CrossRef](#)]
242. Van Cuyk, S.; Siegrist, R.L.; Lowe, K.; Harvey, R.W. Evaluating Microbial Purification during Soil Treatment of Wastewater with Multicomponent Tracer and Surrogate Tests. *J. Environ. Qual.* **2004**, *33*, 316–329. [[CrossRef](#)] [[PubMed](#)]
243. Axler, R.; Henneck, J.; McCarthy, B. Residential subsurface flow treatment wetlands in northern Minnesota. *Water Sci. Technol.* **2001**, *44*, 345–352. [[CrossRef](#)] [[PubMed](#)]
244. Powelson, D.K.; Mills, A.L. Transport of *Escherichia coli* in sand columns with constant and changing water contents. *J. Environ. Qual.* **2001**, *30*, 238–245. [[CrossRef](#)] [[PubMed](#)]
245. Zapater, M.; Gross, A.; Soares, M.I.M. Capacity of an on-site recirculating vertical flow constructed wetland to withstand disturbances and highly variable influent quality. *Ecol. Eng.* **2011**, *37*, 1572–1577. [[CrossRef](#)]
246. Ginn, T.R.; Wood, B.D.; Nelson, K.E.; Scheibe, T.D.; Murphy, E.M.; Clement, T.P. Processes in microbial transport in the natural subsurface. *Adv. Water Resour.* **2002**, *25*, 1017–1042. [[CrossRef](#)]