Disinfection Processes and Mechanisms in Wastewater Stabilization Ponds: A Review

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1 **Disinfection Processes and Mechanisms in Wastewater Stabilization Ponds: A**
2 **Review**

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Draft

9 **Abstract**

10 Wastewater stabilization ponds (WSPs) have been proven to be economical alternatives to
11 conventional wastewater treatment technologies due to their unique advantages, including ease
12 of operation, minimal energy input and minimal maintenance requirements. Their reported high
13 pathogen removal efficiencies have made WSPs a popular choice for wastewater treatment,
14 especially as tertiary lagoons. This paper provides a critical overview of the various disinfection
15 processes and mechanisms that occur in WSPs. A thorough review of the removal or attenuation
16 mechanisms for bacterial, viral, protozoan and helminthic pathogens is presented. Factors that
17 impact the removal efficiency of pathogenic organisms may include sunlight, pH, dissolved
18 oxygen (DO), temperature, sedimentation, attachment, hydraulic retention time (HRT), pond
19 depth, predation and nutrient availability; the relationship between these factors is also discussed.
20 The purpose of this review paper is to utilize the current understanding of pathogen removal
21 mechanisms in pond systems to improve the operation and design of WSPs, and more
22 importantly, to provide guidance for the definition of regulations with respect to pathogen
23 removal in eco-engineered wastewater treatment systems such as WSPs.

24
25 **Keywords:** wastewater treatment, WSPs, disinfection mechanisms, environmental factors,
26 indicator organisms

28 **Introduction**

29 Wastewater management plays an important role in ensuring our future environmental
30 sustainability, especially with growing demands on water resources and the subsequent impacts
31 on receiving environments (Moumouni et al. 2016). Pressured by water scarcity and increases in

water demand worldwide, treated wastewater has been reused for a variety of purposes over the past few decades, such as for agricultural irrigation and other industrial, environmental and municipal uses (Mo and Zhang 2012; Cornejo et al. 2013; Mosteo et al. 2013; Verblyla et al. 2016). The target 6.3 of the United Nations Sustainable Development Goals is to increase water reuse globally by 2030 (United Nations 2015; Verblyla et al. 2016). The potential for water reuse is dependent on effective pathogen removal (Sharafi et al. 2015).

Passive or semi-passive systems, such as constructed wetlands and WSPs (Al-Hashimi and Hussain 2013), are considered economical alternatives to conventional wastewater treatment approaches. They are also known being effective in pathogen removal, so commonly applied as tertiary treatment. In the United States and New Zealand, more than half of the wastewater treatment facilities involve passive treatment systems (Mara 2003; US EPA 2011). Approximately 90% of pond systems in the United States are used in small communities with populations of less than 10,000 and have been shown to be very effective in treating wastewater (Gray 2004). The removal of a wide spectrum of pathogenic microorganisms, such as bacterial, viral, protozoan and helminthic pathogens, is commonly achieved in WSPs. Conversely, disinfection methods (UV irradiation, chlorination and ozone) applied in conventional treatments often only target pathogenic bacteria and viruses, as helminth eggs and protozoan (oo)cysts are resistant to these disinfection methods (Jiménez 2007a; 2009; Drechsel et al. 2009). Some studies have reported removals of up to 6 log units of bacteria and practically all protozoan and helminth eggs in WSP systems, producing final effluents that not only meet discharge to the environment requirements in most jurisdiction, but also meet the WHO guidelines for the use of treated wastewater in unrestricted agricultural irrigation (Bolton et al. 2010). The performance of

WSP systems has been reported to be superior to that of conventional treatment processes, such as activated sludge or primary treatments, for which reductions of 1 to 2 log units for bacteria and 70–99% for protozoan and helminth eggs have been noted (Ansa et al. 2012).

This paper provides a review of the operational conditions and mechanisms contributing to disinfection in WPS systems. Physical, chemical and biological parameters that impact the efficiency of pathogen removal are characterized and discussed. In addition, consideration is given to alternative indicators and their potential benefits in the context of public and environmental safety. Moreover, the information summarized herein can be used to assess microbial risk in treated wastewater in order to develop wastewater discharge guidelines and regulations, as well as safe water reuse policies.

Pathogen removal in WSPs

There are three different pathways by which pathogenic microorganisms can enter a WSP: influent wastewater, regrowth with the WSPs and animal activities. There are approximately 60 known types of waterborne human pathogens (Wu et al. 2016) and it would be very cost-intensive to assess each of them in a timely manner in the effluent of a WSP. Therefore, treated wastewater is routinely assessed through the monitoring of bacterial indicator organisms (typically *E. coli*, FC or TC). In addition, turbidity and total suspended solids (TSS) are often used to indicate the potential presence of pathogenic microorganisms, as these parameters that have been demonstrated to be correlated with the presence of pathogens (Rose et al. 2004). Although WSPs are considered to be a relatively well-established biological method for the removal of pathogens, there remains some knowledge gaps regarding the importance of mechanisms such as sunlight, pH, DO and other environmental factors by which pathogens could

be effectively removed (Reinoso et al. 2011; Mosteo et al. 2013). Removal efficiencies of pathogenic and indicator microorganisms during wastewater treatment can be inconsistent and have been shown to vary, depending on the type of treatment process, as a function of HRT, solids retention time (SRT), sunlight, DO concentration, pH, temperature and the efficacy in TSS removal (Tyagi et al. 2011). Generally, an optimally functioning eco-engineered system could be expected to achieve 90–99% microbial reductions, but in some cases, poor reductions have been observed and treated effluents could contain high numbers of fecal microorganisms (George et al. 2002; Koivunen et al. 2003; Reinoso et al. 2008).

Table 1-4 summarize the log/percentage removal of bacteria, viruses, protozoa and helminthes from WSP systems operating world-wide. It shows a wide variation in performance, as pathogen removal in WSPs can be location and climate specific. Hence, disinfection efficiency can be influenced greatly by environmental conditions as well as different disinfection mechanisms factor.

Factors affecting pathogen removal in WSPs

WSPs have generally been shown to be effective in the reduction or removal of pathogens. Reinoso et al. (2011) reported that *Cryptosporidium* oocysts could effectively be reduced by an average of 96%, *Giardia* cysts by 98% and helminth eggs by 100% in a Spanish pond system consisting of two anaerobic ponds running in parallel, a facultative pond followed by a maturation pond operated under both winter and summer season operations (from December 2003 to September 2004). In other studies world-wide, WSPs have been reported to remove up to 7 log units of bacteria, up to 5 log units of viruses and almost 100% helminth ova (Zanotelli et al. 2002; Awuah 2006; Jimenez 2007c; Tyagi et al. 2011; Reinonso et al. 2011; Liu et al., 2018).

A number of studies have attempted to explain the factors responsible for pathogen reduction in passive wastewater treatment systems. As shown in Figure 1, pathogen removal within WSPs is considered to be a complex process involving various physical, chemical and biological factors, alone or in combination, including sunlight, pH, DO, temperature, predation, attachment/sedimentation and starvation (Maynard et al. 1999; Awuah et al. 2001; Oilgae 2009; Fisher et al. 2012).

Sunlight

Sunlight has a lethal impact on coliform bacteria and the die-off rate has been reported to be proportional to sunlight intensity (Curtis et al. 1992a; 1994; Kadir and Nelson 2014; Champagne et al. 2017). As shown in Figure 2, different wavelength regions of the solar spectrum, such as the ultraviolet (UV) spectrum (290–400nm) and photosynthetically active radiation (PAR) (400–700nm), have been shown to contribute differently to disinfection in wastewater (Bolton 2010).

Sunlight damage in pond systems has been attributed to three mechanisms: direct damage to DNA by UV-B wavelengths (280–320nm); indirect endogenous (part of the virion) damage caused by UV-B; and indirect exogenous (in solution) damage involving UV-A (320 – 400 nm), UV-B and PAR (Davies- Colley et al. 1999; 2000; Kadir and Nelson 2014).

Studies by Sarikaya and Saatci (1987) and Mayo (1989) each indicated that using light alone might not achieve significant FC removals. In a more recent study, Kadir and Nelson (2014) also found that direct damage by UV-B light was not a significant removal mechanism for *E. coli* and *E. faecalis*. These findings suggest that indirect endogenous and exogenous photo-oxidation may play a more important role in sunlight-mediated inactivation of pathogens. Photo-oxidation is the process by which photo-sensitizers absorb light and transfer its energy to other molecules, leading to the formation of reactive oxygen species (ROS). ROS react with membranes of microorganisms and consequently cause damage. Photo-oxidation occurs when ROSs are produced by endogenous and exogenous photo-sensitizers as well as by other reactions, such as Fenton's reaction (Curtis et al. 1992b; Kohn and Nelson 2007; Bolton 2010). Endogenous photo-sensitizers, such as flavins and porphyrin derivatives, are found inside the cell of microbes, while exogenous photo-sensitizers, such as humic substances, photosynthetic pigments and dissolved organic matter (DOM), are present outside the cell in the aquatic environment (Curtis et al. 1992b; Kohn and Nelson 2007).

Sunlight, however, is attenuated with depth as it penetrates into the water column. In particular, shorter wavelengths, such as UV, may be only effective at pond surfaces (Curtis et al. 1994; Kadir and Nelson 2014; Verbyla and Mihelcic 2015). This may explain the limited effect of UV-

B in directly damaging DNA. Conversely, longer wavelengths (PAR spectrum) can penetrate much deeper into the water column, hence their effect can potentially be more important for disinfection (Kadir and Nelson 2014). Kohn and Nelson (2007) reported that more than 99% of UV-B light at 290 nm was absorbed in the first 2.5 cm depth of a WSP, while over 99% of visible light at 556 nm was absorbed in the first 8 cm. Sunlight gets attenuated more readily in turbid water due to the higher solids and/or microbial and/or organic matter concentrations.

The response of different bacteria to sunlight damage can vary considerably. In studies by Mezrioui et al. (1995a, 1995b), it was observed that sunlight had a much greater effect on *E. coli* survival than on *V. cholerae* as the concentration of *E. coli* was markedly reduced, particularly during the warmer seasons in a WSP operating in Marrakesh, Morocco. Many studies have focused on the sunlight mechanisms contributing to bacterial pathogen removal, including both endogenous and exogenous bacterial inactivation (Berney et al. 2006a; Berney et al. 2006b; Bosshard et al. 2010a; Bosshard et al. 2010b; Fisher et al. 2012; Santos et al. 2012; Sassoubre et al. 2012; Kadir and Nelson, 2014). Kadir and Nelson (2014) concluded that the inactivation of *E. coli* occurs through endogenous mechanisms promoted by UV-B and UV-A, while the inactivation of *E. faecalis* is mainly through exogenous mechanisms facilitated by UV-A and visible wavelengths. Different exogenous photo-sensitizers, such as humic substances, may also induce various degrees of photo-oxidation (Curtis et al. 1992b, MacDougall 2016).

Although the pathways responsible for bacterial inactivation resulting from exposure to various biocides are generally well understood, virus inactivation mechanisms are often contradictory. Photo-sensitizers assist with bacterial inactivation through photo-oxidation; however, they may not have a similar effect on viruses as they lack a bound chromosphere to act as the endogenous

photo-sensitizer (Kohn and Nelson, 2007). Hence, a detailed mechanistic understanding does not yet exist for viruses. The development of a thorough understanding of virus inactivation is complicated by the fact that related viruses can often exhibit different disinfection kinetics when treated with the same disinfectant under similar conditions (Baxter et al. 2007; Cromeans et al. 2010). These different responses suggest that even minor variations in structural or genomic components can have a marked impact on viral resistance to inactivation. It has been demonstrated that exposure to inactivating oxidants and radiation can result in modifications to viral proteins (Sano et al. 2009; Hotze et al. 2009; Wigginton et al. 2010) and nucleic acids (Nuanualsuwan et al. 2002; Li et al. 2004), leading to inactivation of viruses.

Sunlight-mediated inactivation may not be the most effective route to protozoan and helminthic disinfection. Reinoso et al. (2011) demonstrated that sunlight exposure increased the rate of *Cryptosporidium parvum* (*C. parvum*) oocyst inactivation compared to dark conditions, reaching a maximum difference of 40% after 4 days of exposure. While UV radiation is widely used for water disinfection, its efficacy against FLA has not been widely reported when compared to other protozoa such as *C. parvum*. Hijnen et al. (2006) provides a comprehensive overview on the role of UV radiation in cysts inactivation and reported that *Acanthamoeba spp.* is highly UV resistant. *Acanthamoeba spp.* cysts were demonstrated to be resistant to exposure to UV-C at 253.7 nm at $1.1 \text{ mJ s}^{-1} \text{ cm}^{-2}$ (Hwang et al. 2004), while *Acanthamoeba castellanii* (*A. castellanii*) cysts were shown to be resistant to UV-B irradiation at 800 mJ cm^{-2} and *Balamuthis mandrillaris* (*B. mandrillaris*) cysts to exposure at 200 mJ cm^{-2} UV irradiation (Siddiqui and Khan 2008). Another study reported a 4 log reduction of *Acanthamoeba polyphaga* (*A. polyphaga*) cysts after exposure to 40 mJ cm^{-2} (Loret et al. 2008). Exposure of *A. polyphaga* cysts to simulated global solar irradiance in water ($85 \text{ mJ s}^{-1} \text{ cm}^{-2}$) did not achieve cyst inactivation when the temperature

was maintained $< 40^{\circ}\text{C}$, but yielded better results at 45°C ($1.2 \log_{10}$ reduction after 6 h), 50°C ($> 3.6 \log_{10}$ reduction after 6 h) and 55°C ($> 3.3 \log_{10}$ reduction after 4 h) (Heaselgrave et al. 2006). Sixty one percent of *Naegleriagruberi* cysts were still alive after UV irradiation with 21.6 mJ cm^{-2} , but treating amoebae with DNA repair mechanism inhibitors was noted to improve UV irradiation efficacy (Hillebrandt and Muller 1991). Similarly, photo-sensitized inactivation of *Acanthamoeba palestinensis* in the cystic stage has also been demonstrated by incubating cysts with tetracationic Zn(II)-phthalocyanine prior to exposure to 600–700 nm wavelength light sources (Ferro et al. 2006). Protozoa in cyst or oocyst form are generally considered resistant to UV irradiation, but there are likely differences in UV sensitivity depending on the strain or species. UV mechanisms could be more effective in protozoa inactivation with the application of longer exposures (days) or other operational factors, such as higher temperatures. Very few studies have examined the effects of visible wavelengths or photo-oxidation in protozoa inactivation.

pH

pH plays an important role in pathogen removal in WSPs, and can be highly influenced by the presence of algae in the system (Wallace et al. 2016; Champagne et al. 2017). Photosynthesis is the process by which light energy is transformed into chemical energy by the chloroplast in autotrophs. In general, this process converts carbon dioxide and water into organic molecules. As shown in Figure 3, in aquatic photosynthesis, algae uptake CO_2 and release O_2 , leading to an increase in pH and DO concentration (Fallowfield et al. 1996). Therefore, high pH levels are often observed in WSPs, with values frequently varying diurnally within a range of 7 – 9.4 (Curtis et al. 1992a; Sweeney et al. 2007; Bolton et al. 2010; Wallace et al. 2016). Neutral to

slightly acidic pH conditions (between 6.5 and 7.5) have been reported as optimal for fecal bacteria growth (Awuah 2006), while pH levels higher than 8.5 have been noted to be effective for the removal of indicator organisms (Davies-Colley et al. 1999; 2000; Awuah et al. 2002; Ansa et al. 2011; 2012; Liu et al. 2015). Fluctuations in pH have been shown to negatively affect the survival of *E. coli* (Awuah 2006).

Some bacteria behave differently under different pH conditions. David-Colley et al. (1999) found that the inactivation rate of *E. coli* increased when pH was above 8.5. *Enterococci* were found to be more tolerant to high pH (>9) than to acidic conditions (Awuah et al. 2001). Inactivation of *E. coli* increased with increasing pH while in contrast, *Enterococci* was noted to exhibit similar rates of inactivation at all pH levels (Davies Colley et al. 1999; Nelson et al. 2009; Ouali et al. 2014). In a study by Mezrioui et al. (1995a), it was found that pH of 8 and 8.8 (tested pH include 6.6, 7.3, 8 and 8.8) inhibited the survival of *E. coli* and prolonged the survival of *V. cholera*. The contrast in the behavior of these organisms in response to their exposure to elevated pH levels could be due to a different reaction mechanisms or sensitivity to sunlight (Maynard et al. 1999; Kadir and Nelson 2014), where damage to the cytoplasmic membranes of bacteria caused by sunlight could render the organisms more vulnerable to the effects of other factors such as high pH (Curtis et al. 1992a). In their study on WSPs, Davies-Colley et al. (1999) reported that *Enterococci* were immune to sunlight when pH was increased from 7.5 to 9.5 and under low DO levels. However, a number of studies have demonstrated that light-induced inactivation of *E. coli* was dependent on pH (Davies-Colley et al. 1997; 1999; Ouali et al. 2014). Under moderate pH conditions, *E. coli* was inactivated by endogenous photo-sensitizers, but when the pH was raised to above 8.5, *E. coli* was inactivated more rapidly by exogenous mechanism (Davies-Colley et al. 1997; 1999).

Information on the manner in which pH inactivation impacts viruses, protozoa and helminthes is limited. Viral attachment was observed to be a function of water pH in ground water studies (Bales et al. 1993; 1997; Guan et al. 2003). It was shown that pH levels above 12 could inactivate helminth ova (Jimenez 2007c). However, pH levels above 12 may not be desirable in most WSP environments, particularly when these systems discharge into receiving environments. There is much debate in the literature on whether sunlight or pH is the most important mechanism for pathogen removal, and there are conflicting results on the contribution of these two mechanisms as previously discussed, hence, more targeted and systematic research is required in this area.

DO

Oxygen concentrations above 0.5 mgL^{-1} have been shown to contribute to the removal of fecal bacteria (Van Buuren and Hobma 1991; Ansa et al. 2011). As previously noted, oxygen is produced as a by-product of algal photosynthesis, which is a source of oxygen supply in WSPs. Sweeney et al. (2007) observed DO concentration as high as 30 mg/L in a single pond system at the Bolivar Wastewater Treatment Plant in Adelaide, South Australia during the summer period. Photo-oxidation requires the presence of oxygen to form ROC, therefore, an increase in DO concentration would likely increase the effect of photo-oxidation. As an example, the light inactivation of *E. coli* and *Enterococci* has been reported to increase with increasing DO concentrations (Curtis et al. 1992a; David-Colley et al. 1994; Ouali et al. 2014). David-Colley et al. (1994) found that the inactivation rates of *Enterococci* increased significantly with increasing DO levels when pH and light intensity remained constant. In their study, David-Colley et al.

(1994) also observed a synergistic increase in inactivation of *E. coli* when both DO and pH increased. This may imply that endogenous photo-inactivation of *E. coli* and *Enterococci* were strongly dependent on DO (Davies Colley et al. 1999; Ouali et al. 2014).

As facultative anaerobes, FC are able to survive over a wide range of oxygen concentrations. However, under anaerobic conditions, FC were found to survive for longer periods than under aerobic conditions (Klock 1971). In their separate studies Klock (1971) and Marais (1974) found that aeration enhanced FC die-off rates. Kaneko (1997) also observed that the removal of polioviruses, bacteriophages and Coxsackie virus B3 was enhanced by aeration. Davies-Colley et al. (1999) noted that the inactivation of the F-DNA virus was independent of DO concentration, while the inactivation of the F-RNA virus increased with increasing DO concentrations (Awuah 2006). To date, there has been very little information regarding the effect of DO concentration on protozoa and helminth egg removal and/or inactivation.

Indicator microorganisms respond differently to pH, water temperature and DO levels (Love et al. 2010; Ouali et al. 2014; Champagne et al. 2017). Bolton et al. (2011) reported that the effect of DO concentration on inactivation rates was organism-dependent. Significant correlations between kinetic disinfection coefficient (K) and pH, DO, temperature and light intensity were observed. The kinetic coefficient (K) values for *E. coli* and *Enterococci* were found to be dependent on physicochemical parameters (pH, DO, water temperature and light intensity), where K has generally been reported to increase with increasing pH, light intensity and temperature (Ouali et al. 2014).

Temperature

Although optimal rates of bacterial growth are typically restricted to small temperature ranges, bacterial organisms are able to survive within broader temperature limits. Some researchers have stated that the minimum water temperature required for the treatment of wastewater using WSPs is 7°C with higher temperatures stimulating bacterial growth, where growth rates doubles with approximately every 10°C increase in temperature until optimal growth is reached (USEPA 1983; Metcalf and Eddy 2003). Several researchers have reported increased removal efficiencies of FC at higher temperatures (Polprasert et al. 1983; Pearson et al. 1987a;b; Barzily and Kott 1991; Ouali et al. 2014; Champagne et al. 2017). In a study by Mezrioui et al. (1995b), fecal coliforms were reduced by 98.95% during the warmer seasons and by 94.91% during the cold seasons in a WSP system, operating in Marrakesh, Morocco. This system consisted of an anaerobic and an aerobic pond in series. They also found that when temperature was increased from 8°C to 23°C, the removal rate of *E. coli* also increased.

It has been noted that bacterial removal with increasing temperature in horizontal flow constructed wetlands (CWs) (Winward et al. 2008). In a study by Boutilier et al. (2009), the first order rate constants for *E. coli* removal were 0.09 day⁻¹ at 7.6 °C and 0.18 day⁻¹ at 22.8 °C in a laboratory experiment using domestic wastewater collected from a surface flow wetland. Ulrich et al. (2005) reported that higher temperatures improved the removal of bacterial indicator organisms by 1 log (CFU/100mL), when comparing the operation of a CW in summer and winter seasons. Jokerst et al. (2011) found that the wetland was able to remove an average of 1.7 log CFU/100mL *E. coli* in spring, summer and fall, but an average of 1.0 log CFU/100mL in winter.

Different bacteria have been reported to respond differently to temperature change. Mezrioui et al. (1995a) found that *V. cholera* did not survive as well as *E. coli* at a temperature of 8°C, and a Spearman correlation between temperature and the concentrations of *V. cholera* in a WSP in the arid region of Marrakesh, Morocco was 0.91. Conversely, Mara and Pearson (1986) concluded that there was no direct relationship between FC die-off and temperature and that a tertiary pond exhibited higher levels of FC removal than anaerobic and facultative ponds when operated at the same temperature. Factors, other than temperature, may have contributed to the higher removal efficiencies observed in this case. This supports the theory that pathogen removal in WSP is driven by several mechanisms and operational factors. However, to date, temperature effects on viral, protozoan and helminthic pathogens have not been investigated extensively.

Attachment & Sedimentation

Sedimentation has been reported to be effective in removing some microorganisms, such as helminth eggs as they have a higher settling velocities (~0.1mm/s) (Sengupta et al. 2011, Wu et al. 2016). However, bacteria, viruses and protozoa have much lower settling velocities, so they can be removed through sedimentation only if they attach to larger particles (Jasper et al. 2013).

In a study by Ansa et al. (2012), investigating the attachment of FC in pond systems, FC attachment to suspended matter was determined to significantly contribute to FC removal only in the first two maturation ponds (71% - 99% removal efficiencies), and was generally more pronounced at the bottom of the ponds. Mahler et al. (2000) observed that a significant proportion of bacteria were associated with suspended solids in wastewater. Bacteria in the aqueous media attach to solid surfaces by secreting extracellular polysaccharides and the

quantity and composition of these extracellular polysaccharides may affect the attachment properties of the bacteria (Sanin et al. 2003). This would suggest that bacterial pathogen attachment to algae and suspended solids could be an important mechanism for bacteria removal (Ansa et al. 2011).

However, a study by Boutilier et al. (2009) examining sedimentation of *E. coli* reported that settling did not appear to significantly contribute to *E. coli* removal in 5-day sedimentation experiments during which *E. coli* remained in suspension and 10% and 50% of *E. coli* in domestic dairy wastewater and septic tank effluent, respectively, associated with particulate matter. The role of sedimentation in pathogen removal has been reported to range from 0 to 100% (Maynard et al. 1999). Some studies demonstrated that the performance of WSPs in removing protozoan cysts and helminth eggs could primarily be attributed to the process of sedimentation favoured by the adsorption of the pathogens onto settleable solids, as well as relatively long retention times (Amahmid et al. 2002; Mara 2003). Conversely, Reinoso et al. (2011) noticed a negligible influence on oocyst removal through sedimentation in facultative and maturation ponds with 0% and 5.56% of *Cryptosporidium* oocysts removed through sedimentation in the facultative and maturation ponds, respectively.

Reinoso et al. (2011) suggested that anaerobic ponds were the most effective in parasite removal followed by facultative ponds and then maturation ponds. These findings were consistent with those previously reported, which indicated that the majority of (oo)cysts, eggs and suspended solid material are generally removed in primary ponds (Jimenez et al. 2001; Reinoso et al. 2011). However, contrary to previous studies crediting sedimentation as a main mechanism for

parasites removal in stabilization ponds, results presented by Reinonso et al. (2011) indicated that the physicochemical environment (light, pH, DO, ammonia concentration, etc.) of the pond could also be a significant factor in the removal of parasites from these systems.

Retention time & Pond depth

HRT in WSPs is defined as the average hydraulic period that wastewater remains in pond systems prior to discharge into a receiving environment. It is an important factor that impacts the die-off and the removal of pathogens. Sedimentation in ponds is dependent on HRT, since longer HRTs allow more time for the settling of suspended particles to which pathogens may be attached (Metcalf and Eddy 2003). Also, longer retention times provide a longer period of time for pathogen inactivation or removal via factors that contribute to disinfection such as sunlight, pH, DO and temperature.

In a constructed wetland study, the *E. coli* removal efficiency was 91% for wetlands with 11.6 day HRT, while for wetlands with a HRT of 0.9 day, the *E. coli* removal efficiency was 66% (Diaz et al. 2010). Moreover, of all the parameters (pH, total nitrogen (TN), nitrate, total phosphorus (TP), dissolved organic carbon (DOC), total suspended solids (TSS), temperature, DO, Chlorophyll-a, *E. coli* and *Enterococci*) compared in the study, HRT appeared to be the factor having the greatest effect on the efficiency of *E. coli* and *Enterococci* removal (Diaz et al. 2010). HRT and the loading rate were two of the most important factors for removing fecal coliforms and total coliforms in a pilot-scale combined constructed wetland system (Tuncsiper et al. 2012). In domestic wastewater, Feacham et al. (1983) proposed a time period of 30-60 days for pathogenic bacteria removal. Jiménez et al. (2001) indicated that a minimum retention time

of 5–20 days, depending on the initial concentration, was required to efficiently remove helminth eggs, while 38 days would be necessary to remove protozoan (oo)cysts. Grimason et al. (1996a; b) showed that an HRT of 25.3 days would be sufficient for the removal of *Ascaris* eggs and *Giardia* cysts in Eldoret, Kenya. However, 40 days would be required to accomplish the same performance in Meze, France. It should be noted, that these retention times are longer than the 8–10 days recommended in the WHO guidelines, to achieve a standard of 1 helminth ovum per liter (WHO 1989; Awuah 2006). The different concentrations and sizes of protozoa and helminthes, which yielded different settling velocities, may explain these variations in recommended retention times (Reinoso et al. 2007).

A baffle applied to a WSP system can extend HRT. Only the baffled maturation pond showed a significant removal of fecal bacteria, viruses and protozoa when four maturation ponds (one with baffle and three without baffle) were compared (Sheludchenko et al. 2016). After introducing baffles to the first maturation pond, the HRT was increased by 14 hours, and the removal efficiency of both *E. coli* and *Enterococci* was raised by 0.2 to 0.7 log unit at Korba wastewater treatment plant which consisted of a conventional activated sludge system followed by three maturation ponds (Ouali et al. 2012).

Depth may increase retention period, but it may also decrease sunlight penetration in algal ponds which would present confounding results with respect to disinfection mechanisms. Mayo (1989) found that, while fecal bacteria reduction in wastewater was observed to increase with solar intensity and hydraulic retention time, it was also noted to decrease with depth. Agunwamba (1991) and Pearson et al. (1995) also reported reductions in pond efficiencies with respect to

bacteria die-off rates in deeper ponds. Many pond system designers have indicated that increasing pond depth, and thus retention time, would lead to better overall pond performance, however, this may not be applicable for disinfection (Pearson et al. 1995; Mara et al. 2001). Buchauer (2007) found that shallower maturation ponds were generally more efficient for FC removal.

Predation & Presence of other microorganisms

Another way to remove pathogens from wastewater is through the presence of other microorganisms that consume or compete with the pathogenic organisms. Protozoa prey on organisms such as bacteria, yeasts, algae and other protozoa (Storer et al. 1979). Predation is an important factor in the removal of bacteria and protozoan (oo)cysts from wastewaters in CWs (Jasper et al. 2013; Wu et al. 2016). MacCambridge and MacMeekin (1979; 1980) demonstrated the importance of protozoan predation on *E. coli* removal. Kim and Unno (1996) showed that the predation of viruses by protozoa was more effective than that of bacteria by protozoa. Some protozoa have been identified to feed on FC, as well as diptherial, cholera, typhal and streptococcal bacteria species (MacCambridge and MacMeekin 1979; Wu et al. 2016). FC number in a duckweed pond system correlated well with total ostracod numbers in the pond, suggesting that macro-invertebrate feedings could contribute to FC removal in duckweed ponds (Ansa et al. 2012). In constructed wetlands, predation of *Cryptosporidium* oocysts by ciliates such as *Euplotes patella* and *Paramecium caudatum* has been reported as a mechanism for pathogen removal (Stott et al. 2003; Awuah 2006).

The presence of other bacteria might affect pathogen removal through the competition for nutrients. According to Atlas and Bartha (1981), intestinal bacteria and other pathogen populations were reduced and eventually eliminated through competition with aquatic autochthonous bacteria populations. It has been suggested that understanding nutrient availability and competition for these nutrients in heterotrophic bacteria communities are important factors in determining fecal bacteria die-off rates (Klock 1971; Wu and Klein 1976; Legendre et al. 1984). Competition between heterotrophic bacteria for nutrients and its effects on pathogen removal in pond systems have not been thoroughly investigated.

Nutrients and organic loading

The nutrient level and organic loading of a WSP will affect the survival and regrowth of pathogenic microorganisms. A number of studies have reported on the importance of nutrient availability to the survival and growth of microorganisms (Almasi and Pescod 1996; Kaneko 1997; Gagliardi and Karns 2000; Diaz et al. 2010). Boutilier et al. (2009) reported that *E. coli* survived for a longer period of time in dairy wastewaters, which normally have higher nutrient concentrations than municipal wastewaters. High nutrient concentrations have been shown to generally decrease the rate of pathogen removal in wastewater systems (Gagliardi and Karns 2000; Diaz et al. 2010). Diaz et al. (2010) observed a positive correlation between indicator organisms and DOC, TP, TN and nitrate concentration in a constructed surface flow wetland. In their study, *E. coli* concentration was positively correlated with TP ($r=0.521$; $p<0.01$), while *Enterococci* concentrations were positively correlated with TN and nitrate ($r=0.357$ and $r=0.378$, respectively; $p<0.01$).

439 **Conclusions**

440 It has been commonly noted in literature that traditional indicator organisms (*E. coli*, TC and FC)
441 do not accurately represent the potential presence of all pathogens, which may respond
442 differently to various environmental or exposure factors. These may be subject to different
443 removal mechanisms than indicator organisms. It is also unclear to what extent fecal indicator
444 organisms are representative of the pathogenic organisms of concern. More research is required
445 to gain a better understanding of the distribution of pathogenic organisms in WSPs and to select
446 appropriate additional or alternative indicator organisms. A number of different bacterial, viral,
447 protozoan and helminthic pathogens have been proposed as additional or alternative indicators
448 for monitoring wastewater effluent quality. However, the correlation between these indicators
449 has rarely been studied for the purpose of minimizing the number of pathogen indicators. This
450 will become increasingly relevant to municipalities with the growing number of persistent
451 drug/disinfection resistant microorganisms are introduced into wastewater systems and
452 subsequently discharged into receiving environments.

453
454 Recent work has considered pathogen removal as a complex mechanism involving interactions
455 between the different physical, chemical and biological processes present in WSP systems. Many
456 factors and mechanisms, including sunlight, pH, DO, temperature, sedimentation, retention time,
457 predation and nutrients, contribute to pathogen removal, however, the literature presents
458 conflicting information with respect to the governing mechanism. These results reinforce the
459 idea that pathogen removal may not rely simply on one or two factors, but instead, on multiple
460 factors, with many of these removal mechanisms interdependent or interrelated. Some research
461 has demonstrated correlations between these factors, but further studies are necessary to gain a

better understanding of the removal factors as well as their correlations, particularly in colder climates where a number of these natural disinfection mechanisms, direct and indirect, will be governed by variations climate, particularly sunlight and temperature.

Algae play a unique role in pond systems. They are able to uptake nutrients, such as nitrogen and phosphorus in both inorganic and organic forms. In addition to their role in nutrient removal, algae have been recognized as an indirect disinfection factor, especially in natural wastewater treatment processes. For example, algae are able to raise pH and DO concentrations through photosynthesis. pH levels above 9 have been demonstrated to be bactericidal in WSPs. DO can also inactivate pathogenic organisms at concentrations higher than 0.5 mg/L. The photo-oxidation that occurs in pond systems is enhanced by the presence of high DO concentrations, which is a product of algal photosynthesis. Therefore, it is essential to study the role of algae in the disinfection process in WSPs in order to better understand and correlate important pathogen removal factors such as sunlight, pH and DO. This would provide invaluable insights to operators of WSP systems. The design and operation of the eco-engineered systems could be tailored to promote natural disinfection conditions as a function of anticipated climate and seasonal variations, which would enable small, remote and rural communities – where these systems are largely operated – to better predict their effluent water quality and assist them in meeting increasingly stringent effluent discharge requirements.

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References

- Agunwamba, J. C. 1991. Dispersion Number Determination in Waste Stabilization Ponds. *Water Air Soil Pollut.* **59**(3-4): 241-247. doi:10.1007/BF00211833.
- Al-Hashimi, M., Hussain, H.T. 2013. Stabilization Pond for Wastewater Treatment, *Euro. Sci. J.* **9**(14): 278-294. Available from <file:///Users/Lei/Downloads/1084-3234-1-PB.pdf> [accessed on 27 December 2017].
- Almasi, A., and Pescod, M.B. 1996. Pathogen removal mechanisms in anoxic wastewater stabilization ponds. *Water Sci. Technol.* **33**(7): 133–140. doi:10.1016/0273-1223(96)00348-4.
- Amahmid, O., Asmama, S., and Bouhoum, K. 2002. Urban wastewater treatment in stabilization ponds: occurrence and removal of pathogens. *Urban Water.* **4**(3): 255–262. doi:10.1016/S1462-0758(01)00071-1.
- Ansa, E.D.O., Lubberding, H.J., Ampofo, J.A., and Gijzen, H.J. 2011. The role of algae in the removal of *Escherichia coli* in a tropical eutrophic lake. *Ecol. Eng.* **37**(2011): 317-324. doi:10.1016/j.ecoleng.2010.11.023.
- Ansa, E.D.O., Lubberding, H.J., Ampofo, J.A., Amegbe, G.B., and Gijzen, H.J. 2012. Attachment of fecal coliform and macro-invertebrate activity in the removal of fecal coliform in domestic wastewater treatment pond systems. *Ecol. Eng.* **42**(2012): 35-41.
- Atlas, R.M., and Bartha, R. 1981. *Microbial ecology; Fundamentals and applications*. Addison – Wesley Publishers Co. London. pp. 560.
- Awuah, E. 2006. Pathogen removal mechanisms in waste stabilisation ponds. Ph.D. thesis, Wageningen University/UNESCO-IHE Institute for Water Education, The Netherlands.

- 509 Awuah, E., Anohene, F., and Asante, K. 2001. Environmental conditions and pathogen removal
510 in macrophyte- and algal-based domestic wastewater treatment systems. *Water Sci. Technol.*
511 **44**(6): 11-18.
- 512 Awuah, E., Lubberding, H.J., and Asante, K. 2002. The effect of pH on enterococci removal in
513 Pistia-, duckweed- and algae-based stabilization ponds for domestic wastewater treatment.
514 *Water Sci. Technol.* **45**(1): 67-74.
- 515 Bales, R.C., Li, S.M., Maguire, K.M., Yahya, M.T. and Gerba, C.P. 1993. MS-2 and poliovirus
516 transport in porous medium: Hydrophobic effects and chemical perturbations. *Water Resour.*
517 *Res.* **29**(4): 957-963.
- 518 Bales, R.C., Li, S., Yeh, T.C.J., and Lenczewski, M.E.C.P. 1997. Bacteriophage and microsphere
519 transport in saturated porous medium: Force-gradient experiment at Borden, Ontario. *Water*
520 *Resour. Res.* **33**: 639-648.
- 521 Barzily, A., and Kott, Y. 1991. Survival of pathogenic bacteria in an adverse environment. *Water*
522 *Sci. Technol.* **24**(2): 395-400.
- 523 Baxter, C.S., Hofmann, R., Templeton, M.R., Brown, M., and Andrews, R.C. 2007. Inactivation
524 of adenovirus types 2, 5, and 41 in drinking water by UV light, free chlorine, and
525 monochloramine. *J. Environ. Eng.* **133**(1): 95-103.
- 526 Berney, M., Weilenmann, H.U., and Egli, T. 2006a. Flow-cytometric study of vital cellular
527 functions in *Escherichia coli* during solar disinfection (SODIS). *Microbiol. Sgm.* **152**: 1719-
528 1729.
- 529 Berney, M., Weilenmann, H.U., and Egli, T., 2006b. Gene expression of *Escherichia coli* in
530 continuous culture during adaptation to artificial sunlight. *Environ. Microbiol.* **8**: 1635-1647.
- 531 Bolton, N.F., Cromar, N.J., Hallsworth, P., and Fallowfield, H.J. 2010. A review of the factors

- 532 affecting sunlight inactivation of microorganisms in waste stabilization ponds: preliminary
533 results for enterococci. *Water Sci. Technol.* **61**: 885-890. doi: 10.2166/wst.2010.958.
- 534 Bolton, N.F., Cromar, N.J., Buchanan, N.A., and Fallowfield, H.J. 2011. Mechanisms of sunlight
535 inactivation of common microbial indicators in waste stabilization ponds. *In* Proceedings of
536 the 9th IWA Specialist Group Conference on Waste Stabilization Ponds: Adelaide, Australia,
537 1–3 August 2011.
- 538 Bosshard, F., Bucheli, M., Meur, Y., and Egli, T. 2010a. The respiratory chain is the cell's
539 Achilles' heel during UVA inactivation in *Escherichia coli*. *Microbiol.* **156**: 2006–2015.
540 doi:10.1099/mic.0.038471-0.
- 541 Bosshard, F., Riedel, K., Schneider, T., Geiser, C., Bucheli, M., and Egli, T. 2010b. Protein
542 oxidation and aggregation in UVA irradiated *Escherichia coli* cells as signs of accelerated
543 cellular senescence. *Environ. Microbiol.* **12**(11): 2931–2945. doi:10.1111/j.1462-
544 2920.2010.02268.x.
- 545 Boutilier, L., Jamieson, R., Gordon, R., Lake, C., and Hart, W. 2009. Adsorption, sedimentation,
546 and inactivation of *E. coli* within wastewater treatment wetlands. *Water Res.* **43**(17): 4370–
547 4380. doi:10.1016/j.watres.2009.06.039.
- 548 Buchauer, K. 2007. Comparison of model approaches for predicting coliform removal in waste
549 stabilization ponds. *Water Environ. J.* **21**(2): 108-113. doi:10.1111/j.1747-6593.2006.00055.x.
- 550 Champagne, P., Liu, L., and Howell, M. 2017. Aerobic treatment in cold-climate countries. In
551 Current development in biotechnology and bioengineering. Elsevier, Amsterdam,
552 Netherlands. pp. 161-189.
- 553 Cornejo, P.K., Zhang, Q., and Mihelcic, J.R. 2013. Quantifying benefits of resource recovery
554 from sanitation provision in a developing world setting. *J. Environ. Manag.* **131**: 7–15.

- doi:10.1016/j.jenvman.2013.09.043.
- Cromeans, T.L., Kahler, A.M., and Hill, V.R. 2010. Inactivation of adenoviruses, enteroviruses, and murine norovirus in water by free chlorine and monochloramine. *Appl. Environ. Microbiol.* **76**(4): 1028–1033. doi:10.1128/AEM.01342-09.
- Curtis, T.P., Mara, D.D., and Silva, S.A. 1992a. Influence of pH, oxygen, and humic substances on ability of sunlight to damage fecal coliforms in waste stabilization pond water. *Appl. Environ. Microbiol.* **58**(4): 1335-1343.
- Curtis, T.P., Mara, D.D., and Silva, S.A. 1992b. The effect of sunlight on faecal coliforms in ponds: implications for research and design. *Water Sci. Technol.* **26**(7–8): 1724–1738.
- Curtis, T.P., Mara, D.D., Dixo, N.G.H., and Silva, S.A. 1994. Light penetration in waste stabilization ponds. *Wat. Res.* **28**: 1031-1038.
- Davies-Colley, R.J., Bell, R.G., and Donnison, A.M. 1994. Sunlight inactivation of enterococci and fecal-coliforms in sewage effluent diluted in seawater. *Appl. Environ. Microbiol.* **60**: 2049 - 2058.
- Davies-Colley, R.J., Donnison, A.M., and Speed, D.J. 1997. Sunlight wavelengths inactivating faecal indicator microorganisms in waste stabilization ponds. *Water Sci. Technol.* **35**(11–12): 219–225.
- Davies-Colley, R.J., Donnison, A.M., Speed, D.J., Ross, C.M., and Nagels, J.W. 1999. Inactivation of faecal indicator microorganisms in waste stabilization ponds: interaction of environmental factors with sunlight. *Water Res.* **33**(5): 1220–1230.
- Davies-Colley, R.J., Donnison, A.M., and Speed, D.J. 2000. Towards a mechanistic understanding of pond disinfection. *Water Sci. Technol.* **42**: 149-158.
- Diaz, F.J., O'Geen, A.T., and Dahlgren, R.A. 2010. Efficacy of constructed wetlands for removal

- 578 of bacterial contamination from agricultural return flows. *Agric. Water Manag.* **97**(11): 1813–
579 1821. doi:10.1016/j.agwat.2010.06.015.
- 580 Drechsel, P., Scott, C.A., Raschid-Sally, L., Redwood, M., and Bahri, A. 2009. Wastewater
581 irrigation and health-assessing and mitigating risk in low-income countries. International
582 Water Management Institute and International Development Research Centre, London, UK.
- 583 Fallowfield, H.J., Cromar, N.J., and Evison, L.M. 1996. Coliform die-off rate constants in a high
584 rate algal pond and the effect of operational and environmental variables. *Water Sci. Technol.*
585 **34**(11): 141–147. doi:10.1016/S0273-1223(96)00831-1.
- 586 Feachem, R.G., Bradley, D.J., Garelick, H., and Mara, D.D. 1983. Sanitation and Disease: Health
587 Aspects of Excreta and wastewater management. Published for the World Bank by John
588 Wiley and Sons, U.K.
- 589 Ferro, S., Coppellotti, O., Roncucci, G., Ben Amor, T., and Jori, G. 2006. Photosensitized
590 inactivation of *Acanthamoeba palestinensis* in the cystic stage. *J. Appl. Microbiol.* **101**: 206–
591 212. doi:10.1111/j.1365-2672.2006.02893.x.
- 592 Fisher, M.B., Iriarte, M., and Nelson, K.L. 2012. Solar water disinfection (SODIS) of
593 *Escherichia coli*, *Enterococcus* spp., and MS2 coliphage: effects of additives and alternative
594 container materials. *Water Res.* **46**(6): 1745-1754. doi:10.1016/j.watres.2011.12.048.
- 595 Gagliardi, J.V., and J.S. Karns. 2000. Leaching of *Escherichia coli* O157:H7 in diverse soils
596 under various agricultural management practices. *Appl. Environ. Microbiol.* **66**:877–883.
597 doi:10.1128/AEM.66.3.877-883.2000.
- 598 George, I., Crop, P., and Servais, P. 2002. Faecal coliform removal in wastewater treatment
599 plants studied by plate counts and enzymatic methods. *Water Res.* **36**(10): 2607-
600 2617.

- 601 Grimason, A.M., Smith, H.V., Young, G., and Thitai, W.N. 1996a. Occurrence and removal of
602 *Ascaris* sp. ova by waste stabilisation ponds in Kenya. *Water Sci. Technol.* 33(7): 75-82.
603 doi:10.1016/0273-1223(96)00341-1.
- 604 Grimason, A.M., Wiandt, S., Baleux, B., Thitai, W.N., Bontoux, J., and Smith, H.V. 1996b.
605 Occurrence and removal of *Giardia* sp cysts by Kenyan and French waste stabilisation pond
606 systems. *Water Sci. Technol.* 33(7): 83-89. doi:10.1016/0273-1223(96)00342-3.
- 607 Guan, H., Schulze-Makuch, D., Schaffer, S., and Pillai, S.D. 2003. The effect of critical pH on
608 virus fate and transport in saturated porous medium. *Ground Water* 41(5): 701–708.
- 609 Heaselgrave, W., Patel, N., Kilvington, S., Kehoe, S.C., and McGuigan, K.G. 2006. Solar
610 disinfection of poliovirus and *Acanthamoeba* polyphaga cysts in water - a laboratory study
611 using simulated sunlight. *Lett. Appl. Microbiol.* 43(2):125-30. doi:10.1111/j.1472-
612 765X.2006.01940.x.
- 613 Hillebrandt, S., and Müller, I.1991. Repair of damage caused by UV- and X-irradiation in the
614 amoebflagellate *Naegleria gruberi*. *Radiat. Environ. Biophys.* 30(2): 123-30.
- 615 Hijnen, W.A.M., Beerendonk, E.F., and Medema, G.J. 2006. Inactivation credit of UV radiation
616 for viruses, bacteria and protozoan (oo)cysts in water; a review. *Wat. Res.* 40(1): 3-22.
617 doi:10.1016/j.watres.2005.10.030.
- 618 Hotze, E.M., Badireddy, A.R., Chellam, S., and Wiesner, M.R. 2009. Mechanisms of
619 bacteriophage inactivation via singlet oxygen generation in UV illuminated fullerol
620 suspensions. *Environ. Sci. Technol.* 43(17): 6639–6645.
- 621 Hwang, T.S., Hyon, J.Y., Song, J.K., Reviglio, V.E., Spahr, H.T., and O'Brien, T.P. 2004.
622 Disinfection capacity of PuriLens contact lens cleaning unit against *Acanthamoeba*. *Eye*
623 *Contact Lens* 30:42–43.

- 624 Jasper, J.T., Nguyen, M.T., Jones, Z.L., Ismail, N.S., Sedlak, D.L., Sharp, J.O., Luthy, R.G.,
625 Horne, A.J., and Nelson, K.L. 2013. Unit process wetlands for removal of trace organic
626 contaminants and pathogens from municipal wastewater effluents. *Environ. Eng. Sci.* **30**(8):
627 421–436. doi:10.1089/ees.2012.0239.
- 628 Jiménez, B. 2007a. Helminth ova removal from wastewater for agriculture and aquaculture
629 reuse. *Water Sci. Technol.* **55**(1-2): 485–493. doi: 10.2166/wst.2007.046.
- 630 Jiménez, B. 2007b. Helminth ova control in sludge: a review. *Water Sci. Technol.* **56**(9):147-
631 155.
- 632 Jiménez, B., Maya-Rendón, C., and Salgado-Velázquez, G. 2001. The elimination of helminth
633 ova, faecal coliforms, Salmonella and protozoan cysts by various physicochemical processes
634 in wastewater and sludge. *Water Sci. Technol.* **43**(12): 179–182
- 635 Jiménez, B. 2009. Helminth ova control in wastewater and sludge for agricultural reuse. In
636 book: *Water Reuse New Paradigm towards Integrated Water Resources Management in*
637 *Encyclopedia of Biological, Physiological and Health Sciences*, Edition: 1a, Publisher:
638 EOLSS Publishers Co. Ltd UNESCO, Editors: Grabow W., pp. 429-449.
- 639 Jokerst, A., Sharvelle, S.E., Hollowed, M.E., and Roesner, L.A. 2011. Seasonal performance of
640 an outdoor constructed wetland for graywater treatment in a temperate climate. *Water*
641 *Environ. Res.* **83**(12): 2187–2198. doi:10.2175/106143011X12989211841412.
- 642 Kadir, K., and Nelson, K.L. 2014. Sunlight mediated inactivation mechanisms of *Enterococcus*
643 *faecalis* and *Escherichia coli* in clear water versus waste stabilization pond water. *Water Res.*
644 **50**: 307 – 317. doi: 10.1016/j.watres.2013.10.046.
- 645 Kaneko, J., Kimura, T., Kawakami, Y., Tomita, T., and Kamio, Y. 1997. Panton-valentine
646 leukocidin genes in a phage-like particle isolated from mitomycin C-treated *Staphylococcus*

- 647 aureus V8 (ATCC 49775). Biosci. Biotechnol. Biochem. **61**(11): 1960-1962.
648 doi:10.1271/bbb.61.1960.
- 649 Kim, T.D., and Unno, H. 1996. The roles of microbes in the removal and inactivation of viruses
650 in a biological wastewater treatment system. Water Sci. Technol. **33**(10):243-250.
651 doi:10.1016/0273-1223(96)00426-X.
- 652 Klock, J. W. 1971. Survival of Coliform Bacteria in Wastewater Treatment Lagoons. J. Water
653 Pollut. Control. Fed. **43**(10): 2071-2083.
- 654 Kohn, T., and Nelson, K.L. 2007. Sunlight-mediated inactivation of MS2 coliphage via
655 exogenous singlet oxygen produced by sensitizers in natural waters. Environ. Sci. Technol.
656 **41**(1): 192-197. doi:10.1021/es061716i.
- 657 Koivunen, J., Siitonen, A., and Tanski, H.H. 2003. Elimination of enteric bacteria in biological-
658 chemical wastewater treatment and tertiary filtration units. Water Res., **37**: 690-698. doi:
659 10.1016/S0043-1354(02)00305-6.
- 660 Li, J.W., Xin, Z.T., Wang, X.W., Zheng, J.L., and Chao, F.H. 2004. Mechanisms of inactivation
661 of hepatitis A virus in water by chlorine dioxide. Water Res. **38**(6): 1514–1519.
662 doi:10.1016/j.watres.2003.12.021.
- 663 Liu, L., Hall, G., and Champagne, P. 2015. Effects of environmental factors on the disinfection
664 performance of a wastewater stabilization pond operated in a temperate climate. Water **8**(5):1-
665 11. doi:10.3390/w8010005.
- 666 Liu, L. 2018. The role of algae in disinfection processes in wastewater stabilization ponds. Ph.D.
667 dissertation. Queen's University, Kingston, ON.
- 668 Loret, J. F., Jousset, M., Robert, S., Saucedo, G., Ribas, F., Thomas, V., and Greub, G. 2008.
669 Amoebae-resisting bacteria in drinking water: risk assessment and management. Water Sci.

- Technol. **58**(3): 571-577. doi:10.2166/wst.2008.423.
- Love, D.C., Silverman, A., and Nelson, K.L. 2010. Human virus and bacteriophage inactivation in clear water by simulated sunlight compared to bacteriophage inactivation at a Southern California Beach. Environ. Sci. Technol. **44**(18): 6965–6970. doi:10.1021/es1001924.
- MacDougall, A. 2016. The effects of climate and humic substances on disinfection performance in Arctic wastewater stabilization pond. Master's Thesis, Department of Civil Engineering, Queen's University, Kingston, ON, Canada.
- McCambridge, J., and McMeekin, T.A. 1979. Protozoan predation of Escherichia coli in estuarine waters. Water Res. **13**: 659-664. doi:10.1016/0043-1354(79)90016-2.
- McCambridge, J., and McMeekin, T.A. 1980. Relative effects of bacterial and protozoan predators on survival of Escherichia coli in estuarine water samples. Appl. Environ. Microbiol. **40**: 907-911.
- Mahler, B.J., Personne, J.C., Lods, G.F., and Droque, C. 2000. Transport of free and particulate-associated bacteria in karst. J. Hydrol. **238**(3-4): 179-193. doi:10.1016/s0022-1694(00)00324-3.
- Mara, D.D. 2003. Domestic Wastewater Treatment in Developing Countries. *In* Domestic Wastewater Treatment in Developing Countries Earthscan/James & James, London, UK.
- Mara, D.D., and Pearson, H.W. 1986. Artificial freshwater environments: waste stabilization ponds. *In* Schoernborn W Biotechnology, Weinheim, Germany: VCH Verlagsgesellschaft, pp. 177-206.
- Mara, D.D, Pearson, H., Oragui, J., Arridge, H., and Silva, S.A. 2001. Development of a New Approach to Waste Stabilization Pond Design. Research Monograph No. 5, pp. 1–56. School of Civil Engineering, University of Leeds, Leeds, England.

- 693 Marais, G.V. 1974. Fecal bacterial kinetics in stabilization ponds. J. Sanit. Eng. Div. Am. Soc.
694 Civ. Eng. **100**:119-139.
- 695 Mayo, A.W.1989. Effect of Pond Depth on Bacterial Mortality Rate. J. Environ. Eng. **115**(5):
696 964-976.
- 697 Maynard, H.E., Ouki, S.K., and Williams, S.C. 1999. Tertiary lagoons: a review of removal
698 mechanisms and performance. Water Res. **33**(1): 1–13. doi: 10.1016/s0043-1354(98)00198-5.
- 699 Metcalf and Eddy Inc. 2003. Wastewater Engineering: Treatment and reuse. *In* Wastewater
700 Engineering: Treatment and reuse. 4th ed. McGraw Hill Inc. New York, USA.
- 701 Mezrioui N., Oufdou K., and Baleux, B. 1995a. Dynamics of non-01 *Vibrio cholerae* and faecal
702 coliforms in experimental stabilization ponds in the arid region of Marrakesh, Morocco, and
703 the effect of pH, temperature, and sunlight on their experimental survival. Can. J. Microbiol.
704 **41**(6): 489-498.
- 705 Mezrioui, N., Baleaux, B., and Troussellier, M. 1995b. A microcosm study of the survival of
706 *Escherichia coli* and *Salmonella typhimurium* in brackish water. Wat. Res. **29**: 459-465.
- 707 Mo, W., and Zhang, Q. 2012. Can municipal wastewater treatment systems be carbon neutral? J.
708 Environ. Manag. **112**: 360–367. doi:10.1016/j.jenvman.2012.08.014.
- 709 Mosteo, R., Ormad, M.P., and Goni, P. 2013. Identification of pathogen bacteria and protozoa in
710 treated urban wastewaters discharged in the Ebro River (Spain): water reuse possibilities.
711 Water Sci. Technol. **68**(3): 575–583. doi: 10.2166/wst.2013.201.
- 712 Moumouni, D.A., Andrianisa, H.A., Konate, Y., Ndiaye, A., and Maiga, A. 2016. Inactivation of
713 *Escherichia coli* in a baffled pond with attached growth: treating anaerobic effluent under the
714 Sahelian climate. Environ. Technol. **37**(9): 1054-1064. doi: 10.1080/09593330.2015.1098732.
- 715 Nelson, K.L., Kadir, K., Fisher, M.B., and Love, D. 2009. New insights into sunlight disinfection

- 716 mechanisms in waste stabilisation ponds. 8th IWA Specialist Group Conference on Waste
717 Stabilisation Ponds Belo Horizonte, Brazil, 26–30 April 2009.
- 718 Nuanualsuwan, S., Miriam, T., Himathongham, S., and Cliver, D.O. 2002. Ultraviolet
719 inactivation of feline calicivirus, human enteric viruses and coliphages. *Photochem.*
720 *Photobiol.* **76**: 406–410. doi:10.1562/0031-8655(2002)076<0406:UIOFCH>2.0.CO;2.
- 721 Oilgae 2009. Oilgae guide to algae-based wastewater treatment. *In* Oilgae guide to algae-based
722 wastewater treatment. Tamilnadu, India. pp. 5-21.
- 723 Ouali, A., Jupsin, H., Ghrabi, A., and Vassel, J.L. 2014. Removal kinetic of *Escherichia coli* and
724 enterococci in a laboratory pilot scale wastewater maturation pond. *Water Sci. Technol.* **69**(4):
725 755 – 759. doi: 10.2166/wst.2013.774.
- 726 Ouali, A., Jupsin, H., Vassel, J.L., Marouani, L., and Ghrabi, A. 2012. Removal improvement of
727 bacteria (*Escherichia coli* and Enterococci) in maturation ponds using baffles. *Water Sci.*
728 *Technol.* **65**(4): 589–595. doi: 10.2166/wst.2012.896.
- 729 Pearson, H.W., Mara, D.D., Mills, S.W., and Smallman, D.J. 1987a. Factors determining algal
730 populations in waste stabilization ponds and influence on algae pond performance. *Water Sci.*
731 *Tech.* **19**(12): 131-140.
- 732 Pearson, H.W., Mara, D.D., Mills, S.W., and Smallman, D.J. 1987b. Physiochemical parameters
733 influencing faecal bacteria survival in waste stabilization ponds. *Water Sci. Tech.*, **19**(12):
734 145-152.
- 735 Pearson, H.W., Mara, D.D., and Arridge, H.A. 1995. The Influence of Pond Geometry and
736 Configuration on Facultative and Maturation Waste Stabilization Pond Performance and
737 Efficiency. *Water Sci. Technol.*, **31**(12): 129–139. doi:10.1016/0273-1223(95)00500-M.
- 738 Polprasert, C., Dissanayake, M.G., and Thanh, N.C. 1983. Bacterial die-off kinetics in waste

- 739 stabilization ponds. *J. Water Pollut. Control Fed.* **55**: 285-296.
- 740 Reinoso, R., and Bécares, E. 2008. Environmental inactivation of *Cryptosporidium parvum*
741 oocysts in waste stabilization ponds. *Microb. Ecol.* **56**(4): 585–592. doi:10.1007/s00248-008-
742 9378-7.
- 743 Reinoso, R., Blanco, S., Torres-Villamizar, L., and Bécares, E. 2011. Mechanisms for parasites
744 removal in a waste stabilization pond. *Microb. Ecol.* **61**(3): 648–692. doi:10.1007/s00248-
745 010-9791-6.
- 746 Reinoso, R., Bécares, E., and Smith, H.V. 2007. Effect of various environmental factors on the
747 viability *Cryptosporidium parvum* oocysts. *J. Appl. Microbiol.* **104**: 980–986.
748 doi:10.1111/j.1365-2672.2007.03620.x.
- 749 Rose, J.B., Farrah, S.R., Harwood, V.J., Levine, A.D., Lukasik, J., Menendez, P., and Scott,
750 T.M. 2004. Reduction of pathogens, indicator bacteria, and alternative indicators by
751 wastewater treatment and reclamation processes. Water Environment Research Foundation,
752 Alexandria, VA, US.
- 753 Sanin, S.L., Sanin, F.D., and Bryers, J.D. 2003. Effect of starvation on the adhesive properties of
754 xenobiotic degrading bacteria. *Process Biochem.* **38**: 909–914. doi:10.1016/S0032-
755 9592(02)00173-5.
- 756 Sano, D., Pinto, R.M., Omura, T., and Bosch, A. 2009. Detection of oxidative damages on viral
757 capsid protein for evaluating structural integrity and infectivity of human norovirus. *Environ.*
758 *Sci. Technol.* **44**(2): 808–812. doi:10.1021/es9018964.
- 759 Santos, A.L., Oliveira, V., Baptista, I., Henriques, I., Gomes, N.C.M., Almeida, A., Correia, A.,
760 and Cunha, A. 2012. Wavelength dependence of biological damage induced by UV radiation
761 on bacteria. *Arch. Microbiol.* **195**: 63-74. doi:10.1007/s00203-012-0847-5.

- 762 Sarikaya, H. Z., and Saatci, A. M. 1987. Bacteria die-off in waste stabilization ponds. *J. Environ.*
763 *Eng.* **113**(2): 366-382.
- 764 Sassoubre, L.M., Nelson, K.L., and Boehm, A.B. 2012. Mechanisms for photoinactivation of
765 *Enterococcus faecalis* in Seawater. *Appl. Environ. Microbiol.* **78**: 7776-7785.
766 doi:10.1128/AEM.02375-12.
- 767 Sengupta, M.E., Thamsborg, S.M., Andersen, T.J., Olsen, A., and Dalsgaard, A. 2011
768 Sedimentation of helminth eggs in water. *Water Res.* **45**(15): 4651–4660.
769 doi:10.1016/j.watres.2011.06.017.
- 770 Sharafi, K., Moradi, M., Karami, A., and Khosravi, T. 2015 Comparison of the efficiency of
771 extended aeration activated sludge system and stabilization ponds in real scale in the removal
772 of protozoan cysts and parasite ova from domestic wastewater using Bailenger method: a case
773 study, Kermanshah, Iran. *Desalination water treatment*, **56**(5): 1135-1141.
774 doi:10.1080/19443994.2014.923333
- 775 Sheludchenko, M., Padovan, A., Katouli, M., and Stratton, H. 2016. Removal of Fecal
776 Indicators, Pathogenic Bacteria, Adenovirus, Cryptosporidium and Giardia (oo)cysts in Waste
777 Stabilization Ponds in Northern and Eastern Australia. *Int. J. Environ. Res. Pub. Health* **13**(1):
778 96. doi:10.3390/ijerph13010096.
- 779 Siddiqui, R., and Khan, N.A. 2008. Balamuthia amoebic encephalitis: an emerging disease with
780 fatal consequences. *Microbiol. Pathog.* **44**(2): 89–97. doi:10.1016/j.micpath.2007.06.008.
- 781 Storer, T.I., Usinger, R.L., Stebbins, R.C., and Nybakken, J.W. 1979. Protozoans. *In* General
782 Zoology, 6th Edition. McGraw-Hill, New York, pp. 329–359.
- 783 Stott R., May E., and Mara D.D. 2003. Parasite removal by natural wastewater treatment
784 systems: performance of waste stabilization ponds and constructed wetlands. *Water Sci.*

- Technol. **48**(2): 97–104.
- Sweeney, D.G., Nixon, J.B., Cromar, N.J., and Fallowfield, H.J. 2007. Temporal and spatial variations of physical, biological and chemical parameters in a large waste stabilisation pond, and the implications for WSP modelling. *Water Sci. Technol.* **55**(11): 1–9. doi:10.2166/wst.2007.336.
- Tuncsiper, B., Ayaz, S.C., and Akca, L. 2012. Coliform bacteria removal from septic wastewater in a pilot-scale combined constructed wetland system. *Environ. Eng. Manag. J.* **11**(10): 1873–1879.
- Tyagi, V.K., Sahoo, B.K., Khursheed, A., Kazmi, A.A., Ahmad, Z., and Chopra, A.K. 2011. Fate of coliforms and pathogenic parasite in four full-scale sewage treatment systems in India. *Environ. Monit. Assess.* **181**: 123–135. doi:10.1007/s10661-010-1818-4.
- Ulrich, H., Klaus, D., Irmgard, F., Annette, H., Juan, L.P., and Regine, S. 2005. Microbiological investigations for sanitary assessment of wastewater treated in constructed wetlands. *Water Res.* **39**(20): 4849–4858.
- United Nations. 2015. Resolution Adopted by the General Assembly. NY, USA, New York.
- USEPA, 1983. Process design Manual: Municipal Waste Stabilization Ponds (Report No. EPA 625/1-83-015). Cincinnati, Ohio, USA.
- USEPA, 2011. Principles of Design and Operations of Wastewater Treatment Pond Systems for Plant Operators, Engineers, and Managers. EPA/600/R-11/088, Cincinnati, Ohio, USA.
- Van Buuren, J.C.L., and Hobma, S. 1991. The faecal coliform removal rate at post treatment of anaerobically pre-treated domestic wastewater. Department of Environ. Tech. Agricultural University, Wageningen. Unpublished.
- Verbyla, M.E., Iriarte, M.M., Guzman, A.M., Coronado, O., Almanza, M., and Mihelcic, J.R.

2016. Pathogens and fecal indicators in waste stabilization pond systems with direct reuse for irrigation: Fate and transport in water, soil and crops. *Sci. Total Environ.* **551-552**: 429-437. doi: 10.1016/j.scitotenv.2016.01.159.
- Verbyla, M.E., and Mihelcic, J.R. 2015. A review of virus removal in wastewater treatment pond systems. *Water Res.* **71**: 107-24. doi:10.1016/j.watres.2014.12.031.
- Wallace, J., Champagne, P., and Hall, G. 2016. Multivariate statistical analysis of water chemistry conditions in three wastewater stabilization ponds with algae blooms and pH fluctuation. *Water Res.* **96**: 155-165. doi:10.1016/j.watres.2016.03.046.
- WHO (World Health Organization) 1989. Health guidelines for the use of wastewater in agriculture and aquaculture. WHO Technical Report Series, No.778. World Health Organization, Geneva, Switzerland.
- Wigginton, K.R., Menin, L., Montoya, J.P., and Kohn, T. 2010. Oxidation of virus proteins during UV254 and singlet oxygen mediated inactivation. *Environ. Sci. Technol.* **44**: 5437-5443. doi:10.1021/es100435a.
- Winward, G.P., Avery, L.M., Frazer-Williams, R., Pidou, M., Jeffrey, P., Stephenson, T., and Jefferson, B. 2008. A study of the microbial quality of grey water and an evaluation of treatment technologies for reuse. *Ecol. Eng.* **32(2)**: 187-197. doi:10.1016/j.ecoleng.2007.11.001.
- Wu, S., Carvalho, P.N., Muller, J.A., Manoj, V.R., and Dong, R. 2016. Sanitation in constructed wetlands: A review on the removal of human pathogens and fecal indicators. *Sci. Total Environ.* **541**: 8-22. doi: 10.1016/j.scitotenv.2015.09.047.
- Zanotelli, C.T., Medri, W., Belli Filho, P., Perdomo, C.C., Mullinari, M.R., and Costa, R.H.R. 2002. Performance of a baffled facultative pond treating piggery wastes. *Water Sci. Technol.*

831 **45**(1):49-53.

832 Figure 1: Natural disinfection mechanisms in wastewater stabilization ponds (modified from The
833 Global Water Pathogen Project, 2018).

834 Figure 2: Factors influencing sunlight-mediated disinfection.

835 Figure 3: Algal photosynthesis influences on increases in pH and DO.

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Table 1. Summary of removal of bacteria, viruses, protozoa and helminthes from full/pilot scale WSPs in Africa.

Country	Reference	Retention		Removal bacteria (log)	Removal viruses (%)	Removal helminth eggs			
		time (d)	Scale			Removal protozoa (%)	(%)		
Burkina Faso	Konate <i>et al.</i> , 2013	18	Full scale			>99.99 (<i>Entamoeba coli</i> , <i>Entamoeba histolyca</i> , and <i>Giardia lamblia</i>)	>99.99 (<i>Ascaris lumbricoides</i> , <i>Ancylostoma sp.</i> , <i>Trichuris trichiura</i> , and <i>Trichostrongylus sp.</i>)		
Egypt	El-Deeb <i>et al.</i> , 2008	No data	Full scale	6 (TC)	79.98 (Coliphage)				
				4.6 (FC)					
				3.8 (<i>E. coli</i>)					
				4.9 (FS)	99.66 (Infectious rotaviruses)				
				3.9 (Salmonellae)					
				2.1 (Listeria)					

Ghana	Keraita <i>et al.</i> , 2008	10	Full scale (small)	5.6 (Thermotolerant coliforms)		100
				2.5 (<i>E.coli</i>)	99.92 (Somatic coliphage)	
	Silverman <i>et al.</i> , 2013	No data	Full scale	3.3 (FE)	<90 (F+ coliphage)	
				1.6 (Thermotolerant coliforms)		
Morocco	Amahmid <i>et al.</i> , 2002	16	Pilot scale		100 (<i>Giardia</i> cysts)	100 (<i>Ascaris</i>)
					100 (<i>Entamoeba coli</i> ,	100 (<i>Ascaris</i> , <i>Trichuris</i> ,
	Bouhoum <i>et al.</i> , 2000	16	Pilot scale		<i>Entamoeba histolyca</i> ,	<i>Hymenolepis</i> , <i>Taenia</i> , and
					and <i>Giardia</i> sp.)	<i>Enterobius</i>)
	Kouraa <i>et al.</i> , 2002	57-59	Full scale	6 (FC)		100
	Yagoubi <i>et al.</i> , 2000	No data	Large pilot scale	4.27 (FC) 3.57 (FS)		100

Tunisia	Ouali <i>et al</i> , 2012	7.94-8.51	Full scale	1.18-2 (<i>E. coli</i>)
				1.47-2.14 (Enterococci)
	Ouali <i>et al.</i> , 2014	7.94	Full scale	1.4-2.2 (<i>E. coli</i>)
				1.5-2.6 (FS)

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Table 1. Summary of removal of bacteria, viruses, protozoa and helminthes from full/pilot scale WSPs in Americas.

Country	Reference	Retention time (d)	Scale	Removal bacteria (log)	Removal viruses (%)
Bolivia	Hadley, 2013	24	Full scale		91 (Somatic coliphage)
					99.92 (Culturable enteroviruses)
	Symonds <i>et al.</i> , 2014	25.5	Full scale		99.97 (Norovirus GI)
					99.94 (Rotavirus Group A)
Brazil	Dias <i>et al.</i> , 2014	4.8-14.5	Full scale	4.4-5.4 (<i>E. coli</i>)	
	Pivelli <i>et al.</i> , 2008	20	Full scale	2.58 (TC) 2.89 (<i>E. coli</i>)	
	Von Sperling <i>et al.</i> , 2002	6.3	Full scale	1.64 (<i>E. coli</i>)	
	Von Sperling <i>et al.</i> , 2003	6.1-9.3	Full scale		
	Von Sperling and Mascarenhas, 2005	7.4	Full scale	5.1 (<i>E. coli</i>)	
	Zanotelli <i>et al.</i> , 2002	124.3	Full scale	5 (FC) 7 (TC)	
				3.2-5.5 (Thermotolerant coliforms)	0-99.84 (Somatic coliphage)
Canada	Locas <i>et al.</i> , 2010	16.2-19.6	Full scale	2.8-5.1 (FE)	80-99.984 (Male-specific coliphages)
				0.8-2.3 (CL)	-25.9-90 (Human enteric viruses)
Chile	Herrera and Castillo, 2000	5.3-7.6	Full scale	1.48-1.86 (FC)	

Colombia				3.1-3.5 (FC)	99.21-99.97 (F+ phages <i>Bacterial fragilis</i>)
	Campos <i>et al.</i> , 2002	53	Full scale	3-4.4 (<i>E. coli</i>) 3-3.9 (FS*) 3.3 (CL)	93.69-99.997 (Somatic phages) 87.41 (RYC phages)
	Madera <i>et al.</i> , 2002	12	Full scale	4 (FC) 1.7 (FS)	
	Vorkas and Lloyd, 2000	7.98	Full scale	1.5 (FC)	
Honduras	Oakley, 2005	7.2-34.8	Full scale	2.97 (<i>E. coli</i>) 2.93 (FC)	
Mexico	Escalante <i>et al.</i> , 2000	945	Full scale	3.7 (FC)	
Nicaragua	Buchauer <i>et al.</i> , 2007	16	Full scale	0.54-4 (FC)	

Table 3. Summary of removal of bacteria, viruses, protozoa and helminthes from full/pilot scale WSPs in Asia.

Country	Reference	Retention time (d)	Scale	Removal bacteria (log)	Removal viruses (%)
India	Tyagi <i>et al.</i> , 2008	11	Full scale	1.24 (<i>Salmonella</i>)	
				3 (<i>E. coli</i>)	
				2.35 (FS)	
				2.21 (FC)	
Iran	Sharafi <i>et al.</i> , 2012	No data	Full scale	2.24 (TC)	
				2.30 (TC)	
				2.30 (FC)	
				2.40 (FS)	
Israel	Alcalde <i>et al.</i> , 2003	18	Full Scale	0.82-1.93 (FC)	91.3-99.6 (Somatic coliphages) 98.38-99.97 (F-specific bacteriophages)
	Van der Steen <i>et al.</i> , 2000	5	Mini-ponds	2.2-2.7 (FC)	
Korea	Ham <i>et al.</i> , 2007	11	Pilot scale	2.05 (FC)	
Palestine	Zimmo <i>et al.</i> , 2002	28	Pilot scale	3.3-3.6 (FC)	

Table 4. Summary of removal of bacteria, viruses, protozoa and helminthes from full scale WSPs in Europe.

Belgium	Nameche <i>et al.</i> , 2000	7	Full scale	3.40 (TC)	
				2.62 (FC)	
				3.64 (FS)	
				1.88 (Sulphite-reducing <i>Clostridium</i>)	
France	Brissaud <i>et al.</i> , 2000	2.0-3.0	Full scale	1.7 (FC)	
	Da Silva <i>et al.</i> , 2008	Several weeks	Full scale		98.42 (Norovirus GI) 97.49 (Norovirus GII)
	Picot <i>et al.</i> , 2005	95	Full scale	4.1 (FC) 3.4 (FE)	
Greece	Parissopoulos <i>et al.</i> , 2003	35-106	Full scale	1.68-4 (TC) 1.73-4 (FC)	
	Gratziou and Chalatsi, 2013	No data	Full Scale	2.7- 4 (FC) 1.3 (TC)	
	Amengual-Morro <i>et al.</i> , 2012	69	Full scale	4 (FC)	
Spain	Gomila <i>et al.</i> , 2008	60	Full scale	1.26 (TC)	97.2 (SOMPCPH)
				1.28 (FC)	95.8 (F-specific phages)
				0.86 (<i>E. coli</i>)	73.8 (Enteric viruses)
	Lucena <i>et al.</i> , 2004	49	Full scale	0.54 (FE)	87.2 (BFRYCPH)
				1.25 (<i>Clostridium</i> spp.)	83.3 (Phages of <i>B. thetaiotamicron</i> GA17)
				2.5-4.7 (FC)	93.69-96.02 (BFBRYCPH)
	Mosteo <i>et al.</i> , 2013	50	Full scale	2.5-2.7 (FE)	99.87-99.99 (SOMCPH)
				1.3-1.4 (SRC)	99.37-99.95 (FRNAPH)
				1.38 (<i>E. coli</i>)	
				5.51 (<i>Enterococcus</i> spp.)	
				1.78 (<i>Staphylococcus aureus</i>)	
				0.51 (<i>Pseudomonas</i> sp)	
		24	Full scale	2.14 (<i>E. coli</i>)	

			3.61 (<i>Enterococcus</i> spp.) 0.05 (<i>Pseudomonas</i> sp) 0.7 (CL)	
			2.5-2.8 (TC)	
Reinoso <i>et al.</i> , 2011	5.5	Full scale	3-5 (<i>E. coli</i>) 3-3.9 (FS)	96-99.8 (Coliphage)

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Figure 1. Natural disinfection mechanisms in wastewater stabilization ponds (modified from The Global Water Pathogen Project, 2018).

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Figure 2. Factors influencing sunlight-mediated disinfection.

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Figure 3. Algal photosynthesis influences on increases in pH and DO.