GLOBAL WATER PATHOGEN PROJECT

PART FOUR. MANAGEMENT OF RISK FROM EXCRETA AND WASTEWATER

WASTE STABILIZATION PONDS

Matthew Verbyla
San Diego State University
San Diego, United States

Marcos von Sperling
Federal University of Minas Gerais
Belo Horizonte, Brazil

Ynoussa Maiga
University of Ouagadougou
Ouagadougou, Burkina Faso

Citation:

Verbyla, M., von Sperling, M. and Maiga, Y. (2017). Waste Stabilization Ponds. In: J.B. Rose and B. Jiménez-Cisneros (eds), Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project). (J.R. Mihelcic and M.E. Verbyla (eds), Part 4: Management Of Risk from Excreta and Wastewater - Section: Sanitation System Technologies, Pathogen Reduction in Sewered System Technologies), Michigan State University, E. Lansing, MI, UNESCO. https://doi.org/10.14321/waterpathogens.65

Acknowledgements: K.R.L. Young, Project Design editor; Website Design: Agroknow (http://www.agroknow.com)

Last published: March 13, 2017

Summary

Waste stabilization ponds (WSPs) are sanitation technologies that consist of open basins that use natural processes to treat domestic wastewater, septage, and sludge, as well as animal or industrial wastes. They can be used in centralized or semi-centralized sewerage systems, they can also be used to treat fecal sludge from onsite dry sanitation systems, or as onsite water-based sanitation systems serving a single building or home. The most common types of WSPs are anaerobic ponds, facultative ponds, maturation or polishing ponds, aerated ponds, and high-rate algal ponds (HRAPs). Some pathogen removal is accomplished in anaerobic, facultative, aerated ponds and HRAPs, even though their primary function is to remove and stabilize organic matter. The primary function of maturation and polishing ponds however, is to remove and inactivate pathogens. Under optimal conditions, removal efficiencies in full-scale WSP systems with several units in series can be as high as 6 $\log_{10}$ for fecal bacteria and 4 $\log_{10}$ for viruses, protozoan (oo)cysts, and helminth eggs, however the efficiency of pathogen removal in full-scale systems is highly variable, and in practice many WSP systems achieve only 2 to 3 $\log_{10}$ removal. Some of the most important factors influencing pathogen removal efficiency in WSPs include hydraulic retention time and efficiency, water clarity, pond depth, sunlight exposure and penetration, temperature, and pH. Shallow (<1m) baffled maturation ponds with low turbidity, high pH, and plenty of sunlight exposure will achieve the most efficient pathogen reduction. The sludge/sediments from WSPs (especially anaerobic, facultative and aerated ponds) must be removed periodically, and treated or managed appropriately to limit human exposure. The concentration of viable helminth eggs and protozoan (oo)cysts in this sludge can be as high as 2,000 – 4,000 per gram of total soilds, and helminth eggs in particular can survive in WSP sediments for years. WSP sysetms require large areas of open land, making them ideal in smaller towns and rural settings, though they are used successfully in many urban environments as well, often in combination with other sanitation technologies. One of the biggest advantages of WSPs is that they are easy and inexpensive to operate and maintain, and generally do not rely on mechanized equipment or expensive material or energy inputs.

Waste Stabilization Ponds

1.0 Brief Technology Description

Waste stabilization ponds (WSPs) are open basins enclosed by earthen embankments, and sometimes fully or partially lined with concrete or synthetic geofabrics. They employ natural processes to treat domestic wastewater, septage, and sludge, as well as animal or industrial wastes. They can be used in centralized or semi-centralized sewerage systems, serving cities or towns; they can also be used as onsite systems serving a single entity (e.g., highway rest area, community center, etc.) (Figure 1). WSPs are frequently used in combination with other sanitation technologies. The most common types of WSPs are anaerobic ponds, facultative ponds, maturation ponds, aerated ponds, and high-rate algal ponds (HRAPs). These ponds differ in terms of their function in the overall wastewater treatment system. The main function of anaerobic, facultative and aerated ponds is the removal of carbon-containing organic matter, while the main function of maturation ponds is the removal of pathogens. HRAPs were developed to optimize the efficiency of organic matter removal while simultaneously allowing for the recovery of dissolved nutrients that become incorporated into the algal biomass. These different pond types are distinguished from each other by their depth, hydraulic and organic loading rates, and by whether or not they use mechanized equipment for mixing or aeration. In general, anaerobic ponds are deepest (≥3.0 m) and are used first in series; facultative ponds are shallower (1.5 – 3.0 m) and may be used first or second in series (after anaerobic ponds); maturation ponds are shallowest (≤1.5 m), and are used last in series. Aerated ponds may be used anywhere in a series of ponds, and HRAPs are often used in by themselves or between anaerobic and maturation ponds. For more information about the design of WSP systems, refer to von Sperling (2007), Oakley (2005), Shilton (2006) or Mara (2003). Figure 2 illustrates different types of ponds and one schematic of a typical WSP system design with three different types of ponds (anaerobic, facultative, and maturation) operating in series.

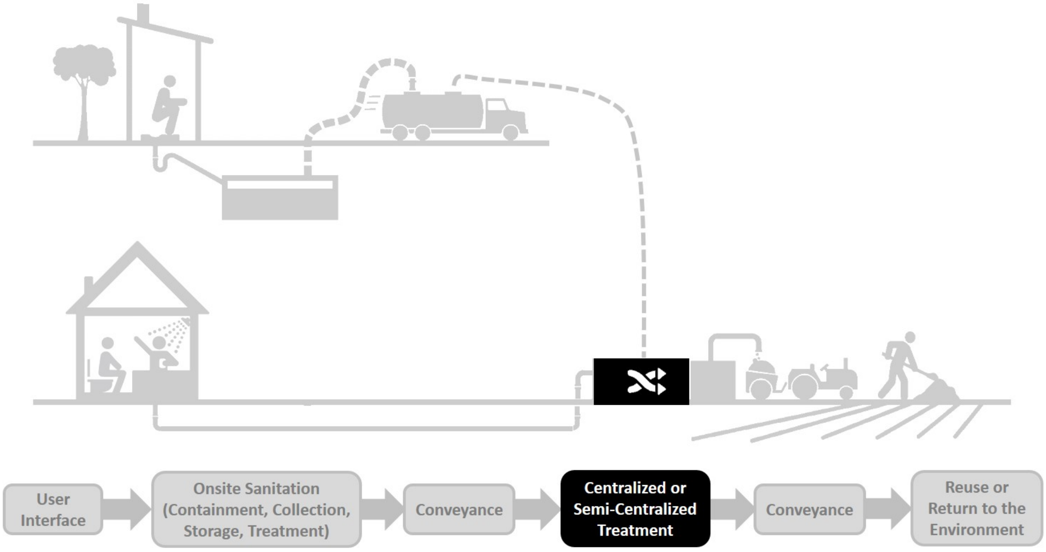
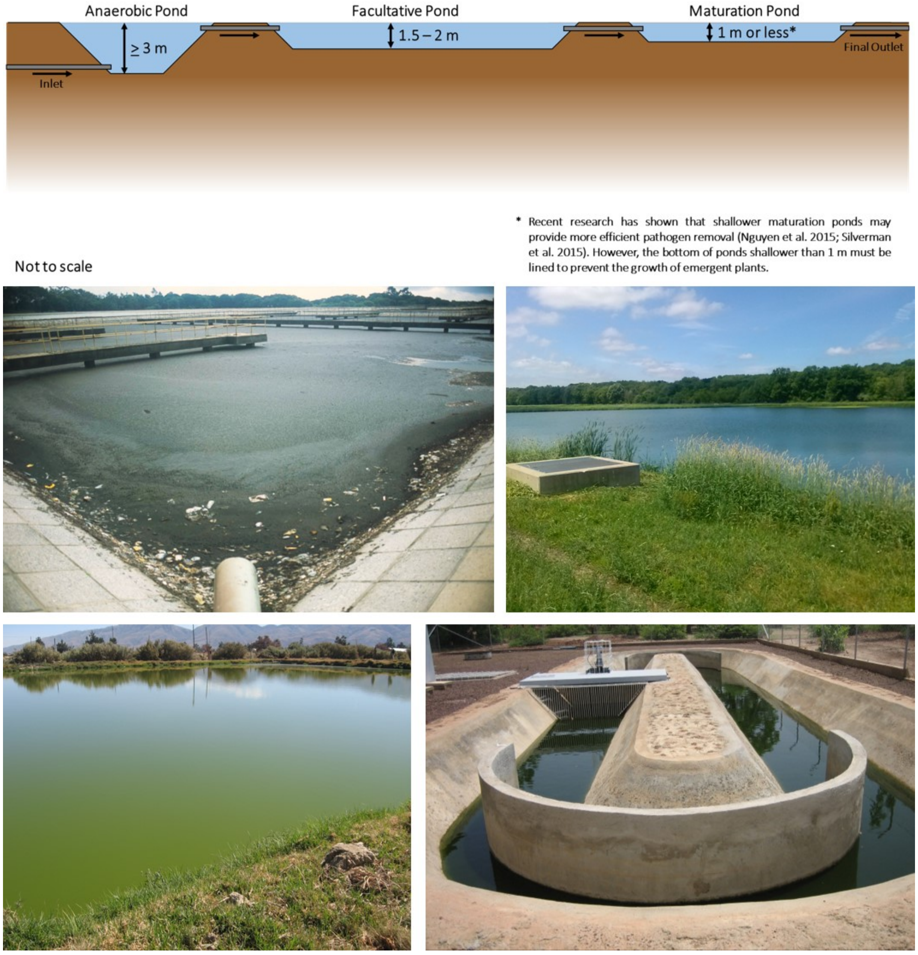


Figure 1. Waste stabilization ponds are a centralized or semi-centralized treatment technology in the overall sanitation service chain, used with sewered sanitation systems and also to treat the contents of onsite systems



* Recent research has shown that shallower maturation ponds may provide more efficient pathogen removal (Nguyen et al. 2015; Silverman et al. 2015). However, the bottom of ponds shallower than 1 m must be lined to prevent the growth of emergent plants.

Figure 2. Schematic of a typical waste stabilization pond system (top); anaerobic pond in Brazil (middle left) (photo from Stewart Oakley); facultative pond in the United States (middle right) (photo from Matthew Verbyla); maturation pond in Bolivia (bottom left) (photo from Matthew Verbyla); high-rate algal pond in Burkina Faso (bottom right) (photo from Ynoussa Maiga)

2.0 Inputs and Outputs for Waste Stabilization Ponds

WSPs can be used to treat a variety of water and waste streams, thus the inputs may include wastewater, septage, latrine pit contents, and/or sludge from other wastewater treatment processes (Figure 3). Some WSP systems also receive landfill leachate. WSPs may receive untreated wastewater that has gone through preliminary treatment (e.g. screening and grit removal), or they may receive secondary effluent from some other treatment process, such as anaerobic reactors, activated sludge, or trickling filters. Typical concentrations for pathogens in wastewater, septage, latrine pit contents, and/or sludge are provided in Part Three of GWPP.

The outputs from WSP systems include the treated effluent (liquid), sludge/sediments (solids), and biogas. The treated liquid effluent from WSPs is often continuously discharged; however, operators of some systems (especially in colder climates) may stop discharging for months at a time, allowing the ponds to fill up and discharging once the temperature gets warmer (this extra retention time makes up for the slower rate of treatment during colder months). Sludge accumulates over time at the bottom of WSPs, and must be removed every few years (anaerobic ponds), every decade (primary facultative ponds), or every few decades (secondary facultative or maturation ponds). Sludge removed from WSPs is contaminated with pathogens and needs to be safely managed (to prevent exposure) or treated (to reduce the concentration of pathogens). Refer to the chapter on Sludge Management.

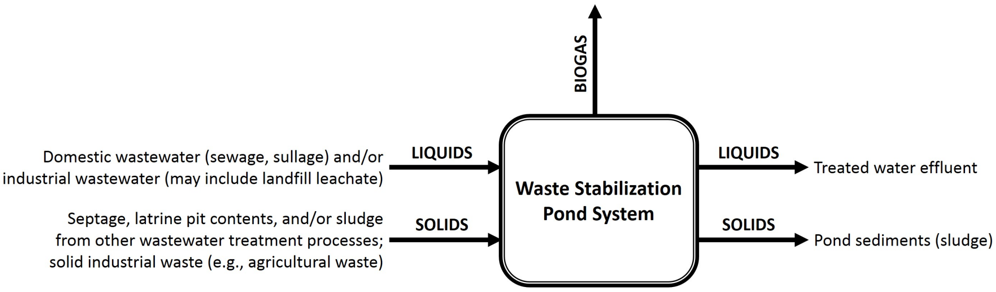


Figure 3. Typical inputs and outputs from waste stabilization pond systems

3.0 Factors Affecting Pathogens in Waste Stabilization Ponds

In WSPs, pathogens are removed from the wastewater or inactivated through a variety of different mechanisms that occur at different rates. The efficiency of these mechanisms is dependent on a number of environmental, design, and operational factors. The most important factors are shown in Figure 4. Systems that are poorly maintained, overloaded or malfunctioning may experience reduced pathogen removal efficiency, regardless of other factors. Likewise, WSPs with poor hydraulic efficiency (where the water entering the pond takes a preferential path short-cut directly to the exit point) will have poorer pathogen removal efficiency simply because the majority of pathogens do not stay long enough in the pond to experience the conditions that will remove or inactivate them.

Different factors affect different types of pathogens in different ways. The most important factor for the removal of viral and bacterial pathogens is sunlight exposure, although other factors such as temperature, dissolved oxygen and pH are also important. Sedimentation, hydraulic efficiency, sunlight exposure, and physical-chemical factors (including temperature and pH) are all important factors for the removal of protozoan pathogens, though sedimentation is perhaps the most important. Helminth eggs are primarily removed by sedimentation, and other factors are less important. Different pathogen types that are removed by the same mechanism are not necessarily removed at the same rate by that mechanism. For example, viruses and bacteria are both damaged by sunlight in WSPs, but viruses are generally more resistant than bacteria (Davies-Colley et al., 2005b; Sinton et al., 2002). Different species of viruses and bacteria are also removed at different rates in WSPs, due to differences in their structural and genetic composition (Silverman et al., 2013; Mattle et al., 2014; Kohn et al., 2016).

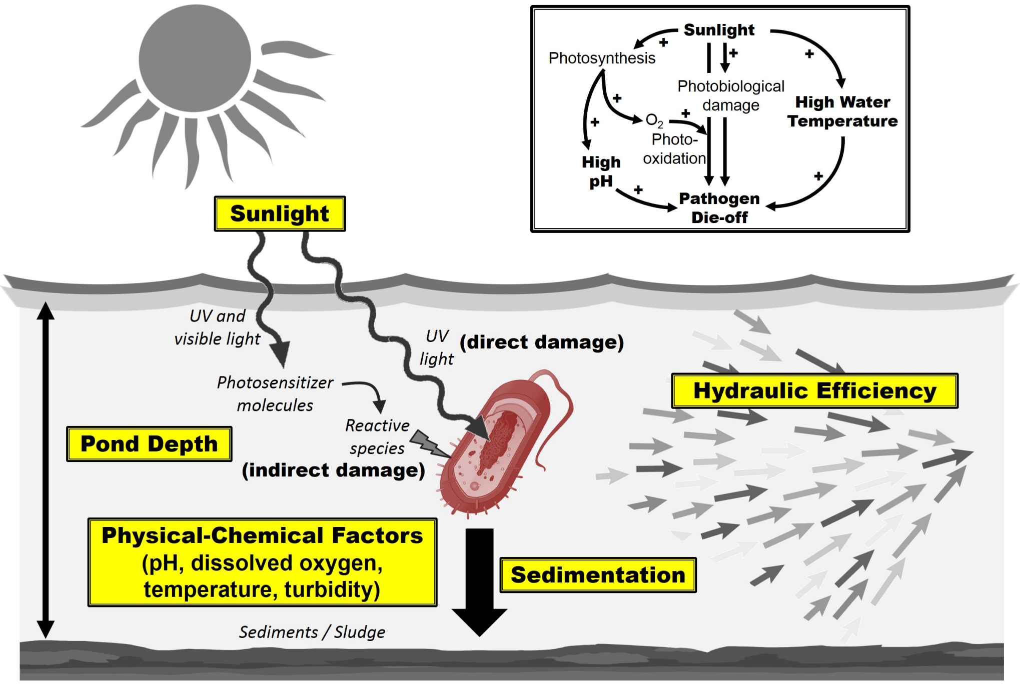


Figure 4. Major factors affecting pathogen removal in waste stabilization ponds and causal loop diagram showing the influence of sunlight on pathogen die-off

3.1 Sunlight and Water Clarity

The UV portion of sunlight directly damages pathogen genomes (photobiological damage), while UV and visible wavelengths can react with photosensitizers in WSPs (such as natural organic matter) or photosensitizer molecules within bacteria (such as NADH/NADPH, flavins and porphyrins) to produce reactive species that indirectly damage pathogens (photo-oxidative damage). Sunlight is stronger at lower latitudes, higher elevations, and in locations with less cloud cover. The composition of WSP water is also important—sunlight is rapidly attenuated within the first few centimeters of WSPs (Davies-Colley et al., 2005a). Research has demonstrated a clear relationship between the amount of direct sunlight that reaches pathogens and their rate of inactivation (Nguyen et al., 2015; Silverman et al., 2015; Dias, 2016). Thus, the clarity of water in WSPs and the amount of sunlight penetration is a very important factor. Sunlight penetration can be improved in maturation ponds by designing them to be shallower than 1 m, a depth that is the current standard (maturation ponds can be as shallow as 20 – 40 cm (Maiga et al., 2009a; Jasper et al., 2013) as long as a liner is used to prevent the growth of emergent vegetation). This is especially true if the pond allows for the growth of periphyton (which grow at the bottom) instead of suspended-growth algae (which contribute to the turbidity of the water) (Jasper et al., 2013). Silverman et al. (2015) found no pathogen removal benefits to making maturation ponds any deeper than 50 cm.

Sunlight is one of the most important factors for viral and bacterial pathogen removal in WSPs. *E. coli* loses viability almost 20 times faster in WSPs with sunlight exposure compared to dark conditions, and it is also inactivated faster in shallower WSPs (Maiga et al., 2009a). *Campylobacter jejuni* is more vulnerable to sunlight damage than *Salmonella enterica*, which is more vulnerable than *E. coli* (Sinton et al., 2007). Enterococci and *E. coli* are equally as vulnerable to sunlight in clear water (Kadir and Nelson, 2014; Maraccini et al., 2016; Silverman et al., 2016), but enterococci were inactivated more rapidly than *E. coli* in a WSP in Ouagadougou, Burkina Faso (Maiga et al., 2009b), perhaps due to their greater vulnerability to sunlight in the presence of external photosensitizer molecules that may be present in WSPs with high concentrations of organic suspended solids (Kadir and Nelson, 2014). Bacteria can repair sunlight damage in the dark (Oguma et al., 2001), while viruses cannot (however, a few viruses may be able to repair sunlight-induced damage after initiating an infection, by utilizing their host cell’s DNA repair machinery (Weitzman et al., 2004).

Protozoan (oo)cysts are much more likely to be removed in a WSP system due to sedimentation than sunlight, but they are susceptible to sunlight damage—viable *Cryptosporidium parvum* oocysts were reduced by 0.2-$\log_{10}$ units in WSP water after four days of sunlight exposure (Reinoso and Bécares, 2008). *C. parvum* is capable of repairing sunlight damage, however it does not necessarily regain infectivity (Oguma et al., 2001). Regarding helminths, *Ascaris* eggs have high resistance to solar disinfection (Bandala et al., 2012; Heaselgrave and Kilvington, 2011). Thus, sunlight exposure is not likely an important factor for the removal of helminth eggs in WSPs.

3.2 Sedimentation

WSP systems have hydraulic retention times on the order of days, weeks, or even months, which allows large, dense particles to settle. Sedimentation is more effective in WSPs with less turbulence. Ponds should be designed to maintain quiescent conditions that approach laminar flow. The size and density of pathogens and particles determines their settling velocities. Bacteria and viruses will not settle in WSPs unless they are attached to larger, denser particles. Only a small percentage of viruses attach to WSP particles, and they mostly attach to particles that are too small to settle (Characklis et al., 2005; da Silva et al., 2008; Krometis et al., 2009; Sobsey and Cooper, 1973; Symonds et al., 2014); this attachment is also reversible (Ohgaki et al., 1986; Sobsey and Cooper, 1973). Likewise, *E. coli* attaches to particles in WSPs, but this does not necessarily result in observable removal via settling (Boutilier et al., 2009). Relative to other factors, sedimentation is not very important for virus and bacteria removal in WSPs.

Protozoan (oo)cysts and helminth eggs are much larger than viruses and bacteria. The settling velocities of free-floating helminth eggs and protozoan (oo)cysts are 5 – 13 m/d and 0.026 – 0.13 m/d, respectively (compared to bacteria and virus settling velocities of 0.012 and 0.00001 m/d, respectively) (Cizek et al., 2008; David and Lindquist, 1982; Kulkarni et al., 2004; Medema et al., 1998; Sengupta et al., 2011). *Cryptosporidium* oocysts are among the smallest of the protozoan (oo)cysts and have low settling velocities, however they are hydrophobic and negatively-charged, which favors their attachment to particles (Characklis et al., 2005). Medema et al. (1998) found that 75% of *C. parvum* oocysts became attached to wastewater particles and settled at greater velocities as a result.

It is important to note that sedimentation does not necessarily result in the loss of pathogen viability, but rather transfers pathogens from the pond water to the sediment/sludge, where they may remain viable; flow velocities of 0.07 to 0.12 m s^{-1}, high turbulence, and the forces generated by rising biogas bubbles can resuspend eggs and (oo)cysts from WSP sediments (Sengupta et al., 2012). Overturning resulting from diurnal or seasonal changes in temperature and the de-stratification of warmer and cooler water layers can also result in the resuspension of potentially viable pathogens in the sediment/sludge layer.

3.3 Physical-Chemical and Microbiological Factors

The most important physical-chemical factors for pathogen inactivation are pH, temperature and dissolved oxygen in the presence of dissolved organic matter. Most bacterial pathogens are vulnerable to high pH, with *Vibrio* spp. as a notable exception (Mezrioui et al., 1995). The sanitizing effect of free ammonia, which becomes more available at higher pH, is even more effective at higher temperatures (Decrey et al., 2014; Emmoth et al., 2011; Burge et al., 1983). Helminth eggs are the most resistant to physical-chemical factors in WSPs; they can survive for years in WSP sludge (Konaté et al., 2010; Nelson et al., 2004). High temperatures and the presence of volatile and organic acids, aldehydes, alcohols, and NH_3 may increase helminth egg die-off in WSP sludge (Fidjeland et al., 2013; Ghiglietti et al., 1997; Nelson and Pecson, 2005; Nelson, 2003; Reimers et al., 1990).

Microbial diversity in WSPs presents situations that are stressful to pathogens. Bacterial pathogens (and viruses to a lesser extent) may be internalized by higher trophic organisms (Miki and Jacquet, 2008), however it is unclear if this affects their viability or transport through WSPs (Scheid and Schwarzenberger, 2012). Starvation affects fecal bacteria in WSPs, especially in maturation ponds with BOD_5 concentrations < 20 mg/L (Almasi and Pescod, 1996). Algae and fungi also excrete metabolites that are harmful to some viruses, bacteria, and protists (Metting and Pyne, 1986; Mille-Lindblom and Tranvik, 2003; Senhorinho et al., 2015).

4.0 Design, Operation, and Maintenance Guidelines for Pathogen Removal

As illustrated in the previous section, the performance of a WSP system depends on a multitude of factors interacting simultaneously, and frequently it is not possible to identify a single factor that explains poor or good performance (Oliveira and von Sperling, 2011).

Nevertheless, there are several design approaches that can help enhance pathogen removal in WSP systems. Systems with longer hydraulic retention times and a greater number of ponds in series are associated with greater removal of helminth eggs (Ayres et al., 1992; Saqqar and Pescod, 1992), protozoan (oo)cysts (Ben Ayed et al., 2009; Reinoso et al., 2008), bacteria (von Sperling, 2005), and viruses (Verbyla and Mihelcic, 2015). The theoretical hydraulic retention time (calculated as the pond volume divided by the flow rate) however, is not the only factor that affects the residence time distribution (the amount of time pathogens actually spend in the system) (Persson, 2000; Shilton and Harrison, 2003a). Other design factors such as the pond depth, the length-to-width ratio, the configurations of inlets and outlets, the speed and direction of the wind, stratification caused by diurnal shifts in the temperature at the surface of the pond, and the presence or absence of baffles can all have an impact on pathogen removal, particularly in maturation ponds. Maturation ponds with higher length-to-width ratios (longer ponds or ponds with baffles) are generally more efficient at reducing pathogens (von Sperling, 2007). The strategic design and positioning of inlet and outlet structures and stub baffles in WSPs can improve hydraulic efficiency and enhance pathogen removal (for more, refer to Shilton and Harrison (2003a, 2003b). Pond depth is also an important factor, particularly for maturation ponds. Anaerobic and facultative ponds are designed to remove organic matter and must be deeper by necessity, but shallower maturation ponds experience better sunlight penetration and increased photosynthetic activity (leading to higher pH and dissolved oxygen), which improves pathogen removal. Pilot studies with extremely shallow maturation ponds (20 cm), installed with a liner to prevent emergent plants that would otherwise shade the water surface, have demonstrated the development of periphyton communities and more efficient pathogen removal than conventional maturation ponds (Nguyen et al., 2015; Silverman et al., 2015). Shallow ponds with full sunlight exposure also experience higher temperatures than deeper ponds, which can enhance pathogen removal (Maiga et al., 2009a).

Proper operation and maintenance are crucial to achieving efficient pathogen removal in WSP systems. Improper maintenance can lead to malfunctions that significantly reduce pathogen removal (Verbyla et al., 2016). Due to their large volumes and long hydraulic retention times, WSPs are usually robust to short-term (daily) fluctuations in the influent, related to flow, concentrations or loads. However, long-term malfunction concerns, such as sludge accumulation, hydraulic short-circuiting, and organic or hydraulic overloading, may reduce the efficiency of pathogen removal. Organic and hydraulic overloading may be especially problematic in cities and towns with rapid population growth or increasing industrial activity. WSPs that are organically overloaded may turn anaerobic, becoming more turbid and allowing less sunlight penetration. The clarity of the water in WSPs is indeed a very important factor for pathogen removal in WSP systems (Davies-Colley et al., 2005; Silverman et al., 2015). Hydraulic overloading can cause hydraulic short-circuiting which reduces the effective time pathogens remain in the system (Verbyla et al., 2013a). Sediments (sludge) will accumulate at the bottom of WSPs (especially anaerobic and facultative ponds) over time, diminishing the effective pond volume, potentially causing overturning and the resuspension of settled pathogens, reducing overall pathogen removal (Verbyla et al., 2016). Modeling has demonstrated that sludge accumulation in ponds can sometimes act as a baffle, potentially improving hydraulic efficiency (Ouedraogo et al., 2016). Nevertheless, sludge should be removed every few years for anaerobic ponds, at least every 10 – 20 years for primary facultative ponds, and every few decades for secondary facultative and maturation ponds. WSP systems should be designed with ponds in parallel to facilitate the process of sludge removal.

Table 1. Summary of key factors and strategies to enhance pathogen removal in waste stabilization ponds

Factor	Pathogen Removal from Wastewater is ↑ Enhanced or ↓ Reduced under the Following Conditions:	Pathogen Groups Primarily Affected			
		Bacteria	Viruses	Protozoa	Helminths
Sunlight Exposure	More Sunlight Exposure = ↑ Pathogen Removal	●●●	●●●	●	●
Water Temperature	Higher Temperature = ↑ Pathogen Removal	●●	●●	●	●
pH	Higher pH = ↑ Pathogen Removal	●●	●	●	●
Hydraulic Retention Time	Longer Retention Time = ↑ Pathogen Removal	●●●	●●●	●●●	●●●
Pond Length/Width Ratio	Greater Length/Width Ratio = ↑ Pathogen Removal	●	●	●	●
Pond Depth	Shallower Ponds = ↑ Pathogen Removal	●●●	●●●	●	●
Number of Ponds in Series	More Ponds in Series = ↑ Pathogen Removal	●●●	●●●	●●	●●
Flow Turbulence	Turbulence = ↓ or ↑ Pathogen Removal [a]	↑	↑	↓	↓
Overturning	Overturning = ↓ or ↑ Pathogen Removal [a]	↑	↑	↓	↓
Sludge Accumulation	More Accumulated Sludge = ↓ Pathogen Removal [b]	●●	●●	●●	●●
Turbidity = Less Sunlight Penetration	Less Sunlight Exposure = ↓ Pathogen Removal	●●●	●●●	●●	●
Hydraulic Short-Circuiting	Hydraulic Short-Circuiting = ↓ Pathogen Removal	●●●	●●●	●●●	●●●
Organic Overloading	Organic Overloading = ↓ Pathogen Removal	●●●	●●●	●	●

● = least affected; ●● = moderately affected; ●●● = most affected

[a] Turbulent flow and overturning due to rapid changes in temperature and vertical stratification/destratification can cause the resuspension of settled protozoan and helminth pathogens, but can also enable bacterial and viral pathogens in the bottom layer of stratified ponds to have a chance to rise to the surface, where they may be more vulnerable to sunlight inactivation.

[b] Ponds with accumulated sludge have lower mean hydraulic retention times and will have less pathogen removal than they would if they did not have any sludge at all (Verbyla et al. 2013a; 2016). However, one modeling study demonstrated that accumulated sludge in a pond can act as a baffle (Ouedraogo et al. 2016), and baffles can improve hydraulic efficiency in a pond. Thus, while accumulated sludge most likely results in lower pathogen removal efficiency, but there may be some exceptions. Also, it may depend on the pathogen content in the sludge, as some pathogens may become re-released from the accumulated sludge.

5.0 Summary of Data on Pathogen Removal in Waste Stabilization Ponds

The removal of pathogens in WSPs clearly depends on a number of environmental factors as well as engineering design and operation and maintenance practices, as demonstrated in the previous section. Data from a review of the literature on pathogen removal in WSPs were collected; if available,data on the following three factors were also collected: 1) theoretical hydraulic retention time, 2) average air or water temperature, and 3) pond depth. The effect of hydraulic retention time on pathogen removal is shown in Figure 5 and the effects of temperature and pond depth on pathogen removal are shown in Figure 6.

Hydraulic retention time certainly influences pathogen removal in WSPs, but it is not the only factor. In general, for facultative and maturation ponds with HRTs up to 80 days, the log10 reduction of coliforms is generally greater than that of viruses (with a few exceptions). The removal of helminth eggs is generally similar to or greater than that of coliforms (Figure 5). There are few studies on the reduction of protozoan parasites (protists) and bacterial pathogens in WSPs, but based on the data available, it appears that their reduction may be similar to that of coliforms.

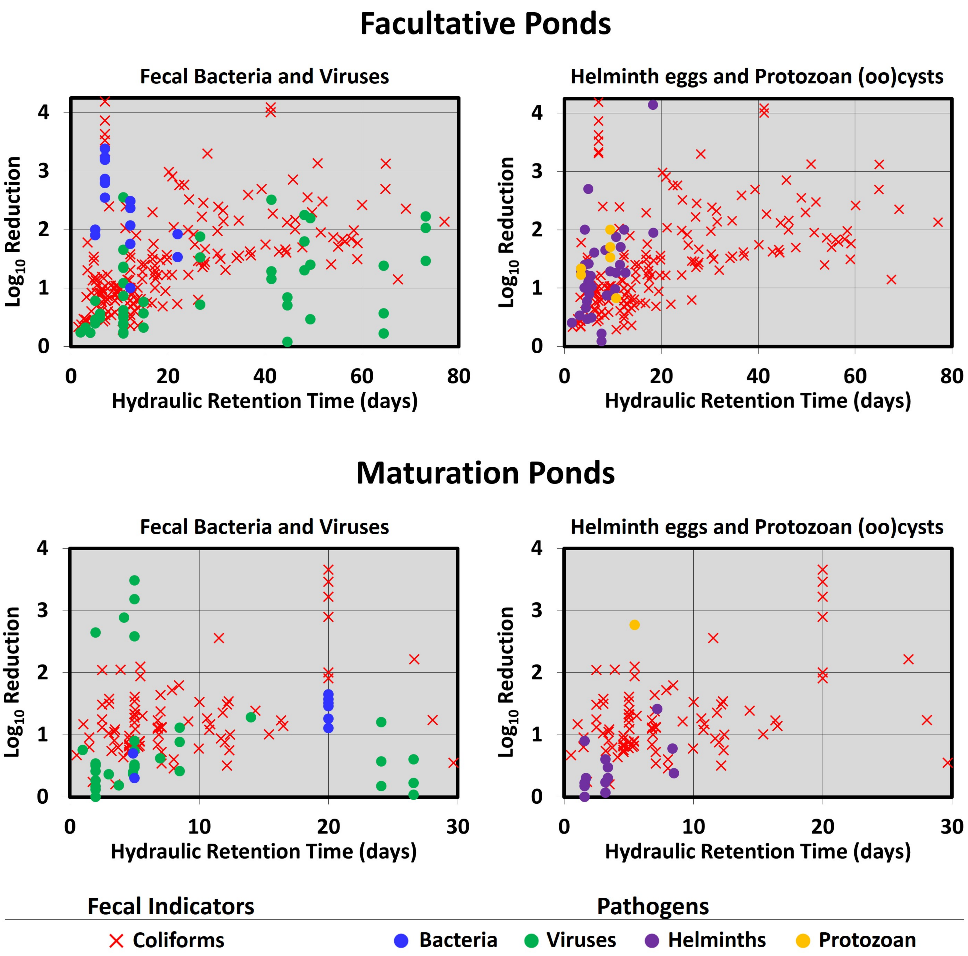


Figure 5. Relationship between the theoretical hydraulic retention time (for individual ponds) and the log$_{10}$ removal of pathogens and coliforms (including *E. coli*) in facultative and maturation ponds. Data for pathogens are shown together with data for traditional fecal indicator bacteria for comparative purposes

A simple way to normalize pathogen removal efficiency in WSPs with different hydraulic retention times is to calculate a ratio of the theoretical hydraulic retention time (HRT) divided by the log$_{10}$ removal. This ratio, which represents the theoretical time required for each log$_{10}$ reduction, may be used as a very simple proxy for planning and design purposes. However, this approach should be used with caution, as the data presented in Figures 5 and 6 have variations that span orders of magnitude. When using this approach, also consider that the data presented in Figure 6 come from studies that found at least 1log$_{10}$ reduction, but generally no more than 2log$_{10}$ reduction of pathogens. Figure 6 shows the relationship between ambient air temperature, pond depth, and the time required for each log10 reduction (note that the data shown in Figure 6 come from studies of ponds that provide between 1 and 2log10 reduction of pathogens). As a general rule, increasing the number of ponds in series should improve hydraulic efficiency and pathogen removal efficiency. For example, one pond with a retention time of 20 days will probably not remove pathogens as well as three ponds in series with an overall retention time of 20 days.

Also, deeper (facultative) ponds generally require more retention time to achieve the same level of pathogen removal as shallower (maturation) ponds, as indicated by Figure 6. There are extremely large variations in the number of days per log10 reduction. This is due to the fact that depth and temperature are only two of many other factors that are also important for pathogen removal in WSPs (e.g., Table 1).

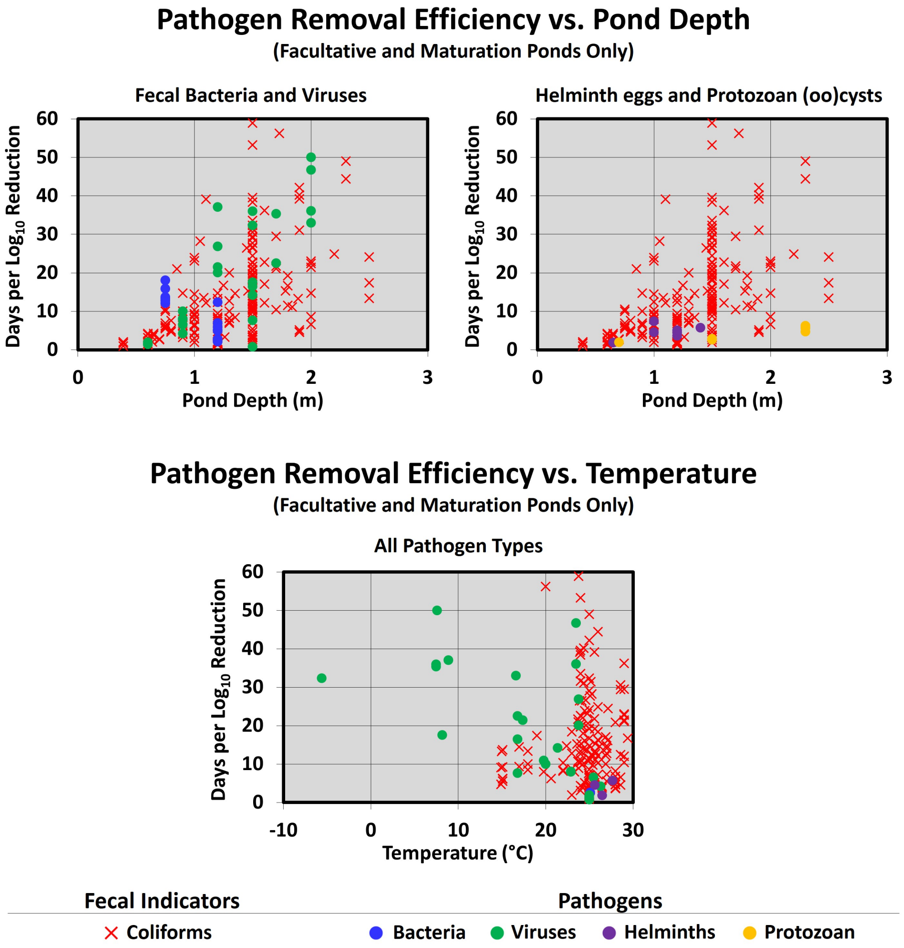


Figure 6. Relationship between temperature, pond depth, and the number of days per unit $\log_{10}$ reduction for waste stabilization ponds achieving at least $1\log_{10}$ removal of pathogens and fecal indicators. Temperatures shown represent average air temperature (for pathogens) and average water temperature (for fecal coliforms)

6.0 Summary of Data on Pathogens in Waste Stabilization Pond Sludge (Sediments)

Over time, some of the pathogens in the wastewater entering WSPs will end up in the sediments that accumulate at the bottom of the pond. Sedimentation does not necessarily result in the inactivation of pathogens, which may remain viable in WSP sludge/sediments (Table 3). The pathogens of greatest concern in WSP sludge, due to their durability and persistence, are helminths and protozoans. Concentrations of helminths reported in WSP sludge generally do not exceed 1,000 eggs per g total solids (TS), and in many cases are less than 100 eggs per g TS (Amahmid et al., 2002; Konaté et al., 2013a, 2010; Nelson et al., 2004; Schwartzbrod et al., 1989, 1987; Verbyla et al., 2013b), however von Sperling et al. (2003) found values close to 1,000 eggs per g TS, Oakley (2004) found more than 4,000 eggs per g TS of WSP sludge from one system in Honduras (which also had relatively higher concentrations of helminth eggs in the raw wastewater entering the system). The concentrations of helminth eggs in WSP sludge likely depend on the incidence of helminth infections in the community. Helminth eggs in particular have been shown to persist and maintain viability for long periods of time in WSP sediments. For example, protozoan cysts and viable helminth eggs were detected at high concentrations even at the bottom layer of WSP sediments in an anaerobic pond that was four years old (Konaté et al., 2013a, 2010); and viable helminth eggs were detected in a WSP sediment layer that was estimated to be 12 years old (Nelson et al., 2004). Konaté et al. (2010, 2013a) reported the following percent viability of helminth eggs in WSP sediments: 36 – 51% (*A. duodenale*), 40 – 100% (*Ascaris*), 22% (*Dicrocoellum spp.*), 14% (*E. vermicularis*), 21% (*H. nana* and *N. americanus*), 6 – 13% (*T. trichiura*). Table 3 provides a summary of typical pathogen concentrations in WSP sediments.

Sediments and sludge removed from WSPs are highly concentrated with potentially viable pathogens, particularly helminth eggs and protozoan (oo)cysts, and therefore, must be managed or treated appropriately to avoid exposure or reduce the concentration of viable pathogens. The recommended treatment method for sludge excavated from WSP sediments is either burial in a nearby field (to limit human contact), or dewatering in an open field or a sludge drying bed, where the material should be stored in the sunlight and out of the rain for a minimum of one year prior to reuse. Engineered systems such as greenhouse solar drying beds may help speed up the process by desiccating the material and raising temperatures during the day. The addition of seeds from wetland plants such as *Ludwigia* spp. can also help accelerate the process of dewatering (Oakley et al., 2012). Lime can be added to sludge removed from WSPs to stabilize the sludge and increase the pH to inactivate pathogens.

7.0 Conclusions

Tables 2 and 3 provide summaries of typical pathogen removal efficiencies in WSPs and presence of pathogens in the pond sediments (sludge). The WSPs used to generate the data in these tables had hydraulic retention times of <10 days (for anaerobic ponds) and <30 days (for facultative and maturation ponds). Therefore, a design engineer sizing anaerobic ponds with 10 days of hydraulic retention and facultative or maturation ponds with 30 days of hydraulic retention can likely anticipate the pathogen removal efficiencies shown in Table 2. Concentrations of pathogens in WSP sludge should not greatly exceed the high end of the ranges reported in Table 3, however concentrations may be lower depending on the prevalence of infection within the community. In general, the compilation of results from the literature (summarized in Figures 5 and 6 and in Table 2) indicates that the efficiency of pathogen removal in full-scale WSP systems can be highly variable, and depends on pond depth and temperature, but also on a number of other factors (e.g., Table 1). Hydraulic efficiency and sunlight penetration are two factors that appear to be very important for maximizing pathogen removal efficiency in WSPs, yet they have not been very well-studied. Community acceptance is another important (non-technological) issue for waste stabilization ponds, as some communities view ponds as outdated technologies, despite their ability to removal pathogens, with negligible energy and material inputs, just as well as many mechanized sanitation technologies.

Table 2. Summary of results from literature review of pathogen removal from wastewater in waste stabilization ponds and waste stabilization pond systems

Pond Type	**Typical Pathogen and Fecal Indicator $\log_{10}$ Removal Values[a] (Typical Ranges Shown in Parentheses)**				
	Bacterial Pathogens	**Viruses**	**Protozoan (Oo)Cysts**	**Helminth Eggs**	**Thermotolerant Coliforms (Including *E. coli*)**
For overall WSP systems with several ponds in series					
Maximum removal in an optimally functioning and well-maintained system with 3 – 4 ponds in series[d]	6	4	4	3 to 4	6
Average removal based on long-term monitoring data from full-scale pond systems in Brazil and the USA[e]	NR	NR	NR	NR	2.2 (*E. coli*) 3.0 (coliforms)
For each unit in the series					
Anaerobic ponds (HRT[b] <10 days)	~0 to 1[f]	0.6 (0.3 to 1.0)	~1 to 2[f]	1.2 (0.6 to 1.8)	0.4 (neg.[c] to 0.8)
Facultative ponds (HRT <30 days)	~1 to 2[f]	0.8 (neg. to 1.6)	1.4 (1.0 to 1.8)	1.2 (0.4 to 2.0)	0.9 (0.5 to 1.3)
Maturation ponds (HRT <30 days)	1.2 (0.7 to 1.7)	0.9 (neg. to 1.8)	~2 to 3[f]	~0 to 1[f,h]	1.4 (0.5 to 2.3)
High rate algal ponds (HRT 3 to 10 days)	1.4 (1.2 to 1.6)	0.9 (neg. to 1.7)	~1 to 2[f,g]	~0 to 1[f,g]	2.2 (1.9 to 2.4)

[a] Sources: (Al-Salem and Lumbers, 1987; Anceno et al., 2007; Araki et al., 2001, 2000; Ayres et al., 1993; Bausum et al., 1983; Bouhoum et al., 2000; Da Silva et al., 2011; De Oliveira et al., 2011; Dixo et al., 1995; García et al., 2008; Grimason et al., 1996a, 1996b; Hachich et al., 2013; Hassani et al., 1992; Hewitt et al., 2011; Joshi et al., 1973; Konaté et al., 2013a, 2013b; Mara and Silva, 1986; Nupen, 1970; Oakley, 2004; Oragui et al., 1995, 1987; Ouazzani et al., 1995; Rao et al., 1981; Saqqar and Pescod, 1992, 1991; Schwartzbrod et al., 1989, 1987; Stott et al., 2003; von Sperling and Mascarenhas, 2005; von Sperling et al., 2005, 2003, 2002; Young et al., 2016). Typical $\log_{10}$ removals are arithmetic mean values of the $\log_{10}$ removals reported in the literature; typical ranges are +/- one standard deviation of the mean. It is important to note that the data on which these typical removal values are based come from studies of ponds with a range of hydraulic retention times, located in many different climates, and with a range of different operating conditions.

[b] HRT = (theoretical) hydraulic retention time; [c] neg. = negligible removal; [d] Source: (Stenström et al., 2011); [e] Source: (Espinosa et al., 2017);

[f] Only approximate ranges are presented because there were fewer than five studies found during the literature review from which removal could be calculated or where removal was reported.

[g] These values are based on studies where the authors placed oocysts and eggs in semi-permeable membranes immersed in HRAP water to study the effect on infectivity; therefore, these estimates do not account for removal via settling in subsequent algal settling ponds.

[h] Note that the removal here is likely an underestimate, due to the fact that in many studies, eggs were not detected in the effluent of maturation ponds, in samples that were typically 10 L; however, in some cases the authors did not report the limit of detection (nor did they report the actual volume of concentrated sample observed under the microscope).

Table 3. Ranges of concentrations of pathogens and fecal indicators in waste stabilization pond sediments (sludge)

Bacterial Pathogens	Viruses	Protozoan (oo)Cysts	Helminth Eggs	Thermotolerant Coliforms
~1 MPN/g TS (*Salmonella* spp.)	0.7 iu /g DS (culturable enteroviruses)	0 to 2,000 (oo)cysts/g TS	0 to 4,000 eggs/g TS	1.0E+03 to 1.0E+06 MPN /g TS

TS = total solids; DS = dry solids

Sources: (Franci, 1999; Gaspard et al., 1997; Konaté et al., 2015a, 2013b, 2010; Nelson, 2003; Nelson et al., 2004; Oakley, 2004; Ponugoti et al., 1997; Schwartzbrod et al., 1987, 1989; Symonds et al., 2014; Verbyla et al., 2013b; von Sperling et al., 2003)

References

Al-Salem, S.S. and Lumbers, J.P. (1987). An initial evaluation of Al Sumra waste stabilisation ponds (Jordan). Water Science and Technology. 19, pp. 33–37.

Almasi, A. and Pescod, M.B. (1996). Pathogen removal mechanisms in anoxic wastewater stabilization ponds. Water Science and Technology. 33, pp. 133–140.

Amahmid, O., Asmama, S. and Bouhoum, K. (2002). Urban wastewater treatment in stabilization ponds: occurrence and removal of pathogens. Urban Water. 4, pp. 255–262. doi: 10.1016/S1462-0758(01)00071-1.

Anceno, A.J., Ozaki, M., Dang, Y.N., Chuluun, B. and Shipin, O.V. (2007). Canal networks as extended waste stabilization ponds: fate of pathogens in constructed waterways in Pathumthani Province, Thailand. Water Science and Technology. 55, pp. 143–156.

Araki, S., González, J.M., De Luis, E. and Becares, E. (2000). Viability of nematode eggs in high rate algal ponds : the effect of physico-chemical conditions. pp. 371–374.

Araki, S., Martín-Gomez, S., Bécares, E., De Luis-Calabuig, E. and Rojo-Vazquez, F. (2001). Effect of High-Rate Algal Ponds on Viability of *Cryptosporidium parvum* Oocysts. Applied and Environmental Microbiology. 67, pp. 3322–3324. doi: 10.1128/AEM.67.7.3322-3324.2001.

Ayres, R.M., Alabaster, G.P., Mara, D.D. and Lee, D.L. (1992). A Design Equation for Human Intestinal Nematode Egg Removal in Waste Stabilization Ponds. Water Research. 26, pp. 863–865.

Ayres, R.M., Mara, D.D., Lee, D.L. and Thitai, W.N. (1993). Monitoring full scale waste stabilisation ponds in Kenya for nematode egg removal. Environmental Technology. 14, pp. 295–300.

Bandala, E.R., González, L., Sanchez-Salas, J.L. and Castillo, J.H. (2012). Inactivation of *Ascaris* eggs in water using sequential solar driven photo-Fenton and free chlorine. Journal of Water and Health. 10, pp. 20–30. doi: 10.2166/wh.2011.034.

Bausum, H.T., Schaub, S.A., Rose, W.E. and Gibbs, P.H. (1983). Enteric Virus Removal in Wastewater Treatment Lagoon Systems. EPA-600/S1-83-012. Research Triangle Park, NC.

Ben Ayed, L., Schijven, J., Alouini, Z., Jemli, M. and Sabbahi, S. (2009). Presence of parasitic protozoa and helminth in sewage and efficiency of sewage treatment in Tunisia. Parasitology Research. 105, pp. 393–406. doi: 10.1007/s00436-009-1396-y.

Bouhoum, K., Amahmid, O. and Asmama, S. (2000). Occurrence and removal of protozoan cysts and helminth eggs in waste stabilization ponds in Marrakech. Water Science and Technology. 42, pp. 159–164.

Boutilier, L., Jamieson, R., Gordon, R., Lake, C. and Hart, W. (2009). Adsorption, sedimentation, and inactivation of *E. coli* within wastewater treatment wetlands. Water Research. 43, pp. 4370–4380. doi: 10.1016/j.watres.2009.06.039.

Burge, W.D., Cramer, W.N. and Kawata, K. (1983). Effect of Heat on Virus Inactivation by Ammonia. Applied and Environmental Microbiology. 46, pp. 446–451.

Characklis, G.W., Dilts, M.J., Simmons, O.D., Likirdopulos, C.A., Krometis, L.A.H. and Sobsey, M.D. (2005). Microbial partitioning to settleable particles in stormwater. Water Research. 39, pp. 1773–82. doi: 10.1016/j.watres.2005.03.004.

da Silva, A.K., Le Guyader, F.S., Le Saux, J.C., Pommepuy, M., Montgomery, M.A. and Elimelech, M. (2008). *Norovirus* removal and particle association in a waste stabilization pond. Environmental Science and Technology. 42, pp. 9151–7.

da Silva, F.J.A., de Souza, R.O., de Castro, F.J.F. and Araújo, A.L.C. (2011). Prospectus of waste stabilization ponds in Ceará, Northeast Brazil. Water Science and Technology. 63, pp. 1265–1270. doi: 10.2166/wst.2011.106.

Davies-Colley, R.J., Craggs, R.J., Park, J. and Nagels, J.W. (2005). Optical characteristics of waste stabilization ponds: recommendations for monitoring. Water Science and Technology. 51, pp. 153–61.

Davies-Colley, R.J., Craggs, R.J., Park, J., Sukias, J.P.S., Nagels, J.W. and Stott, R. (2005). Virus removal in a pilot-scale 'advanced' pond system as indicated by somatic and F-RNA bacteriophages. Water Science and Technology. 51, pp. 107–10.

Decrey, L., Kazama, S., Udert, K.M. and Kohn, T. (2014). Ammonia as an In Situ Sanitizer: Inactivation Kinetics and Mechanisms of the ssRNA Virus MS2 by NH3. Environmental Science and Technology. 49, pp. 1060–1067. doi: 10.1021/es5044529.

De Oliveira, R., Pearson, H.W., Silva, J.V.N., Sousa, J.T., Leite, V.D. and Lopes, W.S. (2011). Baffled primary facultative ponds with inlets and outlets set at different levels treating domestic wastewater in northeast Brazil. Water Science and Technology. 63, pp. 1183–1187. doi: 10.2166/wst.2011.105.

Dias, D.F.C. (2016). Evaluation of *Escherichia coli* inactivation and sunlight penetration in a shallow maturation pond: hydrodynamics and disinfection. Federal University of Minas Gerais, Brazil.

Emmoth, E., Ottoson, J., Albihn, A., Belák, S. and Vinneraas, B. (2011). Ammonia disinfection of hatchery waste for elimination of single-stranded RNA viruses. Applied and Environmental Microbiology. 77, pp. 3960–3966. doi: 10.1128/AEM.02990-10.

Espinosa, M.F., von Sperling, M. and Verbyla, M.E. (2017). Performance Evaluation of 388 Full-Scale Waste Stabilization Ponds Systems with Seven Different Configurations. Water Science and Technology. 75, pp. 916–927. doi: 10.2166/wst.2016.532.

Fidjeland, J., Magri, M.E., Jönsson, H., Albihn, A. and Vinneraas, B. (2013). The potential for self-sanitisation of faecal sludge by intrinsic ammonia. Water Research. 47, pp. 6014–23. doi: 10.1016/j.watres.2013.07.024.

Franci, R. (1999). Gerenciamento do lodo de lagoas de estabilização não mecanizadas.

García, M., Soto, F., González, J.M. and Bécares, E. (2008). A comparison of bacterial removal efficiencies in constructed wetlands and algae-based systems. Ecological Engineering. 32, pp. 238–243. doi: 10.1016/j.ecoleng.2007.11.012.

Gaspard, P., Wiart, J. and Schwartzbrod, J. (1997). Parasitological Contamination of Urban Sludge Used for Agricultural Purposes. Waste Management and Research. 15, pp. 429–436. doi: 10.1177/0734242X9701500409.

Ghiglietti, R., Genchi, C., Di Matteo, L., Calcaterra, E. and Colombi, A. (1997). Survival of *Ascaris suum* eggs in ammonia-treated wastewater sludges. Bioresource Technology. 59, pp. 195–198. doi: 10.1016/S0960-8524(96)00147-2.

Grimason, A.M., Smith, H.V., Young, G. and Thitai, W.N. (1996). Occurrence and removal of *Ascaris* sp. ova by waste stabilisation ponds in Kenya. Water Science and Technology. 33, pp. 75–82.

Grimason, A.M., Wiandt, S., Baleux, B., Thitai, W.N., Bontoux, J. and Smith, H.V. (1996). Occurrence and removal of *Giardia* sp. cysts by Kenyan and French waste stabilisation pond systems. Water Science and Technology. 33, pp. 83–89.

Hachich, E.M., Galvani, A.T., Padula, J.A., Stoppe, N.C., Garcia, S.C., Bonanno, V.M.S. *et al*. (2013). Pathogenic parasites and enteroviruses in wastewater: support for a regulation on water reuse. Water Science and Technology. 67, pp. 1512–1518.

Hassani, L., Imziln, B., Boussaid, A. and Gauthier, M.J. (1992). Seasonal incidence of and antibiotic resistance among *Aeromonas* species isolated from domestic wastewater before and after treatment in stabilization ponds. Microbial Ecology. 23, pp. 227–237. doi: 10.1007/BF00164098.

Heaselgrave, W. and Kilvington, S. (2011). The efficacy of simulated solar disinfection (SODIS) against *Ascaris*, *Giardia*, *Acanthamoeba*, Naegleria, *Entamoeba* and *Cryptosporidium*. Acta Tropica. 119, pp. 138–143. doi: 10.1016/j.actatropica.2011.05.004.

Hewitt, J., Leonard, M., Greening, G.E. and Lewis, G.D. (2011). Influence of wastewater treatment process and the population size on human virus profiles in wastewater. Water Research. 45, pp. 6267–76.

Jasper, J.T., Nguyen, M.T., Jones, Z.L., Ismail, N.S., Sedlak, D.L., Sharp, J.O. *et al*. (2013). Unit Process Wetlands for Removal of Trace Organic Contaminants and Pathogens from Municipal Wastewater Effluents. Environmental Engineering Science. 30, pp. 421–436. doi: 10.1089/ees.2012.0239.

Joshi, S.R., Parhad, N.M. and Rao, N.U. (1973). Elimination of *Salmonella* in stabilization ponds. Water Research. 7, pp. 1357–1365.

Kadir, K. and Nelson, K.L. (2013). Sunlight mediated inactivation mechanisms of *Enterococcus faecalis* and *Escherichia coli* in clear water versus waste stabilization pond water. Water Research. doi: 10.1016/j.watres.2013.10.046.

Kohn, T., Mattle, M.J., Minella, M. and Vione, D. (2016). A modeling approach to estimate the solar disinfection of viral indicator organisms in waste stabilization ponds and surface waters. Water Research. 88, pp. 912–922. doi: 10.1016/j.watres.2015.11.022.

Konaté, Y., Maiga, A.H., Basset, D., Casellas, C. and Picot, B. (2013). Parasite removal by waste stabilisation pond in Burkina Faso, accumulation and inactivation in sludge. Ecological Engineering. 50, pp. 101–106. doi: 10.1016/j.ecoleng.2012.03.021.

Konaté, Y., Maiga, A.H., Basset, D., Picot, B. and Casellas, C. (2013). Occurrence, removal and accumulation in sludge of protozoan cysts and helminth eggs in a full-scale anaerobic pond in Burkina Faso. Water Science and Technology. 67, pp. 193–200. doi: 10.2166/wst.2012.557.

Konaté, Y., Maiga, A.H., Wethe, J., Basset, D., Casellas, C. and Picot, B. (2010). Sludge accumulation in an anaerobic pond and viability of helminth eggs: a case study in Burkina Faso. Water Science and Technology. 61, pp. 919–925. doi: 10.2166/wst.2010.959.

Krometis, L.A.H., Drummey, P.N., Characklis, G.W. and Sobsey, M.D. (2009). Impact of Microbial Partitioning on Wet Retention Pond Effectiveness. Journal of Environmental Engineering. 135, pp. 758–767. doi: 10.1061/(ASCE)EE.1943-7870.0000040.

Maïga, Y., Denyigba, K., Wethe, J. and Ouattara, A.S. (2009). Sunlight inactivation of *Escherichia coli* in waste stabilization microcosms in a sahelian region (Ouagadougou, Burkina Faso). Journal of photochemistry and photobiology. B, Biology. 94, pp. 113–9. doi: 10.1016/j.jphotobiol.2008.10.008.

Maïga, Y., Wethe, J., Denyigba, K. and Ouattara, A.S. (2009). The impact of pond depth and environmental conditions on sunlight inactivation of *Escherichia coli* and enterococci in wastewater in a warm climate. Canadian Journal of Microbiology. 55, pp. 1364–74. doi: 10.1139/W09-104.

Maraccini, P.A., Wenk, J. and Boehm, A.B. (2016). Exogenous indirect photoinactivation of bacterial pathogens and indicators in water with natural and synthetic photosensitizers in simulated sunlight with reduced UVB. Journal of Applied Microbiology. 121(2), pp. 587-597.

Mara, D.D. (2003). Domestic wastewater treatment in developing countries. Earthscan/James and James. London, UK.

Mara, D.D. and Silva, S.A. (1986). Removal of intestinal nematode eggs in tropical waste stabilization ponds. Journal of Tropical Medicine and Hygiene. 89, pp. 71–74.

Mattle, M.J., Vione, D. and Kohn, T. (2015). Conceptual Model and Experimental Framework to Determine the Contributions of Direct and Indirect Photoreactions to the Solar Disinfection of MS2, phiX174, and Adenovirus. Environmental Science and Technology. 49, pp. 334–342. doi: 10.1021/es504764u.

Metting, B. and Pyne, J.W. (1986). Biologically active compounds from microalgae. Enzyme and Microbial Technology. 8, pp. 386–394.

Mezrioui, N., Oufdou, K. and Baleux, B. (1995). Dynamics of non-O1 *Vibrio cholerae* and fecal coliforms in experimental stabilization ponds in the arid region of Marrakesh, Morocco, and the effect of pH, temperature, and sunlight on their experimental survival. Canadian Journal of Microbiology. 41, pp. 489–498.

Miki, T. and Jacquet, S. (2008). Complex interactions in the microbial world: underexplored key links between viruses, bacteria and protozoan grazers in aquatic environments. Aquatic Microbial Ecology. 51, pp. 195–208. doi: 10.3354/ame01190.

Mille-Lindblom, C. and Tranvik, L.J. (2003). Antagonism between Bacteria and Fungi on Decomposing Aquatic Plant Litter. Microbial Ecology. 45, pp. 173–182. doi: 10.1007/s00248-002-2030-z.

Nelson, K.L. (2003). Concentrations and inactivation of *Ascaris* eggs and pathogen indicator organisms in wastewater stabilization pond sludge. Water Science and Technology. 48, pp. 89–95.

Nelson, K.L., Jiménez, B., Tchobanoglous, G. and Darby, J.L. (2004). Sludge accumulation, characteristics, and pathogen inactivation in four primary waste stabilization ponds in central Mexico. Water Research. 38, pp. 111–27. doi: 10.1016/j.watres.2003.09.013.

Nelson, K.L. and Pecson, B.M. (2005). Inactivation of *Ascaris suum* eggs by ammonia. Environmental Science and Technology. 39, pp. 7909–7914.

Nguyen, M.T., Jasper, J.T., Boehm, A.B. and Nelson, K.L. (2015). Sunlight inactivation of fecal indicator bacteria in open-water unit process treatment wetlands: Modeling endogenous and exogenous inactivation rates. Water Research. 83, pp. 282–292. doi: 10.1016/j.watres.2015.06.043.

Nupen, E.M. (1970). Virus studies on the windhoek waste-water reclamation plant (South-West Africa). Water Research. 4, pp. 661–672.

Oakley, S.M. (2005). Lagunas de Estabilización en Honduras: Manual de Diseño, Construcción, Operación y Mantenimiento, Monitoreo y Sostenibilidad. USAID, University of California, Chico. Chico, California.

Oakley, S.M. (2004). Monitoring of wastewater stabilization ponds in Honduras. Congreso Interamericano de Ingenieria Sanitaria y Ambiental, 29; San Juan, 22-27 Ago. 2004. pp. 1–8.

Oakley, S.M., Mendonça, L.C. and Mendonça, S.R. (2012). Sludge removal from primary wastewater stabilization ponds with excessive accumulation: a sustainable method for developing regions. Journal of Water, Sanitation and Hygiene for Development. 2, pp. 68. doi: 10.2166/washdev.2012.093.

Oguma, K., Katayama, H., Mitani, H., Morita, S., Hirata, T. and Ohgaki, S. (2001). Determination of pyrimidine dimers in *Escherichia coli* and *Cryptosporidium parvum* during UV light inactivation, photoreactivation, and dark repair. Applied and Environmental Microbiology. 67, pp. 4630–4637. doi: 10.1128/AEM.67.10.4630.

Ohgaki, S., Ketratanakul, A. and Prasertsom, U. (1986). Effect of sunlight on coliphages in an oxidation pond. Water Science and Technology. 18, pp. 37–46.

Oliveira, S.C. and von Sperling, M. (2011). Performance evaluation of different wastewater treatment technologies operating in a developing country. Journal of Water, Sanitation and Hygiene for Development. 1, pp. 37–56. doi: 10.2166/washdev.2011.022.

Oragui, J.I., Arridge, H., Mara, D.D., Pearson, H.W. and Silva, S.A. (1995). *Rotavirus* removal in experimental waste stabilization pond systems with different geometries and configurations. Water Science and Technology. 31, pp. 285–290.

Oragui, J.I., Curtis, T.P., Silva, S.A. and Mara, D.D. (1987). The removal of excreted bacteria and viruses in deep waste stabilization ponds. Water Science and Technology. 19, pp. 569–573.

Ouazzani, N., Bouhoum, K., Mandi, L., Bouarab, L., Habbari, K., Rafiq, F. *et al*. (1995). Wastewater treatment by stabilization pond: Marrakesh experiment. Water Science and Technology. 31, pp. 75–80.

Ouedraogo, F.R., Zhang, J., Cornejo, P.K., Zhang, Q., Mihelcic, J.R. and Tejada-Martinez, A.E. (2016). Impact of sludge layer geometry on the hydraulic performance of a waste stabilization pond. Water Research. 99, pp. 253–262. doi: 10.1016/j.watres.2016.05.011.

Persson, J. (2000). The hydraulic performance of ponds of various layouts. Urban Water. 2, pp. 243–250.

Ponugoti, P.R., Dehab, M.F. and Surampalli, R. (1997). Effects of different biosolids treatment systems on pathogen and pathogen indicator reduction. Water Environment Research. 69, pp. 1195. doi: 10.2175/106143097X125948.

Rao, V.C., Lakhe, S.B. and Waghmare, S.V. (1981). Virus removal in waste stabilization ponds in India. Water Research. 15, pp. 773–778.

Reimers, R.S., Little, M.D., Akers, T.G., Henriques, W.D., Badeaux, R.C., McDonnell, D.B. *et al*. (1990). Persistence of Pathogens in Lagoon-Stored Sludge. Risk Reduction Engineering Laboratory. US Environmental Protection Agency. Cincinnati, Ohio.

Reinoso, R. and Bécares, E. (2008). Environmental inactivation of *Cryptosporidium parvum* oocysts in waste stabilization ponds. Microbial Ecology. 56, pp. 585–92. doi: 10.1007/s00248-008-9378-7.

Reinoso, R., Bécares, E. and Smith, H.V. (2008). Effect of various environmental factors on the viability of *Cryptosporidium parvum* oocysts. Journal of Applied Microbiology. 104, pp. 980–6. doi: 10.1111/j.1365-2672.2007.03620.x.

Saqqar, M.M. and Pescod, M.B. (1992). Modelling nematode egg elimination in wastewater stabilization ponds. Water Science and Technology. 26, pp. 1659–1665.

Saqqar, M.M. and Pescod, M.B. (1991). Microbiological performance of multi-stage stabilization ponds for effluent use in agriculture. Water Science and Technology. 23, pp. 1517–1524.

Scheid, P. and Schwarzenberger, R. (2012). *Acanthamoeba spp*. as vehicle and reservoir of adenoviruses. Parasitology Research. 111, pp. 479–85. doi: 10.1007/s00436-012-2828-7.

Schwartzbrod, J., Stien, J.L., Bouhoum, K. and Baleux, B. (1989). Impact of Wastewater Treatment on Helminth Eggs. Water Science and Technology. 21, pp. 295–297.

Schwartzbrod, J., Bouhoum, K. and Baleux, B. (1987). Effects of lagoon treatment on helminth eggs. Water Science and Technology. 19, pp. 369–371.

Sengupta, M.E., Andersen, T.J., Dalsgaard, A., Olsen, A. and Thamsborg, S.M. (2012). Resuspension and settling of helminth eggs in water: Interactions with cohesive sediments. Water Research. 46, pp. 3903–12. doi: 10.1016/j.watres.2012.04.033.

Senhorinho, G.N.A., Ross, G.M. and Scott, J.A. (2015). Cyanobacteria and eukaryotic microalgae as potential sources of antibiotics. Phycologia. 54, pp. 271–282. doi: 10.2216/14-092.1.

Shilton, A. (2006). Pond Treatment Technology. IWA Publishing. London, UK. pp. 498.

Shilton, A. and Harrison, J. (2003). Guidelines for the Hydraulic Design of Waste Stabilisation Ponds. Massey University. New Zealand.

Shilton, A. and Harrison, J. (2003). Development of guidelines for improved hydraulic design of waste stabilisation ponds. Water Science and Technology. 48, pp. 173–80.

Silverman, A.I., Nguyen, M.T., Schilling, I.E., Wenk, J. and Nelson, K.L. (2015). Sunlight inactivation of viruses in open-water unit process treatment wetlands: modeling endogenous and exogenous inactivation rates. Environmental Science and Technology. 49, pp. 2757–66. doi: 10.1021/es5049754.

Silverman, A.I. and Nelson, K.L. (2016). Modeling the Endogenous Sunlight Inactivation Rates of Laboratory Strain and Wastewater *E. coli* and *Enterococci* Using Biological Weighting Functions. Environmental Science and Technology. pp. acs.est.6b03721. doi: 10.1021/acs.est.6b03721.

Silverman, A.I., Peterson, B.M., Boehm, A.B., McNeill, K. and Nelson, K.L. (2013). Sunlight inactivation of human viruses and bacteriophages in coastal waters containing natural photosensitizers. Environmental Science and Technology. 47, pp. 1870–8. doi: 10.1021/es3036913.

Sinton, L., Hall, C. and Braithwaite, R. (2007). Sunlight inactivation of *Campylobacter jejuni* and *Salmonella enterica*, compared with *Escherichia coli*, in seawater and river water. Journal of Water and Health. 5, pp. 357–65. doi: 10.2166/wh.2007.031.

Sinton, L.W., Hall, C.H., Lynch, P.A. and Davies-Colley, R.J. (2002). Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. Applied and Environmental Microbiology. 68, pp. 1122–31.

Sobsey, M.D. and Cooper, R.C. (1973). Enteric virus survival in algal-bacterial wastewater treatment systems—I. Water Research. 7, pp. 669–685. doi: 10.1016/0043-1354(73)90085-7.

Stenström, T.A., Seidu, R., Ekane, N. and Zurbrügg, C. (2011). Microbial Exposure and Health Assessments in Sanitation Technologies and Systems. Sustainable Sanitation Alliance, EAWAG, Norwegian University of Life Sciences, Ecological Sanitation Research Programme. Stockholm, Sweden. pp. 1–165.

Stott, R., May, E. and Mara, D.D. (2003). Parasite removal by natural wastewater treatment systems: performance of waste stabilisation ponds and constructed wetlands. Water Science and Technology. 48, pp. 97–104.

Symonds, E.M., Verbyla, M.E., Lukasik, J.O., Kafle, R.C., Breitbart, M. and Mihelcic, J.R. (2014). A case study of enteric virus removal and insights into the associated risk of water reuse for two wastewater treatment pond systems in Bolivia. Water Research. 65, doi: 10.1016/j.watres.2014.07.032.

Verbyla, M.E., Iriarte, M.M., Guzmán, 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. Science of the Total Environment. 551-552, pp. 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 Research. 71, pp. 107–24.

Verbyla, M.E., Oakley, S.M. and Mihelcic, J.R. (2013). Wastewater Infrastructure for Small Cities in an Urbanizing World: Integrating Protection of Human Health and the Environment with Resource Recovery and Food Security. Environmental Science and Technology. 47, pp. 3598–3605. doi: 10.1021/es3050955.

Verbyla, M.E., Oakley, S.M., Lizima, L.A., Zhang, J., Iriarte, M., Tejada-Martinez, A.E. *et al*. (2013). *Taenia* eggs in a stabilization pond system with poor hydraulics: concern for human cysticercosis?. Water Science and Technology. 68, pp. 2698–2703. doi: 10.2166/wst.2013.556.

von Sperling, M. (2007). Waste Stabilisation Ponds. Volume Three: Biological Wastewater Treatment Series. IWA Publishing. London, UK.

von Sperling, M. (2005). Modelling of coliform removal in 186 facultative and maturation ponds around the world. Water Research. 39, pp. 5261–73. doi: 10.1016/j.watres.2005.10.016.

von Sperling, M., Bastos, R.K.X. and Kato, M.T. (2005). Removal of *E. coli* and helminth eggs in UASB: Polishing pond systems in Brazil. Water Science and Technology. 51, pp. 91–7.

von Sperling, M., Chernicharo, C.A.L., Soares, A.M.E. and Zerbini, A.M. (2003). Evaluation and modelling of helminth eggs removal in baffled and unbaffled ponds treating anaerobic effluent. Water Science and Technology. 48, pp. 113–120.

von Sperling, M., Chernicharo, C.A.L., Soares, A.M.E. and Zerbini, A.M. (2002). Coliform and helminth eggs removal in a combined UASB reactor-baffled pond system in Brazil: performance evaluation and mathematical modelling. Water Science and Technology. 45, pp. 237–42.

von Sperling, M. and Mascarenhas, L.C.A.M. (2005). Performance of very shallow ponds treating effluents from UASB reactors. Water Science and Technology. 51, pp. 83-90.

Weitzman, M.D., Carson, C.T., Schwartz, R.a. and Lilley, C.E. (2004). Interactions of viruses with the cellular DNA repair machinery. DNA Repair. 3, pp. 1165–1173. doi: 10.1016/j.dnarep.2004.03.018.

Young, P., Buchanan, N. and Fallowfield, H. (2016). Inactivation of indicator organisms in wastewater treated by a high rate algal pond system. Journal of Applied Microbiology. doi: 10.1111/jam.13180.