GLOBAL WATER PATHOGEN PROJECT

PART FOUR. MANAGEMENT OF RISK FROM EXCRETA AND WASTEWATER

PATHOGEN REDUCTION AND SURVIVAL IN COMPLETE TREATMENT WORKS

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Summary

This chapter examines specific pathogen reduction and survival across all the individual unit processes that make up a complete treatment works as opposed to across specific unit processes discussed in other sanitation chapters. Three types of data obtained from full-scale wastewater treatment plants located around the world are discussed: (1) pathogen influent concentration, (2) pathogen effluent concentration, and (3) $\log_{10}$ reduction.

The chapter is organized around two of the most commonly employed sanitation systems: (1) mechanized systems that use activated sludge and its variants that are combined with advanced or tertiary treatment; and (2) natural systems using either waste stabilization ponds in series or an anaerobic reactor(s) followed by waste stabilization ponds in series. The activated sludge systems assessed in this chapter consisted of: (a) conventional treatment with and without disinfection; and (b) conventional with tertiary processes (coagulation/sedimentation/microfiltration; sand filtration/disinfection; and ultrafiltration/disinfection).

For conventional activated sludge systems that included disinfection, the observed $\log_{10}$ reductions of specific pathogens ranged from 4 – 6 for bacteria, 0.94 – 2.14 for viruses, 0.62 – 2.13 for protozoa, and 1 – 2 for helminths. For conventional activated sludge systems combined with tertiary processes (without ultrafiltration), the observed $\log_{10}$ reductions of specific pathogens ranged from 2.85 – 4.33 for viruses and 0.46 – 3.87 for protozoa (no data were available for bacteria and helminths). For a conventional activated sludge system that used ultrafiltration with disinfection, the effluent concentrations of *Cryptosporidium* and *Giardia* were both below the reported detection limit of 0.033 (oo)cysts/L and the $\log_{10}$ reductions were estimated at > 3.63 for *Cryptosporidium* and > 4.44 for *Giardia*.

The key points for mechanized activated sludge systems are:

- The fundamental basis of design and operation of conventional activated sludge (without or with tertiary processes) is the removal of suspended and dissolved organic matter (TSS and BOD) within a relatively short hydraulic retention time (4 – 20 hours). All unit processes specifically designed to reduce pathogen concentrations (disinfection; sand-, micro- and ultra- filtration) are installed at the end of the treatment train with verification monitoring of pathogen reduction that typically focuses on reporting effluent concentrations of fecal indicator bacteria.
- Disinfection to satisfy reduction of fecal indicator bacteria to meet effluent standards doesn't represent equivalent pathogen reduction for viruses, protozoa, and helminths. Thus even a well-operated activated sludge treatment plant meeting its bacterial effluent requirements with disinfection may contribute to public health risks in both developed and developing countries. This is because

other pathogens may be present in "treated" effluent. As an example, two treatment plants in the U.S. with chlorine disinfection of effluent that satisfied regulatory requirements for indicator bacteria (<200 MPN *E. coli*/100 mL); however, both had effluent concentrations of *Giardia* ranging from <4 – 620 cysts/L and < 4 – 40 oocysts/L for *Cryptosporidium*.

- The unit process that may have the highest pathogen reduction is the activated sludge process (aeration basin + secondary sedimentation) that is located upstream of tertiary processes. For example, in seven treatment plants reviewed here, six in the U.S. and one in China, the reduction of viruses and protozoan (oo)cysts was at least 1.0 $\log_{10}$ greater in the activated sludge unit process than the downstream individual tertiary unit processes of coagulation/flocculation/sedimentation/microfiltration, sand/cloth filtration, or chlorine or UV disinfection. Accordingly, for tertiary processes to have more significant effect on pathogen reduction they must be well-designed and operated, and selected for specific pathogen removal.
- With the exception of one treatment plant in China using ultrafiltration, measurable concentrations of viral and protozoan pathogens were found in all plant effluents examined in both developed and developing countries. It is important to note the authors of the China study mention that the widespread use of ultrafiltration in China (which would significantly reduce pathogen concentrations) is unlikely because of the cost of operation and consumption of energy.
- While it is often assumed helminth eggs will be removed in a conventional activated sludge process, in developing countries high helminth egg concentrations have been measured in final effluents (e.g., in Tunisia four plants had concentrations of *Ascaris* eggs ranging from 49 – 80 eggs/L).
- For a given $\log_{10}$ reduction, higher influent pathogen concentrations, such as may be found in developing countries because of higher prevalence in the population and lower overall water usage (both that lead to higher concentrations), will lead to correspondingly higher effluent concentrations. These higher concentrations in treated effluent may pose health risks where effluents are discharged to surface waters that are used downstream as drinking water sources, recreation or hygiene and/or treated effluent is used directly or indirectly for irrigation of crops.

The natural systems assessed in this chapter encompass tropical, semi-tropical and temperate climates. They systems included a facultative pond followed by a maturation pond in series (F/M); a facultative pond followed by two or three maturation ponds in series (F/M2; F/M3); an anaerobic pond followed by F/M in series (A/F/M); and an upflow anaerobic sludge blanket reactor (UASB) followed by F/M2 in series (UASB/F/M2). Facultative and maturation ponds traditionally have design

water depths from 1.5 - 2.0 meters, but some newer maturation pond designs reported in the literature have depths ≤ 1.0 m.

The $\log_{10}$ reduction results for natural systems show that a properly functioning full-scale waste stabilization pond system and UASB/waste stabilization pond system can remove 2.45 - 5.74 $\log_{10}$ bacterial indicators, and that one three-pond system removed 4.26 $\log_{10}$ *Vibrio cholerae* 01, which correlated well with a 4.89 $\log_{10}$ reduction of fecal coliforms. A four pond system, however, removed 3.00 $\log_{10}$ *E. coli*, but only reduced *Salmonella* by a mean 1.24 $\log_{10}$ during the same monitoring period. For protozoa (oo)cyst reduction, a three pond system removed 2.90 $\log_{10}$ *E. coli* in the cold season and 5.00 $\log_{10}$ in the warm season, but *Giardia* was removed only 1.57 $\log_{10}$ and *Cryptosporidium* was only reduced 1.98 $\log_{10}$. While one system approached the 6 $\log_{10}$ reduction for bacterial pathogens/indicators required for agriculture reuse as recommended by WHO (2006), most were below it in the range 2.90 - 5.00; the reported protozoan (oo)cyst reductions in the cited studies were all $< 2.00 \log_{10}$, well below the maximum 4.0 $\log_{10}$ recommended by WHO (2006) for agricultural reuse. Helminth egg reduction was sufficient in all systems to reduce effluent concentrations below the limit of detection or < 1 egg/L if the detection limit was reported. With the exception of helminth eggs and protozoan (oo)cysts in one system, almost all well-performing natural systems had measurable concentrations of pathogens in the final effluent.

In summary, the $\log_{10}$ reduction results show that properly functioning full-scale waste stabilization pond systems and a system that consists of an Upflow Anaerobic Sludge Blanket (UASB) reactor followed by waste stabilization ponds range from 4.00 - 5.74 for bacterial indicators, 4.26 for specific bacterial pathogens (*Vibrio cholerae* 01), 1.57 - 1.98 or below the detection limit for protozoa, and below the detection limit for helminth eggs (< 1 egg/L). Less data is available for virus reduction in well-functioning waste stabilization pond systems but a recent review article on virus removal in these systems is available that statistically analyzed results from 71 field studies on virus removal (Verbyla and Mihelcic, 2015).

The key points for natural systems are:

- Pathogen reduction is highly dependent on hydraulic retention time (HRT) with hydraulics that simulate plug flow. Systems that show high reduction of select pathogens have HRTs greater than 20 days with baffled maturation ponds, several ponds in series, or high length-to-width ratios.
- Pathogen reduction is a function of water temperature, especially in temperate climates where $\log_{10}$ reduction for bacteria can vary by a factor of 2.0 from winter to summer.
- There are few studies in the literature that have monitored and reported reduction of specific pathogens in relation to indicator bacteria in the same treatment system. For example, the assumption that high *E. coli* or fecal coliform

reduction is comparable to bacterial pathogen reduction has been demonstrated with *Vibrio cholerae* 01 but not *Salmonella*.

- The existing data show that specific pathogen reduction in waste stabilization pond systems, where the pathogens have been monitored, is often not as high as suggested in the literature and design manuals. Reduction is a function of numerous complex variables, including climatic conditions, hydraulic retention time, number of ponds in series, degree of hydraulic short-circuiting associated with improper design or maintenance, sludge accumulation, and pond depth, and many studies do not take into account many of these variables.
- Unlike mechanized systems whose processes can be continuously modified and controlled during routine operation, there is very little operational control of the process in natural wastewater treatment systems after they are constructed. In addition, improper design, hydraulic overloading, and inadequate operation and maintenance in developing countries is a very common occurrence. As an example, a recent study in Peru estimated that only 15.8% of all treated wastewater in Peru meets effluent quality levels that protects public health.
- Reduction of helminth eggs is excellent in all properly designed and operated systems with reported effluent concentrations < 1 egg/L.

The key points for both natural and mechanized systems:

- Data reported only on $\log_{10}$ reduction of bacterial indicators does not necessarily correlate with specific pathogen reduction, which is often lower. Thus apparently well-performing mechanized and natural systems cannot be assumed to be reducing pathogens at the same level of indicators.
- Wastewater discharge and reuse standards assume that the pathogens occur at much lower densities than the indicator and that reductions of the indicator by 3 - 4 $\log_{10}$ results in the elimination of bacterial and other pathogens. This concept is commonly applied in the U.S. and has also been applied to viral and protozoan pathogens in the WHO guidelines for wastewater reuse in agriculture, which recommends using *E. coli* for verification monitoring in lieu of monitoring specific pathogens. This strategy should be revisited in light of recent work showing the reduction and survival of pathogens in various treatment processes around the world: Different pathogens can exhibit vastly different $\log_{10}$ reductions in both natural and mechanized wastewater treatment systems.
- One recommended strategy would be to focus on pathogens that are a local or regional public health concern, such as parasitic infections with protozoa and helminths instead of bacterial or viral pathogens, in the management of wastewater treatment systems where agricultural reuse is an important concern. This strategy can apply to both mechanized and natural wastewater treatment

systems.

the 2006 World Health Organization (WHO) guidelines for wastewater agricultural reuse (WHO, 2006).

1.0 Introduction

While pathogen reduction ($\log_{10}$) has been the focus of the GWPP Chapters in Part IV on specific *sanitation technology unit processes*, this chapter will examine specific pathogen reduction and survival in complete treatment works. As noted in the original work by Feachem, et al. (1983), significant health risks can be posed by the pathogens that survive treatment processes and are discharged in the final effluent. The survival of pathogens is dependent on the reduction characteristics of individual unit treatment processes combined in full-scale treatment plants.

For a given $\log_{10}$ reduction for a complete treatment works, higher influent pathogen concentrations, such as may be found in developing countries (due to infectivity of population and lower overall water usage), will lead to correspondingly higher effluent concentrations. Feachem et al. (1983) in fact stated that complete treatment works using trickling filters or activated sludge processes will "still be heavily contaminated with pathogens". This section will update the sections originally discussed by Feachem et al. (1983) with recent quantitative data reported for complete treatment works using newer mechanical processes as well as more recent data from natural treatment systems, in combination with recent data on specific pathogen concentrations that were not available 35 years ago.

The full-scale sanitation (wastewater treatment) systems examined here are separated into two sections examining the most common wastewater technologies used worldwide: (1) mechanized systems that use activated sludge and its variants with advanced treatment, and (2) natural treatment systems using wastewater stabilization ponds and anaerobic reactors combined with stabilization ponds in series. Only representative studies that have looked at specific pathogens removed in full-scale wastewater treatment facilities are presented; fecal coliform and *E. coli* are also presented in those studies where their $\log_{10}$ reduction can be compared to specific pathogens. Finally, the reduction and survival data presented here will be compared with the original results presented in Feachem et al. (1983) and those presented in

Explanation of Figures used in this Chapter. The results are reported in figures that incorporate a *Process Flow Diagram*. These diagrams are a type of flowchart that visually shows the relationship between individual unit processes in the complete treatment works. Process flow diagrams are fundamental to design and operation, and are required to adequately understand and configure different unit processes in an effective combination. This is important because treatment plants are not designed the same and thus vary by location. Accordingly, because many combined treatment processes may not adequately reduce pathogen concentrations for treatment or reuse scenarios, process diagrams can be used to appreciate the magnitude of the potential problem for all types of unit process combinations.

In the individual figures, the process flow diagram for a particular complete treatment works is presented along with data on plant influent and effluent concentrations of measured indicators and pathogens. These concentrations are reported with the process flow diagram because the higher influent pathogen concentrations (such as may be found in developing countries) (caused by higher infectivity in the population and lower overall water usage) lead to higher effluent concentrations. Several figures for similar unit processes are presented but will incorporate data obtained from different locations in the world (e.g., developed and developing countries). The process flow diagrams clearly show the specific unit processes and whether disinfection is employed or not for a particular data set. Also provided directly on top of the process flow diagram is a table summarizing the overall $\log_{10}$ removal observed for a particular complete treatment works design. The data in the figures are presented in tabular format in Appendices (Tables A1.A to A25) at the end of the chapter.

2.0 Mechanized Wastewater Treatment Systems

2.1 Conventional Secondary Treatment and Advanced Treatment

Figure 1 shows conventional secondary treatment as it has been developed in Europe and the U.S. (Burton et al., 2014). It consists of pretreatment and primary sedimentation of organic suspended solids followed by aerobic treatment of the mostly soluble organic matter. The aerobic treatment can use suspended growth biological processes such as activated sludge, or attached growth processes such as trickling filters. All aerobic

processes need secondary sedimentation to remove the biomass generated by the aerobic process. The primary and secondary sludge are typically treated (e.g., with anaerobic digestion) and dewatered before final disposal or reuse. Historically, secondary treatment plants did not use disinfection although it is now mandatory in various countries and cities worldwide.

Figure 1. Conventional secondary treatment as developed historically and practiced today: a) with primary treatment and sludge stabilization; secondary treatment is most often activated sludge but also can be trickling filters; b) extended aeration activated sludge that does not require primary treatment and separate sludge stabilization.

Advanced (tertiary) treatment processes include various forms of filtration (cloth, media, membrane filters) and/or chemical flocculation/precipitation to improve effluent quality, followed by disinfection with chlorine, chlorine dioxide, ozone, or ultraviolet radiation (Burton et al., 2014). In some parts of the world (e.g., U.S.), advanced treatment processes are becoming more common as more stringent effluent discharge requirements are enacted (Burton et al., 2014).

The fundamental design of all secondary and secondary/advanced treatment systems to this day is based on the removal of suspended and dissolved organic matter, which in turn is based on the historical circumstances in which wastewater treatment was developed in Europe and North America. As Feachem et al. (1983, pp. 63-64) stated:

"...Conventional sewage works were originally developed in order to prevent gross organic pollution in

European and North American rivers; they were never intended to achieve high removal of excreted pathogens..."

Where pathogen reduction is an important consideration in wastewater treatment, secondary and secondary- advanced treatment plants must employ disinfection or filtration/disinfection as the final unit processes in the overall treatment train. This section will examine the effectiveness of the continuation of this historical approach by reviewing representative case studies for the most common treatment trains employed worldwide.

2.2 Conventional Activated Sludge without Disinfection

Many conventional activated sludge plants worldwide are designed and operated without disinfection. In Brazil,

for example, of 56 activated sludge plants studied for a detailed reliability analysis (Oliveira and von Sperling, 2008), none practiced disinfection (von Sperling, 2015). As another typical example, the only municipal activated sludge wastewater treatment plant in Guatemala, which discharges directly into Lake Atitlan (which is the drinking water supply for 100,000 people) was designed and is operated without disinfection. Finally, many activated sludge plants worldwide that discharge to the ocean or coastal waters with outfalls typically do so without disinfection.

An excellent example of an activated sludge plant discharging to the ocean without disinfection and the consequent effects of pathogens is presented by Flannery et al. (2012). The treatment plant served a population equivalent of 91,600, with a daily flow rate of 45,000 m³/d, and discharged to the ocean through a 400-m outfall at a depth of 10 m. The study assessed the reduction of *E. coli* and Norovirus (Genotypes I and II) in the treatment works and the effect of the final effluent on *E. coli* and Norovirus concentrations in tissue taken from oysters in the proximity of the outfall (Figure 2). The results show very low log₁₀ reduction for *E. coli* (1.49), and lower still for Norovirus GI and GII, 0.80 and 0.92 log₁₀ reduction, respectively. The concentration in the effluent is consequently high for the means and high range values (right column of Figure 2).

Of particular note regarding the inadequacy of indicators to assess treatment plant performance in removing pathogens. For the study reviewed in Figure 2, the concentration of *E. coli* was measured in oyster tissue obtained near the treatment plant outfall and it was compared with Norovirus GI and GII. In this case, the mean values of Norovirus GI and GII concentrations were 2.0 and 3.2 times greater than *E. coli*. The authors comment that *E. coli* is used as the indicator organism for the sanitary quality of shellfish in the European Union, and that the concentrations of *E. coli* found in the oyster tissue during the study (mean = 1,660 MPN/100 g) would have passed compliance for human consumption. However, in contrast, the Norovirus concentrations would have been consistent with those that have caused illness in consumers (Flannery et al., 2012). The authors conclude that *E. coli* is an inadequate indicator to assess the Norovirus risks associated with oysters.

Fong et al. (2010) monitored adenovirus reduction for one year in an activated sludge plant discharging to a Michigan (U.S.) river and sampled secondary effluent before disinfection with chlorine. Their data are also shown in Figure 2. They found a mean 1.15 x 10⁶ copies/L of adenovirus in raw wastewater and a mean 2.0 x 10⁴ copies/L in secondary effluent with a mean log₁₀ reduction of 1.77. Their results after disinfection will be discussed in detail in the next section.

^aGC: Gene Copy. Virus concentrations were measured by the qPCR molecular method and not by cell culture. The qPCR method identifies specific viruses, but they do not have to be intact or infective to be detected (Francy et al., 2011) and log₁₀ reduction may be underestimated. Fong et al. (2010) used qPCR for adenovirus although there are routine cell culture methods available; there are no known routine cell culture methods available for viable norovirus.

Figure 2. Adenovirus, norovirus (GI and GII) and *E. coli* reduction and persistence at two conventional activated sludge treatment plants without disinfection. Data from Flannery et al. 2012; Fong et al. 2010. Data are average values with range in parentheses.

Ramo et al. (2017) present a valuable study in north-eastern Spain on protozoan cyst reduction (*Cryptosporidium* and *Giardia*) in 13 out of 23 conventional activated sludge plants that do not practice disinfection.

The treatment plants served populations from 5,000 to over 660,000, with only 3 plants serving populations over 20,000. Most plants discharged their effluents into rivers, but three reused their effluents for irrigation of parks,

residential lawns, and agriculture.

The results of that study are presented in Figure 3 for influent-effluent cyst concentrations and log₁₀ reduction, and for (oo)cyst concentrations found in dewatered sludges. The influent (oo)cyst concentrations were higher for *Giardia* than *Cryptosporidium* (754 – 6,606 versus 22 – 456 (oo)cysts/L, respectively), and log₁₀ reduction ranged from 1.89 for *Giardia* and 0.34 for *Cryptosporidium*, reduction rates that would be considered insufficient if the effluents were directly reused for agricultural irrigation or if they were discharged into surface waters used downstream for irrigation or drinking water supply (Feachem et al., 1983).

The (oo)cyst concentrations measured in dewatered sludges were relatively high, ranging from 23 – 475 cysts/g TS for *Giardia* and 2 – 44 cysts/g TS for *Cryptosporidium*, with cyst viability exceeding 60%. Viability of cysts in effluents ranged 52 – 74% for *Giardia* and 61 – 91% for *Cryptosporidium*. The authors conclude that: (1) *Giardia* and *Cryptosporidium* are ubiquitous pathogens in the study area with a higher prevalence in the population than indicated by diagnostic data; (2) the high numbers of potentially viable (oo)cysts in effluents and dewatered sludge is a consequence of the limited efficacy of wastewater treatment plants; and (3) the results raise concerns regarding environmental impact and public health associated with disposal and reuse of treated wastewater and highlight the need for legislation that includes regulating pathogens during wastewater reuse (Ramo et al., 2017).

Figure 3. *Giardia* and *Cryptosporidium* reduction and persistence in effluents and sludges from 13 conventional activated sludge plants without disinfection (11 systems were extended aeration without primary sedimentation and 2 were conventional activated sludge.) Data are average values with ranges in parentheses. Sampling was done four times per year during different seasons (Ramo et al., 2017).

^aDry solids basis

Tyagi et al. (2011) monitored two activated sludge plants, one waste stabilization pond system, and one Upflow Anaerobic Sludge Blanket (UASB)-polishing pond plant in India for fecal coliforms and helminth eggs; the results for the two activated sludge plants are presented in Figure 4. Concentrations of helminth eggs ranged from 23.4 – 71.2

eggs/L in the influent and from nondetectable to 4.3 eggs/L in the effluent, with a mean log₁₀ reduction of 1.40 and 1.55 respectively in the two plants. Fecal coliform log₁₀ reduction was much higher at 2.22 and 3.10 reported for the two systems.

Figure 4. Helminth egg and fecal coliform reduction and persistence at 2 conventional activated sludge plants without disinfection in India. Data show average influent/effluent concentrations for each plant (AS1 & AS2). Influent/effluent concentration ranges are combined from both plants. Data from Tyagi et al. (2011).

Rose et al. (2004) performed a detailed study of six activated sludge treatment plants in the U.S. by monitoring reduction of indicator bacteria and viruses, enteric viruses, and two protozoan pathogens, *Cryptosporidium* and *Giardia*, in individual unit processes. That study thus enables an analyses of pathogen reduction by unit processes through the activated sludge treatment train. Rose et al. (1996) also provided some of the few available data on helminth egg reduction in an activated sludge plant. The data from these two studies for activated sludge treatment without disinfection are presented in Figure 5. For secondary effluents, the log₁₀ reductions

ranged from 1.62 - 3.11 for fecal coliforms, 1.85 - 2.44 for enterovirus, 1.62 - 3.11 for fecal coliforms, 1.85 - 2.44 for enterovirus, 1.23 - 2.67 for *Giardia* and 1.11 - 1.92 for *Cryptosporidium*, and greater than 2.40 for helminths. Although the low end log₁₀ reduction for *Giardia* and *Cryptosporidium* were similar (1.23 and 1.11 respectively), the corresponding effluent concentration for *Giardia* was an order of magnitude higher than *Cryptosporidium*, 8.3 cysts/L versus 0.84 (oo)cysts/L. These differences underscore the relationship of influent and effluent concentrations for a given log₁₀ reduction; that is, higher influent pathogen concentrations will lead to correspondingly higher effluent concentrations (survival).

Figure 5. Pathogen and fecal coliform reduction and persistence at 7 conventional activated sludge plants without disinfection in the U.S. Helminth data are for one system only; all other data are from six treatment systems. Influent/effluent ranges are from geometric means for each treatment plant; range of log₁₀ reductions calculated with geometric mean influent/effluent concentration for each plant. Data from Rose et al. (1996, 2004).

Ben Ayed et al. (2009) measured protozoan cyst (*Entamoeba coli*, *Entamoeba histolytica*, *Giardia*) and helminth egg (*Ascaris*, *Enterobius vermicularis*, *Taenia*) concentrations in 17 activated sludge plants in Tunisia. Their results for four conventional systems not using disinfection are shown in Figure 6. Their findings are quite different from those of Ramo et al. (2017) and Rose et al. (2004) and show high concentrations of cysts and eggs both in influents and effluents, with total log₁₀ reductions of only 0.73 and 0.90 respectively. The data from this study demonstrates that log₁₀ reduction can vary greatly among different species of protozoa and helminths. Mean log₁₀ reduction of *Entamoeba coli* (1.28) was more than double that of *Entamoeba histolytica* (0.50) and *Giardia* (0.57), and log₁₀ reduction for both *Ascaris* (0.90) and *Enterobius vermicularis* (1.13) were much greater than *Taenia* (0.64).

The authors conclude that wastewater treatment efficiency for parasite reduction needs to be improved to protect public health in Tunisia, where prevalence of protozoan and helminth infections is high (Ben Ayed et al., 2009). Unfortunately the authors did not report on the detailed operation of each plant in terms of BOD and TSS removal, but because they were all operated by National Sanitation Utility it is assumed the plants were functioning adequately in terms of organic matter removal (i.e., BOD and TSS).

Figure 6. Protozoan cyst and helminth egg reduction and persistence at four conventional activated sludge plants without disinfection in Tunisia. *Taenia* data are for one system only; all other data are from the four treatment systems. Influent/effluent ranges are from geometric mean values for each treatment plant; average and range of log₁₀ reductions are calculated with geometric mean influent/effluent concentration for each plant. Data from Ben Ayed et al., 2009.

Tables A1a to A1d in the Appendix at the end of this chapter summarize the data from Ben Ayed et al. (2009, Flannery et al. (2012), Fong et al. (2010), Ramo et al. (2017), Rose et al. (1996, 2004) and Tyagi et al. (2011) in comparison to the original data presented by Feachem et al. (1981, 1983) and the more recent data presented in WHO (2006) on pathogen reduction in conventional

activated sludge treatment plants without disinfection. While the reduction and survival of *E. coli* is similar in the comparison, the newer methods for analysis of specific viruses show higher reductions than originally cited in Feachem et al. (1983): enterovirus can be removed to over 2.0 log₁₀, adenovirus 1.77, and norovirus slightly less than 1.0 log₁₀. Nevertheless, the survival of these viruses ranged as high as 10³ - 10⁴/L in mechanically treated wastewater and can pose public health risks depending on final effluent

disposal or use. The newer data on *Giardia* and *Cryptosporidium* (oo)cysts that was not available to Feachem et al. (1983) show that these pathogens can be removed from 1.0 - 2.5 log₁₀, and from 0.35 - <1.6 log₁₀, respectively. And as with viruses, (oo)cyst survival in treated effluents and produced sludges is a cause for concern as discussed by Ben Ayed et al. (2009) and Ramo et al. (2017). The newer data available on helminth egg reduction show that the effluent concentration of eggs can often be below the limit of detection (which unfortunately is often not reported), but also can be found in very high concentrations in well-operated plants and thus pose a serious public health risk.

2.3 Conventional Activated Sludge with Chlorine Disinfection

Activated sludge plants in countries where disinfection is mandated by regulations rely on final stage disinfection to remove pathogens, and typically do so without monitoring the effectiveness of pathogen reduction in upstream unit processes prior to disinfection. Worldwide mechanized wastewater treatment plants, of which

activated sludge is the most common, continue to be designed principally for removal of organic suspended and soluble solids. Disinfection, if used, is incorporated as the final unit process before discharge.

In the study of Ramo et al. (2017) activated sludge plants with chlorine disinfection were also monitored: 7 out of the 23 plants monitored used disinfection as the final unit process. Figure 7 presents results for influent-effluent cyst concentrations and the log₁₀ reduction, and for cyst concentrations found in dewatered sludges for these 7 systems. The influent (oo)cyst concentrations were higher for *Giardia* than *Cryptosporidium* (22 – 6,703 versus 67 – 134 (oo)cysts/L, respectively), and the reported log₁₀ reduction ranged from 0.62 - 2.13 for *Giardia* and from 0.62 - 1.80 for *Cryptosporidium*. The reduction for *Giardia* is lower than that found for treatment without disinfection (Figures 3, 5, and 6), and *Cryptosporidium* reduction is only slightly different, illustrating the ineffectiveness of chlorine disinfection for (oo)cyst reduction, as would be expected. The (oo)cyst concentrations measured in dewatered sludges ranged from 0 – 593 cysts/g TS for *Giardia* and 0 – 10 cysts/g TS for *Cryptosporidium*.

Figure 7. *Giardia* and *Cryptosporidium* reduction and persistence in effluents and sludges from 7 conventional activated sludge plants with chlorine disinfection (5 systems were extended aeration without primary sedimentation and 2 were conventional activated sludge.) Values shown are ranges of average values for the 7 plants. Sampling was performed four times per year during different seasons (Ramo et al., 2017).

^aDry solids basis

Kitajima et al. (2014a, 2014b) monitored a conventional activated sludge plant with chlorine disinfection for reduction of select pathogens that included enteric viruses

(noroviruses, Aichi virus, sapovirus, rotavirus, enterovirus), *Giardia* and *Cryptosporidium*. The treatment plant had a mean flow rate of 155,000 m³/d and served a population of approximately 500,000; influent and final effluent samples were collected once a month for one year.

The results for Aichi virus, norovirus GI and GII, *Giardia* and *Cryptosporidium* are shown in Figure 8 for influent and effluent concentrations, and log₁₀ reduction for each pathogen. Effluent *E. coli* concentrations are also shown although influent concentrations were not monitored. Mean Aichi virus reduction was low, 0.94 log₁₀, and norovirus GI reduction was reported at 1.65 log₁₀. This is 0.8 log₁₀ reduction greater than the reduction reported by Flannery et al. (2012) without disinfection. Norovirus GII reduction, however, at 2.14 log₁₀, is only 0.22 log₁₀ higher than the 0.92 reported by Flannery et al. (2012) without disinfection. The log₁₀ reduction of *Giardia* and

Cryptosporidium, 2.08 and 0.71 respectively, are within the same range as the reductions found by Ramo et al. (2017) without disinfection (*Giardia*: 1.54 - 2.34 log₁₀, *Cryptosporidium*: 0.35 - 1.63 log₁₀). It should be noted that while effluent concentrations of *E. coli* were below 150 MPN/100mL (range reported as <1 - 134 MPN/100mL) for the study period as a result of disinfection, viruses and protozoan (oo)cysts were much less influenced by disinfection with Aichi and norovirus concentrations as high as 104/L and *Giardia* and *Cryptosporidium* as high as 150 cysts/L and 36 oocysts/L respectively.

^aGC: Gene Copy. Virus concentrations were measured by the qPCR molecular method and not by cell culture. The qPCR method identifies specific viruses but they do not have to be intact or infective to be detected (Francy et al., 2011) and log₁₀ reduction may be underestimated. There are no known routine cell culture methods available for viable Aichi virus and norovirus.

Figure 8. Reduction and persistence of Aichi virus, norovirus GI and GII, *Giardia* and *Cryptosporidium* at a conventional activated sludge plant with chlorine disinfection in Arizona (U.S.) (Kitajima et al., 2014a). Influent and effluent concentrations are average values with ranges in parentheses. *E. coli* was only monitored in effluent samples.

2.4 Trickling Filter with Chlorine Disinfection

Kitajima et al. (2014a, 2014b) also monitored a conventional trickling filter plant with chlorine disinfection for reduction of the same select pathogens as discussed in Section 2.3. The mean flow rate was 94,600 m³/d for a population of approximately 250,000. The results from this plant are shown in Figure 9. The results are similar to those of the activated sludge plant but the authors reported that *Giardia* reduction in the activated sludge plant was statistically different from the trickling filter (2.08 log₁₀ reduction compared to 1.52 log₁₀) but that there was no

statistically significant difference for *Cryptosporidium* reduction (0.71 versus 0.81 log₁₀). Norovirus GI and GII reduction, however, were higher in the trickling filter plant (2.57 and 2.85 log₁₀ reduction respectively) than the activated sludge plant (1.65 and 2.14 log₁₀ reduction). These differences, however, are minor compared to the likely need to remove >3.0 or 4.0 log₁₀ viruses and protozoan cysts if the effluent were reused for agriculture or recreational purposes.

Table A2 in the Appendix shows a comparison of virus and protozoan cyst reduction and survival for the treatment plants that practiced disinfection (Sections 2.3, 2.4) and those that did no (Section 2.2).

^aGC: Gene Copy. Virus concentrations were measured by the qPCR molecular method and not by cell culture. The qPCR method identifies specific viruses but they do not have to be intact or infective to be detected (Francy et al., 2011) and log₁₀ reduction may be underestimated. There are no known routine cell culture methods available for viable Aichi virus and norovirus.

Figure 9. Reduction and persistence of Aichi virus, norovirus GI and GII, *Giardia* and *Cryptosporidium* at a conventional trickling filter plant with chlorine disinfection in Arizona (U.S.) (Kitajima et al., 2014a). Influent/effluent concentrations are average values with ranges in parentheses. *E. coli* was only monitored in effluent samples.

2.5 Activated Sludge with Tertiary Treatment Processes

Tertiary treatment processes are covered in a separate chapter. They are used with activated sludge and include filtration (cloth, sand or membrane filters), chemical processes (precipitation) and physical-chemical processes (coagulation, flocculation) commonly followed by disinfection. Tertiary processes have been developed principally to improve effluent quality in terms of physical-chemical parameters for more stringent effluent guidelines (e.g., nitrogen and phosphorus) with less attention on specific pathogen reduction (Burton et al., 2014)

2.5.1 Activated sludge with coagulation, flocculation, sedimentation and filtration

Figure 10 presents the results of *Giardia* and *Cryptosporidium* reduction in an activated sludge plant in China with a flow rate of 1,000,000 m³/day (Fu et al., 2010). The plant uses the following tertiary processes: coagulation, flocculation, sedimentation, and sand

filtration. *Giardia* reduction was reported at 3.46 log₁₀ and *Cryptosporidium* 3.34 log₁₀, with effluent concentrations ranging from <0.2–2.1 cysts/L for *Giardia* and from 0.2–0.4 oocysts/L for *Cryptosporidium*. Figure 11 presents the log₁₀ reduction in each unit process (activated sludge, coagulation-flocculation-sedimentation, and sand filtration). The conventional activated sludge process has the highest log₁₀ reduction for both *Giardia* and *Cryptosporidium* (1.85 and 1.64 log₁₀ respectively), which is approximately double the log₁₀ reduction for

each of the subsequent unit processes (coagulation/flocculation/sedimentation (C/F/S) and sand filter (SF)) for both *Giardia* and *Cryptosporidium* (Figure 11). The total log₁₀ reduction reported in this study for both *Giardia* and *Cryptosporidium*, however, is among the highest reported in the literature.

Figure 10. *Giardia* and *Cryptosporidium* reduction and persistence in an activated sludge plant with tertiary treatment of coagulation, flocculation, sedimentation, and sand filtration. Influent and effluent concentrations are average values with ranges in parentheses. Data from Fu et al., 2010.

Figure 11. Log₁₀ reduction of *Giardia* and *Cryptosporidium* in individual unit processes in an activated sludge treatment plant using tertiary treatment processes of coagulation, flocculation, and sedimentation with sand filtration. Data from Fu et al., 2010. AS = secondary effluent. C/F/S = coagulation/flocculation/sedimentation. SF = sand filtration.

2.5.2 Activated sludge with filtration and disinfection

Rose et al. (2004), as part of the study discussed previously in Section 2.2 present reduction data for six

activated sludge plants employing filtration followed by disinfection. Five of the plants used sand filters for filtration and one used a cloth filter (the filter pore size was not provided); five plants used chlorine disinfection and one used UV radiation.

The results for these treatment plants are presented in Figure 12. Log₁₀ reduction of fecal coliforms ranged from 5.94–7.31, enterovirus from 2.85–4.33, Giardia from 2.15–3.87, and Cryptosporidium from 1.68–3.00. Data from Chaoua et al. (2018) on helminth reduction in an activated sludge plant with sand filtration and UV/chlorine disinfection in Morocco is also shown in Figure 12; total

helminth egg influent concentrations averaged 173.8 eggs/L (86.75% Ascaris) and no eggs were ever detected in the final effluent. Effluent concentrations of all pathogens were relatively low as seen in Figure 12. Rose et al. (2004), however, concluded that all monitored effluents contain measureable concentrations of pathogens (except helminths) and exposure to these pathogens carries some risk to public health (Rose et al. 2004).

^aAuthors presented helminth effluent values as 0.

Figure 12. Pathogen and fecal coliform reduction and persistence at seven wastewater treatment plants (six in the U.S. and one in Morocco) using activated sludge with tertiary process of filtration plus disinfection (6 plants using sand filters and 1 a cloth filter), and disinfection (5 plants using chlorine and 2 UV). Helminth data are from Chaoua et al., 2018 in Morocco; all other data are from Rose et al. 2004 in the US. Helminth influent data are average values; effluent values were reported as 0 and log₁₀ reduction could not be calculated. Bacteria, virus and protozoa influent/effluent data are ranges of geometric means for six treatment plants.

The study by Rose et al. (2004) allows an analysis of the cumulative log₁₀ reduction per unit process along the overall treatment train. This is shown in Table 1 and Figure 13. Figure 13 shows that although the mean values for the unit processes of secondary effluent, filtration, and filtration with disinfection each have a >2 log₁₀ reduction for fecal coliforms, this is not the case for enterovirus, Giardia, or Cryptosporidium: for these pathogens the conventional activated sludge process contributes the

greatest log₁₀ reduction over the tertiary processes. As seen in Figure 14, the mean log₁₀ reduction in the individual unit processes for enterovirus, Giardia, and Cryptosporidium is highest in the conventional activated sludge process, which is approximately 2.0, 1.0, and 1.0 log₁₀ higher than filtration, respectively. Disinfection, not surprisingly, has very little effect on the protozoan (oo)cysts. Surprisingly, (oo)cyst reduction by filtration is roughly 1.0 log₁₀ less than conventional activated sludge.

In summary, the principal unit process for reduction of enterovirus, *Giardia*, and *Cryptosporidium* in a system that employ tertiary treatment is conventional activated sludge,

which is at least 1.0 log₁₀ greater than filtration or disinfection.

Table 1. Ranges of geometric mean log₁₀ reduction for unit processes within the treatment train for six activated sludge plants that employed tertiary treatment. Values are ranges of mean log₁₀ reduction for the six plants. Data from Rose et al., 2004.

Pathogen/Indicator	Range of Log ₁₀ Reduction for Unit Process			
	Secondary Effluent	Sand/Cloth Filtration ^a	Cl ₂ or UV Disinfection ^b	Total Processes
Fecal Coliforms	1.62 to 3.11	0.06 to 4.54	0.79 to 3.22	5.94 to 7.31
Enterovirus	1.85 to 2.44	0.27 to 1.20	0.10 to 1.63	2.85 to 4.33
<i>Giardia</i>	1.23 to 2.67	2.03 to 3.98	-0.10 to 0.35	2.15 to 3.87
<i>Cryptosporidium</i>	1.11 to 1.92	0.11 to 1.04	-0.28 to 0.55	1.68 to 3.00

^aOne plant used a cloth filter; ^bOne plant used UV.

Figure 13. Reduction of select pathogens as a function of sequential unit processes in an activated sludge treatment train that employed tertiary treatment. AS = activated sludge secondary effluent. AS/F = Activated sludge with filtration. AS/F/D = activated sludge with filtration and disinfection. Data points are mean values with range of values for six treatment plants. Adapted from Rose et al. 2004.

Figure 14. Pathogen reduction in individual unit process in activated sludge treatment plants with and without tertiary processes. AS = secondary effluent. F = media filtration. D = disinfection. Data points are geometric means with range of values. Adapted from Rose et al. 2004.

Figure 15 presents the results of the study of Ramo et al. (2017) for three activated sludge plants that employed tertiary treatment using microfiltration and disinfection, with two plants using UV and one chlorine disinfection. The authors do not specify the microfilter pore size used. Microfilter pore size can range from 0.05–2.0 µm (Burton et al., 2014) which should filter protozoan cysts if operated properly. The results in Figure 16 for pathogen reduction).

as a function of sequential unit processes used, however, show that the plants with tertiary treatment processes did not operate any better, and perhaps worse, than those with only conventional activated sludge without disinfection. As an example, Figure 16 shows the log10 reduction for *Giardia* in conventional activated sludge (AS) is as high or higher than the plants with disinfection (AS/D) or microfiltration/disinfection (AS/MF/D or AS/MF/UV

Figure 15. *Giardia* and *Cryptosporidium* reduction and persistence at three wastewater treatment plants in Spain using activated sludge, microfiltration and disinfection (two plants used UV and one chlorine disinfection). Influent and effluent ranges are from all three plants. Data from Ramo et al., 2017.

Figure 16. *Giardia* and *Cryptosporidium* (oo)cyst reduction as a function of sequential treatment processes. AS = activated sludge secondary effluent. AS/D = activated sludge with disinfection. AS/MF/D = activated sludge with microfiltration and chlorine disinfection. AS/MF/UV = activated sludge with microfiltration and UV disinfection. Data show average and range of log10 reduction for number of plants in in each category (shown in parentheses). Adapted from Ramo et al., 2017.

Figure 17 plots the results of Fong et al. (2010) on adenovirus reduction in activated sludge with disinfection/filtration; reduction without disinfection from their study was discussed previously in Section 2.1. The treatment plant had chlorine disinfection followed by rapid sand filtration, which is an atypical design, especially so if the design intent were to remove pathogens. As seen in Figure 17, the average concentration measured after disinfection/filtration was higher than the secondary effluent, with an average of 83,000 copies/L and range of 13,500 – 428,000 copies/L,

for a $\log_{10}$ reduction in the complete treatment works of 1.14. The authors state there was no statistical difference in adenovirus concentrations between secondary effluent and the disinfected/filtered effluent for the data after one year of monitoring, and that disinfection/filtration had no effect on adenovirus reduction. The authors detected adenoviruses in the river downstream of the wastewater effluent discharge and concluded that wastewater treatment is inadequate to remove viruses, and as a result adenovirus may represent a public health risk (Fong et al., 2010).

Figure 17. Influence of wastewater unit process treatment train on adenovirus reduction. Data are plotted as average with range of values during study period. Adapted from Fong et al., 2010.

Fu et al. (2010) report on *Giardia* and *Cryptosporidium* reduction in an anaerobic-anoxic-oxic activated sludge plant using ultrafiltration and ozone/chlorine disinfection at another treatment plant in China. The treatment facility had a average flow rate of 400,000 m³/day. The authors did not report the membrane pore sizes, but they can range from 0.005 – 0.1 μ m for ultrafiltration (Burton et al., 2014). Figure 18 shows the results of 18 sampling groups over a two year period for the treatment plant. Influent *Giardia* and *Cryptosporidium* concentrations ranged from 170–3,600 cysts/L and 33–430 oocysts/L respectively, and no effluent concentrations were detected for the duration of the study; effluent concentrations for both pathogens were reported as <0.033 (oo)cysts/L, which is interpreted as the

limit of detection of the analytical methods used by the authors (Fu et al., 2010).

Table 2 shows the average $\log_{10}$ reduction for each unit process in the treatment train. The authors concluded that the effluent from ultrafiltration can meet any reuse standard in China, and that treatment plants using this process require less use of disinfectants and produce less disinfection byproducts. They cautioned, however, that the expensive cost of operation is a great obstacle to widespread use of ultrafiltration in China (Fu et al., 2010). Ultrafiltration requires 0.2 – 0.3 kWh/m³ of wastewater treated, which is approximately double the energy used in a conventional activated sludge plant with chlorine disinfection (Burton et al., 2014).

Table 2. Mean $\log_{10}$ reduction for unit processes within the treatment train for an anaerobic/anoxic/oxic activated sludge treatment plant using ultrafiltration. Data from Fu et al., 2010.

Pathogen/Indicator	Log ₁₀ Reduction for Unit Process		
	Secondary Effluent	Membrane Ultrafiltration	Total Processes
<i>Giardia</i>	2.04	>2.40	>4.44
<i>Cryptosporidium</i>	1.79	>1.84	>3.63

Figure 18. *Giardia* and *Cryptosporidium* reduction and persistence in an anaerobic/anoxic/oxic activated sludge plant with ultrafiltration and ozone/chlorine disinfection. Data from Fu et al., 2010. Data are average values with ranges in parentheses.

2.6 Summary of Pathogen Reduction and Survival in Mechanized Wastewater Treatment

Tables 3a, 3b, and 3c present a summary of pathogen reduction and survival in mechanized wastewater treatment plants without disinfection, with disinfection, and with tertiary processes.

Table 3a. Summary of *E. coli* / fecal coliforms pathogen reduction and survival in conventional activated sludge with and without chlorine disinfection, a conventional trickling filter with disinfection, and activated sludge with tertiary processes. It is assumed all sludges contain concentrations of pathogens potentially posing health risks.

Process	Influent	Effluent	Log ₁₀ Reduction
Conventional Activated Sludge no disinfection	5.4E+04 to 1.5E+09	1.1E+04 to 9.3E+07	1.49 to 3.10
Conventional Activated Sludge with disinfection	NR	E+01 to 1.3E+03	NR
Trickling Filter with disinfection	70 to 2.2E+03	7E+01 to 2.2E+03	NR
Activated Sludge with Tertiary Processes: Coagulation/Flocculation Sedimentation/Filtration	NR	NR	NR

Process	Influent	Effluent	Log₁₀ Reduction
Activated Sludge with Tertiary Processes: Sand/Filtration Disinfection	NR	NR	NR
Activated Sludge with Tertiary Processes: Ultrafiltration Disinfection	NR	NR	NR

NR: Not Reported

Table 3b. Summary of viral pathogen reduction and survival in conventional activated sludge with and without chlorine disinfection, a conventional trickling filter with disinfection, and activated sludge with tertiary processes. It is assumed all sludges contain concentrations of pathogens potentially posing health risks.

Virus	Process	Influent	Effluent	Log₁₀ Reduction
Adenovirus ^a	Conventional Activated Sludge no disinfection	2.6E+05 to 2.8E+06 ^c	1.0E+03 to 4.4E+04 ^c	1.77
Aichi virus ^a	Conventional Activated Sludge with disinfection	9.7E+04 ^c	1.1E+04 ^c	0.94
Aichi virus ^a	Trickling Filter with disinfection	2.0E+06 ^c	2.0E+05 ^c	0.99
Enterovirus	Conventional Activated Sludge: No disinfection	4.6E+03 to 9.4E+04 ^b	3.0E+01 to 1.3E+03 ^b	1.85 to 2.44
Enterovirus	Activated Sludge with Tertiary Processes Sand/Filtration Disinfection	4.6E+02 to 9.4E+03 ^b	3.1 to 9.1	2.85 to 4.33
Norovirus GI & GII ^a	Conventional Activated Sludge no disinfection	6.5E+02 to 2.2E+08 ^c	1.8E+02 to 1.2E+04 ^c	0.80 to 0.92
Norovirus GI & GII ^a	Conventional Activated Sludge with disinfection	ND ^d to 6.5E+06 ^c	ND ^d to 1.0E+04 ^c	1.65 to 2.14
Norovirus GI & GII ^a	Trickling Filter with disinfection	1.0E+02 to 3.2E+05 ^c	ND ^d to 1.6E+03 ^c	2.57 to 2.85

^aVirus concentrations were measured by the qPCR molecular method and not by cell culture. The qPCR method identifies specific viruses but they do not have to be intact or infective to be detected (Francy et al., 2011) and log₁₀ reduction may be underestimated. ^bMPN/L; ^cCopies/L; ^dND: Non-detect

Table 3c. Summary of protist and helminth pathogen reduction and survival in conventional activated sludge with and without chlorine disinfection, a conventional trickling filter with disinfection, and activated sludge with tertiary processes. It is assumed all sludges contain concentrations of pathogens potentially posing health risks.

Virus	Process	Influent	Effluent	Log₁₀ Reduction
<i>Giardia</i> cysts ^a	Conventional Activated Sludge no disinfection	6.1E+01 to 6.6E+03	0.65 to 8.1E+01	1.23 to 2.67
<i>Giardia</i> cysts ^a	Conventional Activated Sludge with disinfection	2.2E+01 to 9.9E+03	<4 to 1.5E+02	0.62 to 2.13

Virus	Process	Influent	Effluent	Log ₁₀ Reduction
<i>Giardia</i> cysts ^a	Trickling Filter with disinfection	1.4E+03 to 1.5E+04	5.6E+01 to 6.2E+02	1.52
<i>Giardia</i> cysts ^a	Activated Sludge with Tertiary Processes: Coagulation/Flocculation Sedimentation/Filtration	8.3E+02 to 2.7E+03	<0.2 to 2.1	3.46
<i>Giardia</i> cysts ^a	Activated Sludge with Tertiary Processes: Sand/Filtration Disinfection	6.1E+01 to 9.8E+03	0.04 to 2.7E+02	1.40 to 3.87
<i>Giardia</i> cysts ^a	Activated Sludge with Tertiary Processes: Ultrafiltration Disinfection	1.7E+02 to 3.6E+03	<0.033	>4.44
<i>Cryptosporidium</i> oocysts ^b	Conventional Activated Sludge no disinfection	6.5 to 4.6E+02	0.28 to 3.0E+02	0.35 to 1.92
<i>Cryptosporidium</i> oocysts ^b	Conventional Activated Sludge with disinfection	4.0E+01 to 1.6E+02	0.28 to 3.0E+02	0.62 to 1.80
<i>Cryptosporidium</i> oocysts ^b	Trickling Filter with disinfection	<4.0E+01 to 7.2E+02	<4 to 4.0E+01	0.81
<i>Cryptosporidium</i> oocysts ^b	Activated Sludge with Tertiary Processes: Coagulation/Flocculation Sedimentation/Filtration	1.0E+02 to 4.0E+02	0.2 to 0.4	3.34
<i>Cryptosporidium</i> oocysts ^b	Activated Sludge with Tertiary Processes: Sand/Filtration Disinfection	6.5 to 38.6E+02	0.03 to 4.7E+01	0.46 to 3.00
<i>Cryptosporidium</i> oocysts ^b	Activated Sludge with Tertiary Processes: Ultrafiltration Disinfection	3.3E+01 to 4.3E+02	<0.033	>3.63
<i>Entamoeba coli</i> ^b	Conventional Activated Sludge: No disinfection	1.12E+03 to 1.5E+03	5.2E+01 to 9.8E+01	1.15 to 1.40
<i>Entamoeba histolytica</i> ^a	Conventional Activated Sludge no disinfection	6.40E+02 to 9.14E+02	1.85E+02 to 3.20E+02	0.30 to 0.66
Helminth eggs ^c	Conventional Activated Sludge no disinfection	<2 to 1.04E+03	ND ^d to 2.4E+02	0.81 to ≤2.40

^aCysts/L; ^bOocysts/L; ^cEggs/L; ^dND: Non-detect

3.0 Natural Wastewater Treatment Systems

Natural wastewater treatment systems utilize natural physical and biological processes such as gravity flow, natural convection of air, photosynthesis (Crites et al., 2006), and anaerobic digestion. They are able to treat wastewaters without external energy and chemical inputs. Natural systems are considered the best options for wastewater treatment in developing countries and small cities worldwide because of their low construction cost, simple operation and maintenance requirements, and contribution to sustainability by using natural energy-

producing processes and producing effluents with nutrients that can be reused in agriculture and aquaculture (Muga and Mihelcic, 2008; Kumar and Asolekar, 2016; Verbyla et al., 2015). Natural wastewater treatment systems are discussed in detail in other chapters. They include waste stabilization ponds, hyacinth and duckweed ponds, constructed wetlands, gravity flow anaerobic reactors such as UASBs and anaerobic filters, and gravity flow trickling filters with natural convection for aeration (Arceivala and Asolekar, 2007; Mendonça and Mendonça, 2016). This section presents representative case studies of the most common natural system technologies used worldwide.

3.1 Waste Stabilization Ponds

Waste stabilization ponds (see *Waste Stabilization Ponds* chapter) are one of the most common wastewater treatment technologies in both developed and developing countries in small to large cities. Unlike mechanized technologies where disinfection is applied as the final unit process because the systems were designed for treatment of organic matter, pathogen reduction in facultative and maturation ponds, if desired, is incorporated into the fundamental design of the system (Ayres et al., 1992; von Sperling, 2007).

3.1.1 Facultative and maturation ponds in series

The simplest of all treatment technologies designed to remove pathogens (and organic matter) is a facultative pond followed by a maturation pond. In this configuration the facultative pond is designed to remove helminth eggs for example, by hydraulic retention time or hydraulic overflow rate, and the maturation pond to further remove helminths and other pathogens (von Sperling, 2007). While helminth eggs can be routinely monitored throughout a pond system, fecal coliforms and *E. coli* are the commonly used indicators of bacterial, protozoan and viral pathogen reduction.

Figure 19 presents the reduction and survival data of helminth eggs and *E. coli* obtained from ten facultative/maturation waste stabilization pond systems monitored over a two-year period in Honduras (Oakley, 2005). The average flowrates ranged from 218 – 5,150 m³/d, with populations from 1,000 – >23,000 and theoretical hydraulic retention times ranging from 7.2 – 34.8 d. Mean log₁₀ reduction of *E. coli* for the 10 systems was 2.92, but the range varied from a low of 1.87 to a high of 4.84; one system consistently had effluent concentrations during six sampling events in winter and summer <1,000 MPN/100mL with a geometric mean of 271 MPN/100mL.

Helminth egg concentrations, principally *Ascaris*, were always measured in the influent but never detected in the effluent; unfortunately the level of detection was not reported and log₁₀ reduction could not be calculated. Facultative pond sludges all contained helminth eggs ranging from 1.0 – 4,473 eggs/g TS; the viability of eggs in one facultative pond sludge sampled at different locations ranged from 0.4 – 25.0 eggs/g TS (Oakley et al., 2012). The presence of *Shigella* was monitored throughout the study but was never detected (Oakley, 2005). Oakley (2005) concluded that although several pond systems performed well, others did not and a major problem was adequate design and construction coupled with sustainable operation and maintenance on the part of the municipalities.

^aGeometric mean for bacteria; ^bAverage for helminths; ^cReported as 0 by the author; ^dDry weight basis

Figure 19. *E. coli* and helminth egg reduction and persistence in effluent and sludges from 10 wastewater stabilization pond systems in Honduras. Influent and effluent data are mean values (geometric for *E. coli* and arithmetic for helminths) for the 10 systems with ranges in parentheses. Helminth egg concentrations were not detected in effluent samples but the detection limit was not presented by the author and therefore log₁₀ reduction could not be calculated. Data from Oakley, 2005.

3.1.2 Facultative pond followed by two maturation ponds in series (F/M/M)

A common configuration where the effluent is to be reused for unrestricted irrigation is a facultative pond followed by two maturation ponds in series (F/M/M); the

design strategy is to maximize pathogen reduction through extended hydraulic retention time for sedimentation of particles and exposure to solar radiation.

Figure 20 shows one of the few studies where bacterial pathogen reduction, in this case *Vibrio cholera* 01, was monitored throughout a F/M/M system and compared with fecal coliform reduction during the 1991 cholera epidemic in Lima, Peru (Castro de Esparza et al., 1992). During the

peak of the epidemic in March 1991, *Vibrio cholera* 01 was detected at concentrations as high as 4.3×10^5 MPN/100mL in one of the principal wastewater collectors in Lima (Castro de Esparza et al., 1992). As a result, a monitoring study of the wastewater stabilization pond system in San Juan de Miraflores, where the final effluent was being reused in aquaculture and agriculture, was implemented from May - August 1991. During the monitoring period the flowrate into the system was controlled to maintain a hydraulic retention time of 50 days, and the raw wastewater influent and the effluent from each pond was monitored for fecal coliforms and *Vibrio cholera* 01; the aquaculture ponds were also monitored for fecal coliforms and *Vibrio cholera* 01, and tissue from tilapia for the presence of *Vibrio cholera*.

The results in Figure 20 show a 4.26 log₁₀ reduction of *Vibrio cholera* 01 and a 4.89 log₁₀ reduction for fecal coliforms, with effluent concentrations of 0.1 MPN/100mL and 2.11×10^4 respectively (geometric means). (It should be noted that while the final effluent concentrations of *Vibrio cholera* 01 are low at 0.1 MPN/100mL, during the peak of the epidemic raw wastewater concentrations measured at 4.3×10^5 MPN/100mL would have given an effluent concentration of 23.5 MPN/100mL for the measured 4.26 log₁₀ reduction.) *Vibrio cholera* 01 was found in the aquaculture ponds at very low concentrations (0.03 MPN/100mL), and the presence of *Vibrio cholera* was detected in tilapia tissue (skin, gills, intestines), but the test did not distinguish between *Vibrio cholera* 01 and *Vibrio cholera* non-01 (Castro de Esparza et al., 1992). Figure 21 shows the serial reduction of fecal coliforms and *Vibrio cholera* 01 through each pond in series during the monitoring period.

^aExpressed as both MPN/100mL for comparison with fecal coliforms and MPN/L for comparison with other pathogens; ^bThe presence test for *Vibrio Cholerae* did not distinguish between *Vibrio Cholerae* 01 and *Vibrio Cholerae* non-0

Figure 20. Reduction and persistence of fecal coliforms and *Vibrio cholerae* 01 in the waste stabilization pond system San Juan de Miraflores, Lima, Peru, from June 9 to August 10, 1991 (Castro de Esparza et al., 1992). Influent and effluent values are geometric means.

Figure 21. Reduction and survival of fecal coliforms and *Vibrio cholerae* 01 at the San Juan wastewater stabilization pond system in Lima, Peru during the cholera epidemic from June - August 1991 (Castro de Esparza et al., 1992). The influent flowrate was controlled during the monitoring period to maintain a hydraulic retention time of 50 days (HRT values shown in the figure are for each pond in series which add up to 50 days). Mean water temperature in the system averaged 17.5°C.

An excellent example of an F/M/M system with agricultural reuse that has operated for decades is the Campo Espejo system in Mendoza, Argentina (Figure 22). The system was built in 1995 and consists of 12 batteries of F/M/M ponds in series; the design flowrate is 146,620 m³/d with a theoretical hydraulic retention time of 21.1 days and a potential irrigated area of 4,000 ha (Anzorana, 2001; Vélez et al., 2002). The system has been continuously monitored for fecal coliforms and helminth eggs to meet the WHO guidelines for agricultural reuse.

Figure 22 shows the mean results for daily monitoring during one year (January - December 2000) (Anzorana, 2001). Mean fecal coliform concentrations were reduced from 1.1×10^7 MPN/100mL to 75 MPN/100mL, a 5.14 log₁₀ reduction, and helminth eggs from a mean of 26 eggs/L to <1 egg/L, thus meeting the WHO effluent guidelines of 1989 of <1,000 fecal coliforms/100mL and <1 helminth egg/L for unrestricted irrigation (Vélez et al., 2002).

^a*E. coli*; ^bHelminth eggs

Figure 22. Reduction and persistence of *E. coli* and helminth eggs at the wastewater stabilization pond system Campo Espejo, Mendoza, Argentina. Values are geometric or arithmetic mean values from daily monitoring for one year (January 1, 2000 to December 31, 2000). The detection limit of helminth eggs was not mentioned by the author and the log₁₀ reduction of >1.41 assumed the detection limit was 1 egg/L. Data from Anzorana, 2001. Approximately 4,000 ha are irrigated with the effluent from this system.

Tyagi et al. (2011) reported on the reduction of *E. coli*, *Salmonella* and helminth eggs in a facultative/maturation pond system in India that was monitored for 2 years; the system had a facultative pond followed by three maturation ponds in series (Figure 23). Mean *E. coli* influent and effluent concentrations were 2.2×10^6 MPN/100mL and 1.8×10^3 MPN/100L respectively, giving a $\log_{10}$ reduction of 3.09. *Salmonella*, in contrast, exhibited a much lower 1.24 $\log_{10}$ reduction, with means of 408 CFU/100mL in the influent and 27.5 in the effluent; this is an important example of how different genera of bacteria can exhibit different $\log_{10}$ reduction values when monitored simultaneously. Helminth eggs averaged 16 eggs/L in the effluent and were detected in the effluent at low concentrations with a mean value of 0.18 eggs/L, giving a $\log_{10}$ reduction of 2.48. The system performed well in terms of BOD and TSS removal, and met the WHO guidelines (WHO, 2006) for restricted reuse. The authors noted that fecal indicator reduction was higher in summer than winter by approximately 2.0 $\log_{10}$ units, and this was best correlated with suspended solids removal. This waste stabilization pond system performed better in helminth egg reduction than the two activated sludge plants discussed previously by Tyagi et al. (2011) (previously shown in Figure 4).

Many existing waste stabilization pond systems in developing countries are improperly designed, lack adequate operation and maintenance, or are abandoned entirely, and Figure 24 provides an example of the performance of an improperly designed, operated and maintained F/M/M system in Bolivia (Symonds et al., 2014; Verbyla et al., 2013a, 2013b). The system did not

have pretreatment and was poorly designed hydraulically, with the mean hydraulic retention time (HRT) based on tracer studies estimated to be 50% of the theoretical HRT (≈ 13 days versus 27 days) (Verbyla et al., 2013b). Composite samples were taken on two different days in June 2012 and analyzed for enteric viruses: mean enterovirus concentrations were 4.2×10^4 infectious units/L (IU/L) for the influent and 37 IU/L in the final effluent for a log₁₀ reduction of 3.06; norovirus GI and rotavirus concentrations, however, were not reduced through treatment and were approximately 1.0×10^6 in both influent and effluent samples (Symonds et al., 2014).

Giardia and *Cryptosporidium* were monitored in one composite sample in June 2012, with influent and effluent concentrations of 160 cysts/L and 23 cysts/L for *Giardia*, for a log₁₀ reduction of 0.84; Influent and effluent *Cryptosporidium* oocyst concentrations were 6.0 and 4.0 oocysts/L respectively, for a 0.18 log₁₀ reduction (Verbyla et al., 2013a). Helminth eggs were measured once in June 2011 and 2012, and both *Ascaris* and *Taenia* were detected in the influent at up to 306 and 3,006 eggs/L respectively, with *Taenia* detected in one effluent sample at 45 eggs/L, for a 1.82 log₁₀ reduction (Verbyla et al., 2013b). The authors conclude that although the system effluent was not being used for irrigation, wastewater reuse is common in Bolivia and the effluent from systems such as this one poses a health risk from viruses, protozoa and helminths if it were to be reused without further treatment or in-field management in agriculture (Symonds et al., 2014; Verbyla et al., 2013a, 2013b).

^a*E. coli* and *Salmonella*; ^bHelminth eggs; ^cExpressed as both CFU/100 ML for comparison with *E. coli* and CFU/L for comparison with other pathogens.

Figure 23. *E. coli*, *Salmonella* and helminth egg reduction and persistence in effluent from a wastewater stabilization pond system in India with one facultative pond followed by three maturation ponds in series. Influent and effluent data are mean values (geometric for *E. coli* and *Salmonella*, and arithmetic for helminths) with ranges in parentheses. Data from Tyagi et al. (2011).

^aEnterovirus units in infectious units/L; ^bGC: Gene Copy. Norovirus and rotavirus concentrations were measured by the RT-qPCR molecular method and not by cell culture, thus concentrations reported in effluents may underestimate reduction.

Figure 24. Reduction and persistence of enterovirus, norovirus, rotavirus, *Giardia*, *Cryptosporidium*, *Taenia* and *Ascaris* in a waste stabilization pond system in Bolivia. Data are average values or ranges from composite samples taken on two different days for viruses (June 2012), twice for helminth eggs (June 2011 and 2012), and once for protozoa (June 2012). Log₁₀ reduction of *Taenia* based on influent of 3,006 eggs/L and effluent of 45 eggs/L.; the detection limit for the final effluent was 22 eggs/L. No *Ascaris* eggs were detected in the final effluent and were only detected in one influent sample, thus log₁₀ reduction could not be estimated. Data from Symonds et al., 2014; Verbyla et al., 2013a, 2013b.

3.1.3 Anaerobic pond followed by facultative and maturation ponds in series (A/F/M)

An anaerobic pond followed by facultative and maturation ponds in series system (A/F/M) is a common design in tropical and semi-tropical climates where the purpose of the anaerobic pond is to remove organic matter (and capture methane in some designs) in order to reduce the total area of the facultative pond (Mara, 2003). Figure 25 presents the results of the A/F/M system at the International Institute for Water and Environmental Engineering in Ouagadougou, Burkina Faso, (Konaté et al., 2013). The system, which had an average flowrate of 55 m³/d with a hydraulic retention time of 18 d, was monitored for protozoan cysts and helminth eggs twice a month for one year (2007-2008) in both wastewater and pond sludges.

The protozoan cysts encountered in raw wastewater included *Entamoeba coli*, *Entamoeba histolytica*, and *Giardia lamblia*, with average influent concentrations of 85.8, 18, and 7 cysts/L for *E. coli*, *E. histolytica*, and *Giardia* respectively, and a total influent cyst concentration of 111 cysts/L with range of 4 – 327 cysts/L; no cysts were

detected in the final effluent, and the log₁₀ reduction was estimated at >1.87 (detection limit was not reported). Average influent helminth eggs were reported at 15.7 eggs/L, with a range of 5 – 36 eggs/L; no eggs were detected in the final effluent, and the log₁₀ reduction was estimated at >2.89 (detection limit was not reported).

As shown in Figure 25, protozoan cysts were found in all pond sludges, gradually decreasing from 120/10/7 cysts/g TS in the A/F/M ponds respectively. Helminth eggs were also found in all pond sludges, decreasing from 556/32/12 eggs/L for the A/F/M ponds respectively, with percent viability of 36%/16%/0% in the A/F/M ponds. The authors conclude that the ponds perform well in protozoan cyst and helminth egg reduction from wastewater with respect to potential wastewater reuse in Burkina Faso, but great caution is needed with the management of pond sludges as a result of high concentrations of cysts and viable helminth eggs (Konaté et al., 2013).

^aReported as 0 by the authors; ^bDry weight basis

Figure 25. Protozoan cyst and helminth egg reduction and persistence in effluent and sludges at a wastewater stabilization pond system in Ouagadougou, Burkina Faso (Konaté et al., 2013). Protozoan cyst and helminth egg concentrations were not detected in any effluent samples from the maturation pond but the detection limit was not presented by the authors and therefore total log₁₀ reduction could not be calculated. The log₁₀ reduction shown is based on average values of measured concentrations from the facultative pond effluent: protozoan cysts = 2 cysts/L; helminth eggs = 0.02 eggs/L.

Reinoso et al. (2011) studied the performance of an A/F/M system in Spain in terms of *E. coli*, *Cryptosporidium* oocyst, *Giardia* cyst, and helminth egg reduction with the goal to improve design, operation and maintenance of waste stabilization pond systems. The system had an average influent flowrate of 3,200 m³/d with a theoretical hydraulic retention time of only 5.5 d, and was monitored weekly from December 2003 to September 2004, with results divided for into cold (T_{influent} = 13.1°C) and hot periods (T_{influent} = 20.1°C). The results are presented in Figure 26.

Mean *E. coli* influent concentrations ranged from 2.0 x 10⁵ CFU/100mL in the cold period to 3.16 x 10⁶ CFU/100mL in the hot period, and final effluent concentrations of 251 to 31.6 CFU/100mL in the cold and hot temperature periods respectively. These results gave a 2.90 log₁₀ reduction for the cold period and a 5.00 log₁₀ reduction for the hot period and is one of the few studies demonstrating the effect of season on *E. coli* reduction; the authors monitored other bacterial and viral indicators (total coliforms, faecal streptococci, and

coliphages) but only found a seasonal variation with *E. coli* (Reinoso et al., 2011). *Cryptosporidium* oocysts had mean influent and effluent values of 14.9 and 0.4 oocysts/L, giving a 1.57 log₁₀ reduction, while *Giardia* influent and effluent concentrations averaged 67.1 and 0.7 cysts/L, for a 1.98 log₁₀ reduction. Helminth egg influent concentrations were low, with a mean of only 1.8 eggs/L, and no eggs were detected in the final effluent.

In a similar A/F/M in Morocco with a mean flowrate of 2,000 m³/d, Chaoua et al. (2018) found mean influent helminth egg concentrations of 95.01 eggs/L and a final effluent concentration of 5.0 eggs/L, for a 1.28 log₁₀ reduction; unfortunately the authors did not report the theoretical hydraulic retention time of the system or provide any details as to its performance in other conventional water quality parameters such as BOD and TSS.

^a*E. coli*; ^bProtozoa and helminths; ^cReported as 0 by the authors; ^dDry weight basis

Figure 26. *E. coli*, protozoan (oo)cyst, and helminth egg reduction and survival in effluent and sludges at a wastewater stabilization pond system in northwestern Spain (Reinoso et al., 2011). *E. coli* influent and effluent ranges are geometric mean values from cold ($T_{\text{water}} = 13.1^{\circ}\text{C}$) and hot ($T_{\text{water}} = 20.1^{\circ}\text{C}$) periods as reported by the authors; *E. coli* log₁₀ reductions are for the cold and hot periods. Helminth egg concentrations were not detected in effluent samples but the detection limit was not reported by the authors and total log₁₀ reduction could not be calculated.

Figure 27 presents the results of two A/F/M pond systems in Bolivia that were abandoned by the responsible municipality but are still in operation, with 100% of the effluents being directly reused for agricultural irrigation during the dry season (Verbyla et al., 2016). One system was an anaerobic pond followed by a facultative and two maturation ponds in series (referred to here as A/F/M1/M2), and the other an A/F/M pond system. The A/F/M1/M2 system had an average flowrate of 750 m³/d, and because of sludge buildup in all the ponds, it was estimated the HRT was approximately 1.0 d during the period of the study; the A/F/M system had an average flowrate of 2,730 m³/d and an estimated HRT of 2.5 d due to sludge buildup (the pond had not been desludged since its construction in 1995), poor construction, and possible modification for diversion to irrigated fields (Verbyla et al., 2016).

Grab samples were collected the influent and final effluent of both systems for 2 months in 2012 and 3 months in 2013 and analyzed for *E. coli*, *Giardia*, *Cryptosporidium*, and helminth eggs (Verbyla et al., 2016). The data presented in Figure 23 show the log₁₀ reductions are minimal for both systems, with effluent concentrations ranging $1.3 \times 10^6 - 2.1 \times 10^7$ CFU/L for *E. coli*, 14 – 2,530 cysts/L for *Giardia*, 0.4 – 11 oocysts/L for *Cryptosporidium*, 31 – 350 eggs/L for helminth eggs. The authors conclude that the wastewater stabilization pond systems may pose a public health risk to farmers using the effluents without further treatment or on-farm management. They also conclude the waste stabilization pond systems, which are the simplest and most cost-effective technologies for developing countries, cannot produce effluents that can be safely reused in agriculture unless they receive adequate operation and maintenance or additional on-farm management techniques to reduce risk to workers and consumers (Verbyla et al., 2016).

Figure 27. *E. coli*, *Giardia*, *Cryptosporidium*, and helminth egg reduction and persistence in effluent from two wastewater stabilization pond systems in Bolivia. (Verbyla et al., 2016). Both systems were very poorly maintained, which caused excessive sludge buildup and extensive hydraulic short-circuiting. As a result, high concentrations of pathogens were found in final effluents. Influent and effluent concentrations are ranges of analyzed data. A/F/M₁/M₂ = anaerobic followed by a facultative and two maturation ponds in series. A/F/M = anaerobic followed by a facultative and maturation pond in series.

3.2 UASB Followed by Waste Stabilization Ponds in Series

UASB reactors (see chapter titled *Anaerobic Sludge Blanket Reactors*) are one of the important natural system technologies that utilize anaerobic processes to degrade organic matter without energy input while producing methane as an alternative source of energy, and they have been widely implemented for domestic wastewater treatment in the last 20 years in countries such as India and Brazil (Arceivala and Asolekar, 2007; Chernicharo, 2007; Mendonça and Mendonça, 2016). UASB effluents, however, require post-treatment to further reduce pathogens and organic matter, and the most common post-

treatment sanitation technology consists of waste stabilization ponds in series (Arceivala and Asolekar, 2007; Chernicharo, 2007).

The term "polishing pond" is frequently used in the literature for UASB-pond systems and to avoid confusion in the discussion below the first pond following the UASB will be termed a secondary facultative pond (referred to here as F2) (Mara, 2003; von Sperling, 2007), which is followed by maturation ponds in series (e.g., referred to as M1, M2, etc.).

3.2.1 UASB reactor followed by a secondary facultative pond in series (UASB/F2)

Tyagi et al. (2011) report on fecal coliform and helminth egg reduction for a UASB/F2 system in Morocco (Figure 28). The system had an average flowrate of 38,000 m³/d and a total hydraulic retention time of only 1.3 d, and used baffles in the secondary facultative pond. Fecal coliform mean influent concentrations were 3.1 x 10⁶ MPN/100mL with mean effluent concentrations of 1.1 x 10⁴ MPN/100mL, giving a 2.45 log₁₀ reduction. Mean influent and effluent helminth egg concentrations were 46.4 eggs/L and 0.13 eggs/L respectively, yielding a mean log₁₀ reduction of 2.55.

Helminth egg reduction data from two full size UASB/F2 systems in Brazil were also reported by von Sperling et al. (2005). The mean total HRT was 4.7 d in one system and 20.9 d in the other. Helminth egg influent data were not reported, and effluent egg concentrations were only detected in one system (high of 1.3 eggs/L) that had a mean effluent concentration of 0.2 eggs/L; helminth eggs were never detected in the final effluent of the other UASB/F2 (von Sperling et al., 2005). The authors conclude that the final effluent mean values of both treatment plants easily met the 1989 WHO guidelines for helminth eggs (<1 egg/L).

^aFecal coliforms; ^bHelminth eggs; ^cThe high range value of 1.1E+04 appears to be in error since it is the same as the mean but was published as such.

Figure 28. Fecal coliform and helminth egg reduction and persistence at a full scale UASB/F₂ system in Morocco (Tyagi et al., 2011). Values are geometric means for fecal coliforms and arithmetic for helminth eggs with ranges in parentheses.

3.2.2 UASB reactor followed by a secondary facultative and maturation pond in series (UASB/F2/M)

Figure 29 shows the pathogen reduction results for a UASB/F2/M system in Bolivia that was monitored for enterovirus, norovirus, rotavirus, *Giardia*, *Cryptosporidium*, and helminth eggs (*Taenia*, *Ascaris*, *Trichuris*, Hookworm) during sampling events in June 2011 and 2012 (Symonds et al., 2014; Verbyla et al., 2013a). The system was poorly maintained, with the measured flowrate increasing from 43 m³/d in 2007 to 124 m³/d in 2012 (Verbyla et al., 2013a).

Two composite samples taken on different days were analyzed for enteric viruses: mean enterovirus concentrations were 6.2 x 10⁴ IU/L in the influent and 9.1 x 10³ IU/L in the effluent, giving a log₁₀ reduction of 0.83; norovirus concentrations did not decrease from influent to effluent; and rotavirus influent concentrations were approximately 1.0 x 10⁵ copies/L and effluent

concentrations approximately 1 x 10⁴ copies/L, giving a 1.0 log₁₀ reduction (Symonds et al., 2014). *Giardia* and *Cryptosporidium* were monitored in one composite sample in June 2012, with mean influent and effluent concentrations of 2.4 x 10² cysts/L and 7.3 x 10¹ cysts/L respectively for *Giardia*, giving a 0.52 log₁₀ reduction; mean influent and effluent *Cryptosporidium* oocyst concentrations were 9.5 and 6.5 oocysts/L, for a 0.16 log₁₀ reduction. Helminth eggs were measured twice in composite samples taken in June 2011 and 2012; *Taenia*, *Ascaris*, *Trichuris* and hookworm were detected in the influent with a mean of 1,809 eggs/L, and only *Taenia* and *Ascaris* were detected in one effluent sample at approximately 1,200 eggs/L. Importantly, 236 helminth eggs/g TS were detected in the UASB sludge, with 33% viability (i.e., 78.7 eggs/g TS) (Verbyla et al., 2013a). The authors conclude that although the system effluent was not being used for irrigation, wastewater reuse is common in Bolivia and the effluent poses a health risk if it were to be reused in agriculture without further treatment or on-farm practices to further reduce risk from pathogen exposure (Symonds et al., 2014; Verbyla et al., 2013a).

^aEnterovirus units in infectious units/L; ^bGC: Gene Copy. Norovirus and rotavirus concentrations were measured by the RT-qPCR molecular method and not by cell culture, thus concentrations reported in effluents may underestimate reduction; ^cHelminth eggs (*Taenia* and *Ascaris*) were detected in one of two effluent composite samples; ^dDry weight basis.

Figure 29. Reduction and persistence of enterovirus, norovirus, rotavirus, *Giardia*, *Cryptosporidium* and helminth eggs from a UASB-maturation pond system in Bolivia. Data are average values from composite samples taken on different days for viruses (June 2012), twice for helminth eggs (June 2011 and 2012), and once for protozoa (June 2012). Data from Symonds et al., 2014; Verbyla et al., 2013a.

3.2.3 UASB followed by a secondary facultative and two maturation ponds in series (UASB/F2/M1/M2)

Días et al. (2014) report on the 10-year monitoring results for a UASB/F2/M1/M2 system in Brazil serving 250 population equivalents (Figure 30). Hydraulic retention times in each pond varied from 1.4 – 6.1 days during the study. Throughout the 10-year monitoring period geometric mean *E. coli* concentrations were 2.46×10^8 MPN/100mL in the influent and 4.50×10^2 MPN/100mL in the final effluent, yielding a 5.74 log₁₀ reduction. Helminth egg concentrations were never

detected in the final effluent (detection limit was not reported) and influent concentrations were not reported; ranges of helminth egg concentrations in raw wastewater and secondary facultative pond sludge reported at treatment plants near this site are presented in Figure 30. Assuming a detection limit of 1 egg/L, the log₁₀ reduction of helminth eggs could range from 1.41 – 2.40 for influent concentrations of 254 and 26 eggs/L respectively. The authors conclude that this system has maintained excellent results for 10 years and meets the WHO guidelines for unrestricted irrigation (Días et al., 2014).

^aData are from other UASB/F2 studies near this site (von Sperling et al., 2003, 2005); ^bEffluent values reported as 0 by the authors; ^cDry weight basis.

Figure 30. Reduction and survival of *E. coli* and helminth eggs from a UASB/F₂/M₁/M₂ system in Brazil. *E. coli* data are geometric mean values from 10 years of monitoring the system shown: a UASB followed by three ponds in series with a rock filter in the third pond (Días et al., 2014). Helminth egg influent data were not reported and eggs were never detected in the final effluent. Range of helminth egg concentrations in influent and facultative pond sludge are from other studies in Brazil near this site (von Sperling et al., 2003, 2005).

3.3 Summary of Pathogen Reduction and Survival in Natural Wastewater Treatment Systems

Tables 4a, 4b, and 4c and Tables 5a, 5b, and 5c present

a summary of different pathogen reduction and survival in the natural wastewater treatment systems discussed in this chapter. Tables 4a to 4c presents the data from systems that are assumed to be well designed, operated and maintained from the descriptions in the literature. Tables 5a to 5c presents the data from systems known to be improperly designed, overloaded, or lacking adequate operation and maintenance.

Table 4a. Summary of pathogen reduction and survival in several properly functioning^a natural wastewater treatment system configurations. Number of systems in parentheses. It can be assumed that all produced sludges will contain pathogens where not noted.

Bacteria	Process	Influent (MPN/L)	Effluent (MPN/L)	Log ₁₀ Reduction
Fecal Coliforms	Waste Stabilization Ponds: F/M1/M2 (2)	3.0E+08 to 1.6E+11	7.5E+02 to 1.1E+06	4.89 to 5.14
Fecal Coliforms	UASB-Waste Stabilization Ponds: UASB/F2 (2)	3.9E+06 to 2.3E+08	2.3E+04 to 1.1E+05	2.45
<i>E. coli</i>	Waste Stabilization Ponds: F/M (3)	1.2E+07 to 3.9E+09	2.7E+03 to 2.1E+05	3.02 to 4.84
<i>E. coli</i>	Waste Stabilization Ponds: F/M1/M2/M3 (1)	1.1E+06 to 1.2E+08	2.1E+03 to 2.3E+05	3.00
<i>E. coli</i>	Waste Stabilization Ponds: A/F/M(2)	2.0E+06 to 3.2E+07	3.2E+01 to 2.5E+02	2.90 to 5.00
<i>E. coli</i>	UASB-Waste Stabilization Ponds: UASB/F2/M1/M2 (1)	2.5E+09	4.5E+02	5.74
<i>Vibrio cholerae</i> 01	Waste Stabilization Ponds: F/M1/M2 (2)	3.1E+03 to 2.4E+05	0.3 to 9.0	4.26
<i>Salmonella</i>	Waste Stabilization Ponds: F/M1/M2/M3 (1)	3.8E+03 to 6.0E+03	1.8E+02 to 5.4E+02	1.24

^aProperly functioning in terms of design, loading rates, or operation and maintenance

Table 4b. Summary of protozoan pathogen reduction and survival in several properly functioning^a natural wastewater treatment system configurations. Number of systems in parentheses. It can be assumed that all produced sludges will contain pathogens where not noted.

Protozoa	Process	Influent	Effluent	Log ₁₀ Reduction	Final Sludge, Cysts or Oocysts/g TS ^e
<i>E. coli</i> , <i>E. histolytica</i> , <i>Giardia</i>	Waste Stabilization Ponds: A/F/M(2)	4.0 to 3.3E+02 ^b	ND ^c	>1.74	1.20E+02 ^f
<i>Giardia</i>	Waste Stabilization Ponds: A/F/M(2)	5.3 to 4.01E+01 ^b	ND to 1.4	1.57	6.8E+02 ^f
<i>Cryptosporidium</i>	Waste Stabilization Ponds: A/F/M(2)	1.75E+01 to 2.17E+02 ^d	ND to 1.4	1.98	1.1E+02 ^g

^aProperly functioning in terms of design, loading rates, or operation and maintenance; ^bCysts/L; ^cND: Non-detect; ^dOocysts/L; ^eTS: Total Solids; ^fCysts/g TS; ^gOocysts/g TS

Table 4c. Summary of helminth egg reduction and survival in several properly functioning^a natural wastewater treatment system configurations. Number of systems in parentheses. It can be assumed that all produced sludges will contain pathogens where not noted.

Process	Influent (Eggs/L)	Effluent (Eggs/L)	Log ₁₀ Reduction	Final Sludge (Eggs/g TS ^b)	% Viable Eggs
Waste Stabilization Ponds: F/M (3)	3 to 4.8E+01	ND ^c	NR ^d	6 to 3.54E+02	NR
Waste Stabilization Ponds: A/F/M(2)	2.6E+01	<1	>1.41	NR	NR
Waste Stabilization Ponds: F/M1/M2/M3 (1)	1.8E+01 to 8.8E+01	ND to 1.0	2.48	NR	NR
Waste Stabilization Ponds: A/F/M(2)	5 to 3.6E+01	ND	>2.89	2.84E+01 to 5.56E+02	36.0
UASB-Waste Stabilization Ponds: UASB/F2 (2)	2.1E+01 to 2.54E+02	ND to 1.5	>1.79 to 2.55	1.0E+01 to 1.058E+03 ^e	44 to 100 ^e
UASB-Waste Stabilization Ponds: UASB/F2/M1/M2 (1)	NR	ND	NR	NR	NR

^aProperly functioning in terms of design, loading rates, or operation and maintenance; ^bTS: Total Solids; ^cND: Non-detect; ^dNR: Not Reported; ^eSamples taken at different locations in pond.

Table 5a. Summary of *E. coli* reduction and survival in several poorly functioning^a natural wastewater treatment system configurations. Number of systems in parentheses.

Process	Influent (CFU/L)	Effluent (CFU/L)	Log ₁₀ Reduction
Waste Stabilization Ponds: F/M (4)	1.2E+08 to 9.0E+09	1.3E+06 to 1.2E+08	1.87 to 1.99
Waste Stabilization Ponds: A/F/M(2)	3.0E+05 to 6.2E+08	1.3E+06 to 2.1E+07	0.7 to 1.1

^aPoorly functioning as a result of design, overloading or lack of operation and maintenance.

Table 5b. Summary of viral pathogen reduction and survival in several poorly functioning^a natural wastewater treatment system configurations. Number of systems in parentheses.

Virus	Process	Influent	Effluent	Log ₁₀ Reduction	Final Sludge
Enterovirus	Waste Stabilization Ponds: F/Ma/M2 (1)	4.2E+04 ^b	3.7E+01 ^b	3.06	Contaminated
Enterovirus	UASB-WSP: UASB/F2/M (1)	6.2E+04 ^b	9.1E+03 ^b	0.83	Contaminated
Norovirus GI ^d	Waste Stabilization Ponds: F/M1/M2 (1)	1.0E+06 ^c	1.0E+06 ^c	0	Contaminated
Norovirus GI ^d	UASB-WSP: UASB/F2/M (1)	1.0E+04 ^c	5.0E+05 ^c	0	Contaminated
Rotavirus ^d	Waste Stabilization Ponds: F/Ma/M2 (1)	1.0E+06 ^c	1.0E+06 ^c	0	Contaminated
Rotavirus ^d	UASB-WSP: UASB/F2/M (1)	1.0E+05 ^c	1.0E+04 ^c	1.0	Contaminated

^aPoorly functioning as a result of design, overloading or lack of operation and maintenance; ^bInfectious Units/L (IU/L); ^cCopies/L; ^dVirus concentrations were measured by the RT-qPCR molecular method and not by cell culture. The RT-qPCR method identifies specific viruses but they do not have to be intact or infective to be detected (Francy et al., 2011) and log₁₀ reduction may be underestimated.

Table 5c. Summary of protozoan pathogen reduction and survival in several poorly functioning^a natural wastewater treatment system configurations. Number of systems in parentheses.

Protozoa	Process	Influent	Effluent	Log ₁₀ Reduction	Final Sludge
<i>Giardia</i> cysts	Waste Stabilization Ponds: F/M1/M2 (1)	1.6E+02 ^b	2.3E+01 ^b	0.84	Contaminated
<i>Giardia</i> cysts	Waste Stabilization Ponds: A/F/M (2)	2.9E+02 to 3.8E+03 ^b	1.4E+01 to 2.5E+03 ^b	0.5 to 1.5	Contaminated
<i>Giardia</i> cysts	UASB-WSP: UASB/F2/M (1)	2.4E+02 ^b	7.3E+01 ^b	0.52	Contaminated
<i>Cryptosporidium</i> oocysts	Waste Stabilization Ponds: F/M1/M2 (1)	6.0 ^c	4.0 ^c	0.18	Contaminated
<i>Cryptosporidium</i> oocysts	Waste Stabilization Ponds: A/F/M (2)	4.0 to 1.64E+02 ^c	0.4 to 1.1E+01 ^c	0.7 to 0.8	Contaminated
<i>Cryptosporidium</i> oocysts	UASB-WSP: UASB/F2/M (1)	9.5 ^c	6.5 ^c	0.16	Contaminated

^aPoorly functioning as a result of design, overloading or lack of operation and maintenance; ^bCysts/L; ^cOocysts/L

4.0 Conclusions

Tables 6, 8a, and 8b summarize the log₁₀ reductions for the cited mechanized and natural wastewater sanitation systems in comparison to the theoretical reductions presented in the WHO guidelines for wastewater use in agriculture, which cite Feachem et al. (1983) as one of the key references (WHO, 2006).

4.1 Mechanized Wastewater Treatment Systems (Table 6)

The results in Table 6 for enterovirus show that the low end of log₁₀ reduction ranges for activated sludge with the tertiary processes of sand filtration/disinfection (2.85 log₁₀) is only slightly higher than the high range reported for conventional activated sludge without disinfection (2.44 log₁₀). Furthermore, reduction of Aichi virus is very low at 0.94 and 0.99 log₁₀ reduction compared to norovirus for activated sludge and trickling filter plants with disinfection.

Virus reduction in all disinfection and tertiary processes reported does not approach the 5 - 6 log₁₀ reduction cited by WHO (2006).

Giardia and *Cryptosporidium* (oo)cyst reduction is low in activated sludge and trickling filter plants with chlorine disinfection as would be expected, and the log₁₀ reduction is in the same range as activated sludge plants without disinfection. The tertiary treatment processes generally performed better, and (oo)cysts were never detected in the final effluent of the system with ultrafiltration; the low range values for sand filtration/disinfection, however, are similar to activated sludge without disinfection (1.40 and 0.46 log₁₀ reduction for *Giardia* and *Cryptosporidium* respectively). The protozoan (oo)cyst data also show that reduction can vary greatly among different pathogens, with, for example, in the study by Ben Ayed et al. (2009) in four activated sludge plants, mean log₁₀ reduction of *Entamoeba coli* was more than double that of *Entamoeba histolytica* and *Giardia* (previously shown in Figure 6).

Table 6. Summary of pathogen and indicator reduction in conventional activated sludge with and without chlorine disinfection, a conventional trickling filter with chlorine disinfection, and activated sludge with tertiary processes. The range of pathogen group reduction (bacteria, viruses, protozoa, helminths) reported by WHO (2006) is shown for comparison

Pathogen/ Indicator	Log ₁₀ Reduction					
	Conventional Activated Sludge ^c		Trickling Filter	Activated Sludge with Tertiary Processes ^c		
	No Disinfection	Chlorine Disinfection	Chlorine Disinfection	Coagulation/ Flocculation Sedimentation/ Filtration	Sand Filtration Disinfection	Ultrafiltration Disinfection
Viruses						
Enterovirus	1.85 to 2.44	NR	NR	NR	2.85 to 4.33	NR
Adenovirus ^a	1.77	NR	NR	NR	NR	NR
Norovirus GI & GII ^a	0.80 to 0.92	1.65 to 2.14	2.57 to 2.85	NR	NR	NR
Aichi virus ^a	NR	0.94	0.99	NR	NR	NR
WHO (2006)	0 to 2	1 to 5	1 to 5	2 to >6	2 to >6	3..5 to >6
Bacteria						
<i>E. coli</i> /Fecal coliforms	1.49 to 3.10	≈4 to 6 ^b	≈4 to 6 ^b	NR	NR	NR
WHO (2006)	1 to 2	3 to >6	3 to >6	1 to 5	1 to >6	4.5 to >6
Protozoa						
Giardia cysts	1.23 to 2.67	0.62 to 2.13	1.52	3.46	1.40 to 3.87	>4.44
<i>Cryptosporidium</i> oocysts	0.35 to 1.92	0.62 to 1.80	0.81	3.34	0.46 to 3.00	>3.63
<i>Entamoeba coli</i> cysts	1.15 to 1.40	NR	NR	NR	NR	NR
<i>Entamoeba histolytica</i> cysts	0.30 to 0.66	NR	NR	NR	NR	NR
WHO (2006)	0 to 1	0 to 2.5	0 to 2.5	1 to 6	0 to 5.5	>6
Helminths						
Helminth eggs	0.81 to ≤2.40	NR	NR	NR	NR	NR
WHO (2006)	1 to <2	1 to <2	1 to <2	3 to >3	2 to 3	>3

NR: Not Reported; ^aVirus concentrations were measured by the qPCR molecular method and not by cell culture. The qPCR method identifies specific viruses but they do not have to be intact or infective to be detected (Francy et al., 2011) and log₁₀ reduction may be underestimated; ^bLog₁₀ reductions are estimated from effluent data presented by Kitajima et al. (2014a) (Figures 8 & 9); ^cIt is assumed all treatment produced sludges contain concentrations of pathogens potentially posing health risks.

Emphasis on bacterial indicator reduction, which is a common regulatory requirement worldwide, can mask the low reduction of pathogens in treatment plants with

disinfection and tertiary processes. The results from the study of Kitajima et al. (2014a) (previously summarized in Figures 8 and 9) are illustrative on this point and are shown in Table 7.

Table 7. Table showing how bacterial indicator removal is typically much greater than pathogen removal in mechanical wastewater treatment. Data from Kitajima et al., 2014a.

Conventional Water Quality Indicator or Pathogen	Activated Sludge w/Disinfection	Trickling Filter w/Disinfection
Effluent BOD, mg/L	11 to 28	8 to 27
Effluent TSS, mg/L	4 to 26	9 to 25
<i>E. coli</i>		
Effluent, MPN/100mL	<1 to 130	7 to 220
log ₁₀ Reduction ^a	≈4 to 6	≈4 to 6
<i>Giardia</i>		
Effluent, cysts/L	<4 to 150	56 to 620
log ₁₀ Reduction	2.08	1.52
<i>Cryptosporidium</i>		
Effluent, oocysts/L	<4 to 36	<4 to 40
log ₁₀ Reduction	0.71	0.81

^aInfluent concentrations were not reported and log₁₀ reductions are estimated from typical influent concentration ranges (10⁶ to 10⁷ MPN/100ml)

The information above shows the two treatment plants (employing activated sludge and trickling filters) in Arizona (U.S.) met their discharge requirements for BOD and TSS and chlorinated the final effluent to meet *E. coli* regulations. However, they never monitored for protozoan (oo)cyst pathogens in the final effluent (Kitajima et al., 2014a) and one can see from the information above the log₁₀ reduction of protozoan (oo)cyst pathogens is not near that what was observed for *E. coli*. With the exception of the activated sludge plant with ultrafiltration in Table 8 (shown in Figure 18), every other mechanical system, including activated sludge and trickling filters with disinfection, and activated sludge with disinfection and various tertiary processes (coagulation/flocculation/sedimentation/sand filtration), had measurable concentrations of pathogens in the final effluent. While effluent concentrations in developed countries may often be low, this is not the case in developing countries where they can be quite high, even in a well-functioning plant, as shown in the study in Tunisia by Ben Ayed et al. (2009) (previously summarized in Figure 6).

Well-operated activated sludge plants meeting their bacterial effluent requirements with chlorine disinfection, however, may still contribute to public health risks in

developed countries. The available evidence suggests that the oocyst source for the 1993 Milwaukee (U.S.) outbreak of cryptosporidiosis, which infected more than 400,000 persons, was of human origin from the Jones Island Wastewater Treatment Plant. At the time of the outbreak, it was a conventional activated sludge plant with chlorine disinfection that discharged its effluent into Lake Michigan, which was the source water for the city's drinking water supply (Zhou et al., 2003; Eisenberg et al., 2005).

As was discussed previously and shown in Figures 14 and 16, the major unit process for pathogen reduction may often be the activated sludge process itself upstream from tertiary processes. For tertiary processes to have a more significant effect on pathogen reduction they must be well-designed and operated, which is likely the case with the high range log₁₀ reduction values shown in Table 8. It is evident, however, that full-scale activated sludge plants without disinfection and tertiary processes can have log₁₀ pathogen reductions similar to some plants using tertiary processes, and tertiary processes are not necessarily a guarantee of increased pathogen reduction under field conditions of otherwise well-functioning plants (in terms of BOD and TSS removal) as shown in Table 7 data from Kitajima et al. (2014a).

Table 8a. Summary of pathogen and indicator log₁₀ reduction in properly functioning^a natural wastewater treatment system configurations. Number of systems in parentheses. The range of pathogen group reduction (bacteria, viruses, protozoa, helminths) reported by WHO (2006) is shown for comparison. It is assumed all treatment produced sludges contain concentrations of pathogens potentially posing health risks.

		Log ₁₀ Reduction					
Pathogen Group	Pathogen/ Indicator	Waste Stabilization Ponds				UASB-Waste Stabilization Ponds	
		F/M (3)	F/M1/M2(2)	F/M1/M2/M3 (1)	A/F/M(2)	USAB/F2(2)	USAB/F2/M1/M2 (1)
Bacteria	Fecal Coliforms	NR	4.89 to 5.14	3.00	NR	2.45	NR
	<i>E. coli</i>	3.02 to 4.84	NR	NR	2.90 to 5.00	NR	5.74
	<i>Vibrio cholerae</i> 01	NR	4.26	NR	NR	NR	NR
	<i>Salmonella</i>	NR	NR	1.24	NR	NR	NR
	WHO (2006)	1 to 6	1 to 6	1 to 6	1 to 6	≥1.5 ^b	1 to 6
Protozoa	<i>E. coli</i> , <i>E. histolytica</i> , <i>Giardia</i>	NR	NR	NR	>1.74	NR	NR
	<i>Giardia</i>	NR	NR	NR	1.57	NR	NR
	<i>Cryptosporidium</i>	NR	NR	NR	1.98	NR	NR
	WHO (2006)	1 to 4	1 to 4	1 to 4	1 to 4	≥1.0 ^b	1 to 4
Helminths	Helminth eggs	NR	>1.41	2.48	>2.89	>1.79 – 2.55	NR
	WHO (2006)	1 to 3	1 to 3	1 to 3	1 to 3	>0.5 to >1 ^b	1 to 3

^aProperly functioning in terms of design, loading rates, or operation and maintenance; ^bEstimated from WHO (2006) data for only one pond following a UASB.

Table 8b. Summary of pathogen and indicator log₁₀ reduction in poorly functioning^a natural wastewater treatment system configurations. Number of systems in parentheses. The range of pathogen group reduction (bacteria, viruses, protozoa, helminths) reported by WHO (2006) is shown for comparison^b.

Pathogen Group	Pathogen/Indicator	Log ₁₀ Reduction			
		Waste Stabilization Ponds			UASB-WSP
		F/M (4)	F/M1/M2 (1)	A/F/M (2)	UASB/F2/M (1)
Bacteria	<i>E. coli</i>	1.87 to 1.99	NR	0.7 to 1.1	NR
	WHO (2006)	1 to 6	1 to 6	1 to 6	1 to 6
	Enterovirus	NR	3.06	NR	0.83
Viruses	Norovirus GI ^b	NR	0	NR	0
	Rotavirus ^b	NR	0	NR	1
	WHO (2006)	1 to 4	1 to 4	1 to 4	1 to 4
Protozoa	<i>Giardia</i> cysts	NR	0.84	0.5 to 1.5	0.52
	<i>Cryptosporidium</i> oocysts	NR	0.18	0.7 to 0.8	0.16
	WHO (2006)	1 to 4	1 to 4	1 to 4	1 to 4
Helminths	Helminth eggs	NR	≤1.82	0.5 to 0.6	NR
	WHO (2006)	1 to 3	1 to 3	1 to 3	1 to 3

^aPoorly functioning as a result of design, overloading or lack of operation and maintenance; ^bIt is assumed all treatment produced sludges contain concentrations of pathogens potentially posing health risks. ^cVirus concentrations were measured by the RT-qPCR molecular method and not by cell culture. The RT-qPCR method identifies specific viruses but they do not have to be intact or infective to be detected (Francy et al., 2011) and log₁₀ reduction may be underestimated.

4.2 Natural Wastewater Treatment Systems

The summary log₁₀ reduction results in Table 8a show that properly functioning full-scale waste stabilization pond systems and UASB/waste stabilization pond systems can remove ≥2.45 log₁₀ bacterial indicators up to 5.74 log₁₀, and that one three-pond system removed 4.26 log₁₀ *Vibrio cholerae* 01, which correlated well with a 4.89 log₁₀ reduction of fecal coliforms (Figure 20). A four pond

system, however, removed 3.00 log₁₀ *E. coli*, but only reduced *Salmonella* by a mean 1.24 log₁₀ during the same monitoring period (Figure 23).

For protozoa (oo)cyst reduction, a three pond system removed 2.90 log₁₀ *E. coli* in the colder season and 5.00 log₁₀ in the hotter season, but *Giardia* and *Cryptosporidium* were only reduced 1.57 and 1.98 log₁₀ respectively. While one system approached the 6 log₁₀ reduction for bacterial

pathogens/indicators needed for agriculture reuse as reported by WHO (2006), most were below it in the range 2.90 – 5.00; the reported protozoan (oo)cyst reductions in the cited studies were all <2.00 log₁₀, well below the maximum 4.0 log₁₀ reported by WHO (2006) for agricultural reuse. Helminth egg reduction was sufficient in all systems to reduce effluent concentrations below the limit of detection or < 1 egg/L if the detection limit was reported.

With the exception of helminth eggs and protozoan

(oo)cysts in one system, almost all well-performing natural systems had measurable concentrations of pathogens in the final effluent. The problem of differential pathogen reduction in relation to indicators and other pathogens has important implications also for apparently well-performing natural systems as with mechanized ones. Table 9 shows the results for reduction of indicators and pathogens in three different waste stabilization pond systems shown in Table 8a.

Table 9. Expected log₁₀ reduction of indicators and pathogens in three different waste stabilization ponds.

Pathogen/Indicator	Log ₁₀ Reduction
Fecal coliforms	4.89
<i>Vibrio cholerae</i> 01	4.26
Fecal coliforms	3.00
<i>Salmonella</i>	1.24
<i>E. coli</i>	2.90 to 5.00
<i>Giardia</i>	1.57
<i>Cryptosporidium</i>	1.98

Table 9 shows that while the reduction in fecal coliforms and *E. coli* is relatively high, the log₁₀ reduction for *Salmonella*, *Giardia*, and *Cryptosporidium* is not, and one cannot assume the effluent would be safe in terms of pathogens if the effluent were to be reused, for example, in agriculture. Even the 4.26 log₁₀ reduction of *Vibrio cholerae* 01, which was achieved with a well-performing F/M1/M2 pond system with a hydraulic retention time of 50 days, would have had an effluent with a concentration of 23.5 MPN/100mL if the influent had entered with the concentration at the peak of the epidemic of 4.3 x 10⁵ MPN/100mL *Vibrio cholerae* 01.

Unlike mechanized wastewater treatment systems, whose processes can be continuously modified and controlled during routine operation, there is very little operational control of the process in natural wastewater treatment systems after they are constructed. Table 8b shows the results for full-scale waste stabilization pond systems and UASB/waste stabilization pond systems that are poorly functioning with low log₁₀ reductions and significant concentrations of measured pathogens in the final effluent, which for several systems was being used for agricultural reuse (Verbyla et al., 2016). Unfortunately, improper design, overloading, and inadequate operation and maintenance of natural wastewater treatment systems in developing countries is very common. For example, a recent study by the National Administration for Water in

Peru estimates that only 15.8% of all treated wastewater in Peru meets effluent quality levels that protects public health in terms of thermotolerant coliforms effluent concentrations (ANA, 2017).

Curtis (2003) has stated that "recreational and wastewater reuse standards assume that the pathogens occur at much lower densities than the indicator and that the reductions of the indicator by 3 – 4 logs results in the elimination of bacterial pathogens." This concept is commonly applied in the U.S. and has also been applied to viral and protozoan pathogens in the WHO guidelines for wastewater reuse in agriculture, which recommends using *E. coli* for verification monitoring in lieu of monitoring specific pathogens, which is much more difficult and likely not possible in much of the developing world (WHO, 2006). This strategy should be revisited in light of recent work showing the reduction and survival of pathogens in various treatment processes around the world: Different pathogens can exhibit vastly different log₁₀ reductions in both natural and mechanized wastewater treatment systems. One recommended strategy would be to focus on pathogens that are a local or regional public health concern, such as parasitic infections with protozoa and helminths instead of bacterial or viral pathogens, in the management of wastewater treatment systems where agricultural reuse is an important concern (Verbyla et al., 2015). This strategy can apply as well to mechanized and natural wastewater treatment systems alike.

Appendix A.

The following Appendices (Tables A1.A to A25) present the data that were presented in Figures previously shown in this chapter.

Table A1a. Bacterial pathogen reduction and survival in conventional activated sludge without disinfection

Bacteria	Influent	Effluent	Log ₁₀ Reduction	Final Sludge
Fecal Coliforms ^{a,b}	2.1E+06 to 1.5E+09	1.1E+04 to 9.3E+07	2.22; 3.10 ^f	NR ^g
<i>E. coli</i> ^{a,b}	5.4E+04 to 3.5E+08	3.5E+04 to 1.6E+07	1.49	Contaminated
<i>E. coli</i> ^{c,d}	1.0E+03 to 1.0E+06	1.0E+03 to 1.0E+05	0.30 to 1.00	Contaminated
<i>E. coli</i> ^e	NR	NR	1 to 2	NR

^aSources: Ayed et al., 2009, Flannery et al., 2012, Fong et al., 2010, Ramo et al., 2017, Rose et al., 1996, 2004, Tyagi et al., 2011; ^bMPN/L; ^cSources : Feachem et al., 1981, 1983; ^dCFU/L; ^eSource : WHO, 2006; ^fLog₁₀ reduction shown are two mean values from two activated sludge plants; ^gNR: Not Reported

Table A1b. Viral pathogen reduction and survival in conventional activated sludge without disinfection

Virus	Influent	Effluent	Log ₁₀ Reduction	Final Sludge
Adenovirus ^{a,b,d}	2.6E+05 to 2.8E+06	1.0E+03 to 4.4E+04	1.77	Contaminated
Enterovirus ^{a,c}	4.6E+03 to 9.4E+04	3.0E+01 to 1.3E+03	1.85 to 2.44	Contaminated
Norovirus GI & GII ^{a,b,d}	6.5E+02 to 2.2E+08	1.8E+02 to 1.2E+04	0.80 to 0.92	Contaminated

^aSources: Ayed et al., 2009, Flannery et al., 2012, Fong et al., 2010, Ramo et al., 2017, Rose et al., 1996, 2004, Tyagi et al., 2011; ^bCopies/L; ^cMPN/L; ^dVirus concentrations were measured by the qPCR molecular method and not by cell culture. The qPCR method identifies specific viruses but they do not have to be intact or infective to be detected (Francy et al., 2011) and log₁₀ reduction may be underestimated. Fong et al. (2010) used qPCR for adenovirus although there are routine cell culture methods available; there are no known routine cell culture methods available for viable norovirus.

Table A1c. Protozoan pathogen reduction and survival in conventional activated sludge without disinfection

Protozoa	Influent	Effluent	Log ₁₀ Reduction	Final Sludge	Effluent Cyst/Oocyst Viability %	Sludge Cyst/Oocyst Viability %
<i>E. histolytica</i> ^{a,b}	6.40E+02 to 9.14E+02	1.85E+02 to 4.32E+03	0.30 to 0.66	Contaminated	NR ^f	NR
<i>E. histolytica</i> ^{b,c}	1.0E+01 to 1.0E+04	0.5E+01 to 1.0E+04	0.04 to 0.30	Contaminated	NR	NR
<i>E. histolytica</i> ^d	NR	NR	0 to 1	NR	NR	NR
<i>Giardia</i> cysts ^{a,b}	6.12E+01 to 6.6E+03	0.5 to 4.2E+02	0.34 to 2.67	2.3E+01 to 4.75E+02 ^g	52 to 74	>60
<i>Cryptosporidium</i> oocyst ^{a,e}	6.5 to 4.56E+02	0.28 to 3.0E+02	0.35 to 2.17	2 to 4.4E+01 ^h	61 to 91	>60

^aSources: Ayed et al., 2009, Flannery et al., 2012, Fong et al., 2010, Ramo et al., 2017, Rose et al., 1996, 2004, Tyagi et al., 2011; ^bCysts/L; ^cSources: Feachem et al., 198, Feachem et al., 1983; ^dSource: WHO, 2006; ^eOocysts/L; ^fNR: Not Reported; ^gCysts/g TS; ^hOocysts/g TS

Table A1d. Helminth egg pathogen reduction and survival in conventional activated sludge without disinfection

Influent (Eggs/L)	Effluent (Eggs/L)	Log ₁₀ Reduction	Final Sludge
<2 to 1.04E+03 ^a	<0.7 to 2.4E+02 ^a	0.81 to ≤2.40 ^a	Contaminated
1 to E+03 ^b	0 to E+02 ^b	0.30 to 3.00 ^b	Contaminated
NR	NR	1 to <2 ^c	NR ^d

^aSources: Ayed et al., 2009, Flannery et al., 2012, Fong et al., 2010, Ramo et al., 2017, Rose et al., 1996, 2004, Tyagi et al., 2011; ^bSources: Feachem et al., 1981, 1983; ^cSource: WHO, 2006; ^dNR: Not Reported

Table A2a. Virus pathogen reduction and survival in conventional activated sludge plants with and without chlorine disinfection and a conventional trickling filter plant with disinfection.

Virus	Process	Influent	Effluent	Log ₁₀ Reduction	Final Sludge
Adenovirus ^a	Conventional Activated Sludge no disinfection	2.6E+05 to 2.8E+06 ^b	1.0E+03 to 4.4E+04 ^b	1.77	Assumed Contaminated
Aichi virus ^a	Conventional Activated Sludge with disinfection	9.7E+04 ^b	1.1E+04 ^b	0.94	Assumed Contaminated
Aichi virus ^a	Trickling Filter with disinfection	2.0E+06 ^b	2.0E+05 ^b	0.99	Assumed Contaminated
Enterovirus	Conventional Activated Sludge no disinfection	4.6E+03 to 9.4E+04 ^c	3.0E+01 to 1.3E+03 ^c	1.85 to 2.44	Assumed Contaminated
Norovirus GI & GII ^a	Conventional Activated Sludge no disinfection	6.5E+02 to 2.2E+08 ^b	1.8E+02 to 1.2E+04 ^b	0.80 to 0.92	Assumed Contaminated
Norovirus GI & GII ^a	Conventional Activated Sludge with disinfection	NDd to 6.46E+06 ^b	ND to 1.0E+04 ^b	1.65 to 2.14	Assumed Contaminated
Norovirus GI & GII ^a	Trickling Filter with disinfection	1E+02 to 3.2E+05 ^b	ND to 1.6E+03 ^b	2.57 to 2.85	Assumed Contaminated

^aVirus concentrations were measured by the qPCR molecular method and not by cell culture. The qPCR method identifies specific viruses but they do not have to be intact or infective to be detected (Francy et al., 2011) and log₁₀ reduction may be underestimated. Fong, et al. (2010) used qPCR for adenovirus although there are routine cell culture methods available; there are no known routine cell culture methods available for viable Aichi virus and norovirus.; ^bCopies/L; ^cMPN/L; ^dND: Non-detect; Data from Flannery et al., 2012; Fong et al., 2010; Kitajima et al., 2014a, 2014b; Ramo et al., 2017; Rose et al., 2004.

Table A2b. Protozoa pathogen reduction and survival in conventional activated sludge plants with and without chlorine disinfection and a conventional trickling filter plan with disinfection.

Protozoa	Process	Influent	Effluent	Log ₁₀ Reduction	Final Sludge	Effluent Cyst/Oocyst % Viability	Sludge Cyst/Oocyst % Viability
<i>Giardia</i> cysts	Conventional Activated Sludge no disinfection	6.12E+01 to 6.6E+03 ^a	0.65 to 8.1E+01 ^a	1.23 to 2.67	2.3E+01 to 4.75E+02 ^c	52 to 74	>60
	Conventional Activated Sludge with disinfection	2.2E+01 to 9.9E+03 ^a	<4 to 1.5E+02 ^a	0.62 to 2.13	ND ^b to 5.93E+02 ^c	52 to 74	>60
	Trickling Filter with disinfection	1.4E+03 to 1.5E+04 ^a	5.6E+01 to 6.2E+02 ^a	1.52	ND to 5.93E+02 ^c	NR ^d	NR
	Conventional Activated Sludge no disinfection	6.5 to 4.56E+02 ^e	0.28 to 3.0E+02 ^e	0.35 to 1.92	2 to 4.4E+01 ^f	61 to 91	>60
	Conventional Activated Sludge with disinfection	4.0E+01 to 4.2E+03 ^e	0.3 to 3.0E+02 ^e	0.62 to 1.80	<4 to 3.6E+01 ^f	61 to 91	>60
<i>Cryptosporidium</i> oocysts	Trickling Filter with disinfection	<4.0E+01 to 7.2E+02 ^e	<4 to 4.0E+01 ^e	0.81	Assumed Contaminated	NR	NR

^aCysts/L; ^bND: Non-detect; ^cCysts/g TS; ^dNR: Not Reported; ^eOocysts/L; ^fOocysts/g TS; Data from Flannery et al., 2012; Fong et al., 2010; Kitajima et al., 2014a, 2014b; Ramo et al., 2017; Rose et al., 2004.

Table A3. Adenovirus, norovirus (GI and GII) and *E. coli* reduction at two conventional activated sludge treatment plants without disinfection. Data are mean values with range in parentheses. Oyster tissue concentrations are mean values. (Data from Flannery, et al. 2012; Fong, et al. 2010)

Indicator or Pathogen	Influent/ Input Mean (Range) MPN/100ml GC ^a /L	Effluent Concentrations Post Conventional Treatment No Disinfection Mean (Range) MPN/100ml or /g GC/L or /g	Mean Log ₁₀ Reductions
BACTERIA			
<i>E. coli</i> MPN/100mL	3.47E+06 (5.37E+03 to 3.47E+07)	1.15E+05 (3.47E+03 to 1.58E+06)	1.49
VIRUSES			
Adenovirus GC/L	1.17E+06 (2.63E+05 to 2.82E+06)	2.00E+04 (1.00E+03 to 4.42E+04)	1.77
Norovirus Genotype I GC/L	2.09E+04 (1.12E+03 to 5.75E+05)	3.39E+03 (1.82E+02 to 1.15E+05)	0.8
Norovirus Genotype II GC/L	3.55E+04 (6.46E+02 to 2.19E+06)	4.27E+03 (3.24E+02 to 1.2E+05)	0.92
BACTERIA			
<i>E.coli</i> MPN/100g	Concentration in oysters. Harvested from coastline where secondary effluent was discharged	1.66E+03	NR ^b
VIRUSES			
Norovirus Genotype I GC/g	Concentration in oysters. Harvested from coastline where secondary effluent was discharged	3.39E+03	NR
Norovirus Genotype II GC/g	Concentration in oysters. Harvested from coastline where secondary effluent was discharged	5.37E+03	NR

^aGC; Gene copies, Virus concentrations were measured by the qPCR molecular method and not by cell culture. The qPCR method identifies specific viruses but they do not have to be intact or infective to be detected (Francy et al., 2011) and log₁₀ reduction may be underestimated. Fong et al. (2010) used qPCR for adenovirus although there are routine cell culture methods available; there are no known routine cell culture methods available for viable norovirus; ^bNR: Not Reported

Table A4. *Giardia* and *Cryptosporidium* reductions in effluents and occurrence in sludges from 13 conventional activated sludge plants without disinfection. Eleven systems were extended aeration without primary sedimentation and 2 were conventional activated sludge. Values shown are ranges of arithmetic mean values for the 13 plants. Sampling was done four times per year during different seasons.

Protozoa	Influent/Input Range Cysts or Oocysts/L	Effluent Range Cysts or Oocysts/L	Sludge Range Cysts or Oocysts/g TS ^a	Log ₁₀ Reductions by Secondary Treatment
<i>Giardia</i> Cysts/L or /g TS	7.64E+02 to 6.606E+03	22 to 81	23 to 4.75E+02	1.50 to 2.34
<i>Cryptosporidium</i> Oocysts/L or /g	22 to 4.56E+02	2 to 44	2 to 44	0.35 to 1.63

^aTS: total solids; Data from Ramo et al., 2017

Table A5. Helminth egg and fecal coliform reduction and survival at 2 conventional activated sludge treatment plants without disinfection in India. Influent/effluent ranges are combined from both plants; log₁₀ reductions were calculated from mean influent/effluent concentrations for each plant. Data from Tyagi et al. (2011).

Indicator or Pathogen	Influent/Input Average (Range)	Survival Average (Range)	Log ₁₀ Reductions AS1 AS2
BACTERIA			
Fecal Coliforms (MPN/100ml)	2.1E+05 to 1.5E+08	1.1E+03 to 9.3E+06	2.22 to 3.10
HELMINTHS			
Helminth eggs (eggs/L)	23.4 to 71.2	ND ^a to 4.3	1.40 to 1.55

^aND: Non-detect

Table A6. Pathogen and fecal coliform reduction and survival at 7 conventional activated sludge treatment plants without disinfection in USA. Helminth data are for one system only; all other data are from six treatment systems. Influent/effluent ranges are from geometric means for each treatment plant; range of log₁₀ reductions calculated with geometric mean influent/effluent concentration for each plant.

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions
BACTERIA			
Fecal Coliforms (MPN/100ml)	2.1E+06 to 5.3E+06	2.5E+03 to 1.3E+05	1.62 to 3.11
VIRUSES			
Enterovirus (MPN/100ml)	4.64E+02 to 9.37E+03	3.0 to 1.33E+02	1.85 to 2.44
PROTOZOA			
<i>Giardia</i> (cysts/L)	61.2 to 7.94E+02	0.65 to 8.34	1.23 to 2.67
<i>Cryptosporidium</i> (oocysts/L)	6.5 to 37.8	0.28 to 0.84	1.11 to 1.92
HELMINTHS			
Helminth eggs (eggs/L)	<2 to 1.10 E+02	ND ^a (<2)	<2.40

^aND: Non-detect; Data from Rose et al. (1996, 2004).

Table A7. Protozoan cyst and helminth egg reduction and survival at four conventional activated sludge treatment plants without disinfection in Tunisia. *Taenia* data are for one system only; all other data are from the four treatment systems. Influent/effluent ranges are from mean values for each treatment plant; mean and range of log₁₀ reductions are calculated with geometric mean influent/effluent concentration for each plant.

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions
PROTOZOA (cysts/L)			
<i>Entamoeba coli</i>	1.120E+03 to 1.447E+03	52 to 98	1.28 (1.15 to 1.41)
<i>Entamoeba histolytica</i>	6.40E+02 to 9.14E+02	1.85E+02 to 3.20E+02	0.50 (0.30 to 0.66)
<i>Giardia</i>	7.11E+02 to 8.80E+02	1.36E+02 to 4.00E+02	0.57 (0.34 to 0.75)
HELMINTHS (eggs/L)			
<i>Ascaris</i>	3.31E+02 to 5.69E+02	49 to 80	0.90 (0.78 to 1.07)
<i>Enterobius vermicularis</i>	2.46E+02 to 5.60E+02	9 to 80	1.13 (0.85 to 1.41)
<i>Taenia</i>	74	17	0.64
Total helminth eggs	NR ^a	NR	0.95 (0.81 to 1.11)

^aNR: Not Reported; Data from Ben Ayed et al., 2009.

Table A8. *Giardia* and *Cryptosporidium* reduction in effluents and sludges from 7 conventional activated sludge plants with disinfection (5 systems were extended aeration without primary sedimentation and 2 were conventional activated sludge.) Values shown are ranges of arithmetic mean values for the 7 plants. Sampling was performed four times per year during different seasons.

Indicator or Pathogen	Influent/Input Range (Oo)Cysts/L	Effluent Range (Oo)Cysts/L	Sludge Range (Oo)Cysts/g TS ^a	Log ₁₀ Reductions Range
<i>Giardia</i> Cysts/L	22 to 6.703E+03	4 to 1.34E+02	ND ^b to 5.93E+02	0.62 to 2.13
<i>Cryptosporidium</i> Oocysts/L	67 to 1.34E+02	1 to 17	ND to 10	0.62 to 1.80

^aTS: Total solids; ^bND: Non-detect; Data are from Ramo et al., 2017

Table A9. Reduction of Aichi virus, norovirus GI and GII, *Giardia* and *Cryptosporidium* at an activated sludge treatment plant using chlorine disinfection in Arizona (U.S.). Influent and effluent concentrations are mean values with ranges in parentheses. *E. coli* was only monitored in effluent samples.

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions
BACTERIA			
<i>E. coli</i> MPN/100mL	NR ^b	(<1 to 1.34 E+02)	NR
VIRUSES			
Aichi Virus1 GC ^a /L	9.7E+04	1.1 E+04	0.94
Norovirus GI GC/L	(ND ^c to 1E+06)	(ND to 1E+04)	1.65
Norovirus GII ^d GC/L	NR	NR	2.14
PROTOZOA			
<i>Giardia</i> cysts/L	4.8E+03 (2.7E+03 to 9.9E+03)	33 (<4 to 1.5 E+02)	2.08
<i>Cryptosporidium</i> oocysts/L	74 (40 to 1.6 E+02)	13 (<4 to 36)	0.71

^aGC: Gene copies; ^bNR: Not reported; ^cND: Non-detect; ^dVirus concentrations were measured by the qPCR molecular method and not by cell culture. The qPCR method identifies specific viruses but they do not have to be intact or infective to be detected (Francy et al., 2011) and log₁₀ reduction may be underestimated. There are no known routine cell culture methods available for viable Aichi virus and norovirus; Data from Kitajima et al., 2014

Table A10. Reduction and survival of Aichi virus, norovirus GI and GII, *Giardia* and *Cryptosporidium* at a trickling filter treatment plant using chlorine disinfection in Arizona (U.S.) (Kitajima et al., 2014).

Influent and effluent concentrations are mean values with ranges in parentheses. *E. coli* was only was only monitored in effluent samples.

Indicator or Pathogen	Influent/Input Geometric Mean (Range)	Effluent Geometric Mean (Range)	Log ₁₀ Reductions
BACTERIA			
<i>E. coli</i> MPN/100ml	NR ^a	7 to 2.25E+02	NR
VIRUSES^b			
Aichi Virus copies/L	2.0E+06	2.0E+05	0.99
Norovirus GI & GII copies/L	(1E+02 to 3.2E+05)	ND to 1.6E+03	NR
Norovirus GI	NR	NR	2.57
Norovirus GII	NR	NR	2.85
PROTOZOA			
<i>Giardia</i> cysts/L	6.4E+03 (1.4E+03 to 1.5E+04)	1.9E+02 (56 to 6.2E+02)	1.52
<i>Cryptosporidium</i> oocysts/L	100 oocysts/L (<40 to 7.2E+02)	12 oocysts/L (<4 to 40)	0.81

^aNR: Not Reported; ^bVirus concentrations were measured by the qPCR molecular method and not by cell culture. The qPCR method identifies specific viruses but they do not have to be intact or infective to be detected (Francy et al., 2011) and log₁₀ reduction may be underestimated. There are no known routine cell culture methods available for viable Aichi virus and norovirus

Table A11. *Giardia* and *Cryptosporidium* reduction and survival in an activated sludge plant with tertiary treatment of coagulation/flocculation/sedimentation and sand filtration. Data from Fu et al., 2010.

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions
<i>Giardia</i> cysts/L	8.3E+02 to 2.7E+03	<0.2 to 2.1	3.46
<i>Cryptosporidium</i> oocysts/L	1E+02 to 4E+02	0.2 to 0.4	3.34

Table A12. Pathogen and fecal coliform reduction at seven wastewater treatment plants (six in the U.S. and one in Morocco) using activated sludge, and tertiary processes of filtration (6 plants using sand filters and 1 cloth filter), and disinfection (5 plants using chlorine and 2 UV).

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions		
Bacteria					
Fecal coliforms MPN/100mL	(2.1E+06 to 5.3E+06)	(0.20 to 3.73)	5.94	to	7.31
Viruses					
Enterovirus MPN/L	(4.64E+03 to 9.37E+04)	(3.1 to 9.1)	2.85	to	4.33
Protozoa					
<i>Giardia</i> cysts/L	(61.2 to 794)	(0.04 to 1.87)	2.15	to	3.87
<i>Cryptosporidium</i> oocysts/L	(6.5 to 37.8)	(0.03 to 0.33)	1.68	to	3.00
Helminths					
<i>Ascaris</i> eggs/L	153.5	ND ^{a,c}	NR ^b		
<i>Ancylostoma duodenale</i> eggs/L	8.6	ND	NR		
<i>Taenia</i> spp. eggs/L	5.3	ND	NR		
<i>Capillaria</i> spp. eggs/L	1.6	ND	NR		
<i>Trichuris trichiura</i> eggs/L	1.7	ND	NR		
<i>Hymenolepis</i> spp. eggs/L	3.2	ND	NR		

^aND: Non-detect; ^bNR: Not Reported; ^cAuthors presented helminth effluent values as 0; Helminth influent data are mean values; effluent values were reported as 0 and log₁₀ reduction could not be calculated. Bacteria, virus and protozoa influent/effluent data are ranges of geometric means for six treatment plants. (Data from Chaoua et al., 2018 in Morocco for helminths; all other data are from Rose et al., 2004)

Table A13. *Giardia* and *Cryptosporidium* reduction at three wastewater treatment plants in Spain using activated sludge, microfiltration and disinfection (two plants used UV and one chlorine). Influent and effluent values are ranges and log₁₀ reduction are based on means. (Data from Ramo et al., 2017).

Pathogen	Influent/Input Range (Oo)Cysts/L	Effluent Range (Oo)Cysts/L	Log ₁₀ Reductions ^a UV Disinfection Cl ₂ Disinfection
<i>Giardia</i> cysts/L	2.04E+02 to 9.833E+03	3 to 2.66E+02	1.40 1.86
<i>Cryptosporidium</i> oocysts/L	11 to 8.57E+02	0.55	1.22 0.46

^aPotential viability as reported;

Table A14. *Giardia* and *Cryptosporidium* reduction and survival in an anaerobic/anoxic/oxic activated sludge plant with ultrafiltration and disinfection. Data from Fu et al., 2010.

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions
<i>Giardia</i> cysts/L	1.7E+02 to 3.6E+03	<3.3E-02	>4.44
<i>Cryptosporidium</i> oocysts/L	33 to 4.3E+02	<3.3E-02	>3.63

Table A15. *E. coli* and helminth egg reduction in effluent and sludges from 10 wastewater stabilization pond systems in Honduras

Indicator or Pathogen	Influent/Input Mean (Range) MPN/100 eggs/L	Effluent Mean (Range) MPN/100 eggs/L	Log ₁₀ Reductions Average (Range)
BACTERIA			
<i>E. coli</i> MPN/100 ml	2.71 E+07 (1.22E+06 to 8.96E+08)	2.89E+04 (2.71E+02 to 1.17E+07)	2.97 (1.87 to 4.84) ^a
HELMINTHS			
<i>Ascaris</i> , <i>Trichuris</i> , Hookworm eggs/L	80.8 (9 to 744)	ND ^b	≥2.40

^aArithmetic mean and range of log₁₀ reduction for 10 systems; ^bND: Non-detect, reported as 0 by the author; Influent and effluent data are mean values (geometric for *E. coli* and arithmetic for helminths) for the 10 systems with ranges in parentheses. Helminth egg concentrations were not detected in effluent samples but the detection limit was not presented by the author and therefore log₁₀ reduction could not be calculated. (Data from Oakley, 2005)

Table A16. Reduction and survival of fecal coliforms and *Vibrio cholerae* 01 in the waste stabilization pond system San Juan de Miraflores, Lima, Peru, from June 9 to August 10, 1991 (Castro de Esparza et al., 1992). Influent and effluent values are geometric means.

Sample Type	Indicator or Pathogen	Influent/Input Average (Range) MPN /100ml	Effluent Average (Range) MPN /100ml	Log ₁₀ Reductions
Treatment plant	Fecal Coliforms	1.63E+09	2.11E+04	4.89
Treatment plant	<i>Vibrio cholerae</i> 01	1.88E+03	0.10	4.26
Aquaculture pond	Fecal Coliforms	NR ^a	70.6	NR
Aquaculture pond	<i>Vibrio cholerae</i> 01	NR	0.03	NR
Aquaculture: Tilapia Tissue ^b	NR	NR	NR	NR

^aNR: Not Reported; ^bPresence of *Vibrio cholerae* was detected on skin, and in intestines and gills. The presence test for *Vibrio Cholerae* did not distinguish between *Vibrio Cholerae* 01 and *Vibrio Cholerae* non-01.

Table A17. Reduction and survival of *E. coli* and helminth eggs at the wastewater stabilization pond system Campo Espejo, Mendoza, Argentina. Values are mean values from daily monitoring for one year (January 1, 2000 to December 31, 2000). Data from Barbeito Anzorena, 2001. Approximately 4,000 ha are irrigated with the effluent from this system.

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions
BACTERIA			
<i>E. coli</i> MPN /100ml	1.11E+07	74.8	5.17
HELMINTHS			
Helminth eggs /L	26	<1 ^a	>1.41 ^a

^aThe detection limit of helminth eggs was not mentioned by the author and the log₁₀ reduction of >1.41 assumed the detection limit was 1 egg/L.

Table A18. *E. coli*, *Salmonella* and helminth egg reduction and survival in effluent from a wastewater stabilization pond system in India with one facultative pond followed by three maturation ponds in a series. Influent and effluent data are mean values (geometric for *E. coli* and *Salmonella*, and arithmetic for helminths) with ranges in parentheses. Data from Tyagi et al., 2011.

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions
BACTERIA			
<i>E. coli</i> MPN/100ml	2.2E+06 (1.1E+05 to 1.2E+07)	1.8E+03 (2.1E+02 to 2.3E+04)	3.00
<i>Salmonella</i> CFU/100ml	4.9E+02 (3.8E+02 to 6.0E+02)	27 (18 to 54)	1.24
HELMINTHS			
Helminth eggs eggs/L	48.3 (18 to 88)	0.18 (ND ^a to 1.0)	2.48
^a ND: Non-detect			

Table A19. Reduction and survival of enterovirus, norovirus, rotavirus, *Giardia*, *Cryptosporidium*, *Taenia* and *Ascaris* in a waste stabilization pond system in Bolivia. Data are mean values or ranges from composite samples taken on two different days for viruses (June 2012), twice for helminth eggs (June 2011 and 2012), and once for protozoa (June 2012).

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions
VIRUSES			
Enterovirus IU/L ^a	4.2E+04	37	3.06
Norovirus GI ^b copies/L	≈1E+06	≈ 1E+06	0
Rotavirus ^b copies/L	≈1E+06	≈1E+06	0
PROTOZOA			
<i>Giardia</i> cysts/L	1.6E+02	23	0.84
<i>Cryptosporidium</i> oocysts/L	6.0	4.0	0.18
HELMINTHS^c			
<i>Taenia</i> eggs/L	3.06E+02 to 3.006E+03	<22 to 45	1.82 ^d
<i>Ascaris</i> ^e eggs/L	ND ^f to 3.06E+02	<22	^e

^aIU: Infectious units; ^bConcentrations were measured by the RT-qPCR molecular method and not by cell culture, thus concentrations reported in effluents may underestimate reduction; ^c8.41E+02 helminth eggs/g Total solids (TS) in Facultative Pond Sludge with 37.5% viable; ^dLog₁₀ reduction of *Taenia* based on influent of 3,006 eggs/L and effluent of 45 eggs/L; the detection limit for the final effluent was 22 eggs/L; ^e*Ascaris* eggs were detected in the final effluent and were only detected in one influent sample, thus log₁₀ reduction could not be estimated; ^fND: Non-detect; Data from Symonds et al., 2014; Verbyla et al., 2013a, 2013b.

Figure A20. Protozoan cyst and helminth egg reduction and survival in effluent and sludges at a wastewater stabilization pond system in Ouagadougou, Burkina Faso (Konaté et al., 2013).

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions ^a	Pond Sludges (Percent Viable Eggs)
PROTOZOA				
<i>Entamoeba coli</i> , <i>Entamoeba histolytica</i> , <i>Giardia lamblia</i> cysts/L	1.11E+02 (4 to 327)	ND ^{b,c}	1.74 (>98.1%)	1.20E+02 ^d 10 ^e 7 ^f
HELMINTHS				
Helminth eggs eggs/L	15.7 (5 to 36)	ND ^{b,c}	>2.89 (>99.87%)	5.56E+02 ^d (36) 32 ^e (16.3) 12 ^f (0)

^aProtozoan cyst and helminth egg concentrations were not detected in any effluent samples from the maturation pond but the detection limit was not presented by the authors and therefore total log₁₀ reduction could not be calculated. The log₁₀ reduction shown is based on mean values of measured concentrations from the facultative pond effluent: protozoan cysts = 2 cysts/L; helminth eggs = 0.02 eggs/L; ^bND: Non-detect; ^cReported as 0 by authors; ^dAnaerobic; ^eFacultative; ^fMaturation

Table A21. *E. coli*, protozoan (oo)cyst, and helminth egg reduction and survival in effluent and sludges at a wastewater stabilization pond system in northwestern Spain (Reinoso et al., 2011).

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions	Pond Sludges
BACTERIA				
<i>E. coli</i> ^d CFU/100ml	2.0E+05 to 3.2E+06	31.6 to 25	2.90; 5.00 (Cold; hot periods)	
PROTOZOA				
<i>Cryptosporidium</i> oocysts/L	14.9 (5.3 to 40.1)	0.4 (ND ^b to 1.4)	1.57	1.10E+02 oocysts/g TS ^c
<i>Giardia lamblia</i> cysts/L	67.1 (17.5 to 217)	0.7 (ND to 1.6)	1.98	6.80E+02 cysts/g TS
HELMINTHS				
Helminth eggs	1.8 eggs/L (0.2 to 5.9 eggs/L)	ND ^d	ND ^e	28.4 eggs/g TS

^a*E. coli* influent and effluent ranges are mean values from cold (T_{water} = 13.1°C) and hot (T_{water} = 20.1°C) periods as reported by the authors; *E. coli* log₁₀ reductions are for the cold and hot periods; ^bND: Non-detect; ^cTS: Total solids; ^dReported as 0 by authors; ^eHelminth egg concentrations were not detected in effluent samples but the detection limit was not reported by the authors and total log₁₀ reduction could not be calculated.

Figure A22. *E. coli*, *Giardia*, *Cryptosporidium*, and helminth egg reduction and survival in effluent and sludges from two wastewater stabilization pond systems in Bolivia. (Verbyla et al., 2016). Both systems were very poorly maintained, which caused excessive sludge buildup and extensive hydraulic short-circuiting. As a result, high concentrations of pathogens were found in final effluents.

Indicator or Pathogen	Influent/Input Average (Range) ^a	Effluent ^a Average (Range)	Log10 Reductions A/F/M1/M2 ^b	Log10 Reductions A/F/M ^c
BACTERIA				
<i>E. coli</i> CFU/100ml	3.0E+04 to 6.2E+07	1.3E+05 to 2.1E+06	1.1	0.7
PROTOZOA				
<i>Giardia lamblia</i> cysts/L	2.9E+02 to 3.77E+02	14 to 2.53E+03	1.5	0.5
<i>Cryptosporidium</i> oocysts/L	3.6 to 1.64E+02	0.4 to 11	0.7	0.8
HELMINTHS				
Helminth eggs eggs/L	1.12E+02 to 8.47E+02	31 to 3.50E+02	0.6	0.5

^aInfluent and effluent concentrations are ranges of analyzed data; ^bA/F/M1/M2 = anaerobic followed by a facultative and two maturation ponds in series; ^cA/F/M = anaerobic followed by a facultative and maturation pond in series.

Table A23. Fecal coliform and helminth egg reduction and survival at a full scale UASB/F2 system in Morocco (Tyagi et al., 2011). Values are geometric means for fecal coliforms and arithmetic for helminth eggs with ranges in parentheses.

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions
BACTERIA			
Fecal coliforms MPN/100ml	3.1E+06 (3.9E+05 to 2.3E+07)	1.1E+04 (2.3E+03 to 1.1E+04) ^a	2.45
HELMINTHS			
Helminth eggs eggs/L	26.4 (21 to 57)	0.13 (ND ^b to 4.3)	2.55

^aThe high range values of 1.1E+04 appears to be in error since it is the same as the mean but was published as such; ^bND: Non-detect

Figure A24. Reduction and survival of enterovirus, norovirus, rotavirus, *Giardia*, *Cryptosporidium* and helminth eggs from a UASB-maturation pond system in Bolivia. Data are mean values or ranges from composite samples taken on different days for viruses (June 2012), twice for helminth eggs (June 2011 and 2012), and once for protozoa (June 2012).

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average (Range)	Log ₁₀ Reductions
VIRUSES			
Enterovirus IU/L ^a	6.2E+04	9.1E+03	0.83
Norovirus copies/L ^b	≈ 1E+04	≈ 1E+05	0
Rotavirus copies/L ^b	≈ 1E+05	≈ 1E+04	≈ 1.0
PROTOZOA			
<i>Giardia</i> cysts/L	2.4E+02	73	0.52
<i>Cryptosporidium</i> oocysts/L	9.5	6.5	0.16
HELMINTHS			
Helminth eggs (<i>Taenia</i> , <i>Ascaris</i> , <i>Trichuris</i> , Hookworm) eggs/L	1.809E+03	<i>Taenia</i> , <i>Ascaris</i> ND to ~1.2 E+03 ^c	NR ^d

^aEnterovirus units in infectious units (IU)/L; ^bNorovirus and rotavirus concentrations were measured by the RT-

Table A25. Reduction of *E. coli* and helminth eggs from a UASB/F2/M1/M2 systems in Brazil

Indicator or Pathogen	Influent/Input Average (Range)	Effluent Average	Log ₁₀ Reductions	Secondary Facultative Pond Sludges (Range) Eggs/g TS ^a
BACTERIA				
<i>E. coli</i> ^b MPN/100mL	2.46E+08	4.5 E+02	5.74	NR ^c
HELMINTHS				
Helminth eggs/L ^{b,d} or eggs/g TS ^a (percent viable)	(26 to 2.54 E+02) ^b	ND ^e	NR	(10 to 1.1E+03) ^a (44 to 100%)

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