**Constructed wetlands for greywater recycle and reuse**

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**Highlights**

* Constructed wetlands are a low-tech, low energy water treatment alternative that show potential to adequately treat greywater for nonpotable reuse
* Despite providing the necessary physical, chemical and biological removal mechanisms for greywater contaminants, removal dynamics are complex and less predictable than more engineered systems
* Pathogen reductions provided by constructed wetlands alone are likely not reliable enough to meet regulatory standards for reuse, particularly in the US
* If combined with common disinfection technologies (chlorination, UV) constructed wetlands may be an acceptable, low energy alternative for decentralized, nonpotable reuse

**Abstract**

Concern over dwindling water supplies for urban areas as well as environmental degradation from existing urban water systems has motivated research into more resilient and sustainable water supply strategies. Greywater reuse has been suggested as a way to diversify local water supply portfolios while at the same time lessening the burden on existing environments and infrastructure. Constructed wetlands have been proposed as an economically and energetically efficient unit process to treat greywater for reuse purposes, though their ability to consistently meet applicable water quality standards, microbiological in particular, is questionable. We therefore review the existing case study literature to summarize the treatment performance of greywater wetlands in the context of chemical, physical and microbiological water quality standards. Based on a cross-section of different types of wetlands, including surface flow, subsurface flow, vertical and recirculating vertical flow, across a range of operating conditions, we show that although microbiological standards cannot reliably be met, given either sufficient retention time or active recirculation, chemical and physical standards can. We then review existing case study literature for typical water supply disinfection unit processes including chlorination, ozonation and ultraviolet radiation treating either raw or treated greywater specifically. A comparison of wetland case study effluent quality to disinfection case study influent quality shows that under appropriate conditions these two unit processes together can produce effluent of sufficient quality to meet all nonpotable reuse standards. Specifically, we suggest that recycling vertical flow wetlands combined with ultraviolet radiation disinfection is the best combination to reliably meet standards.

**Keywords**

Greywater, constructed wetland, pathogen removal

**Introduction**

Growing concern in the urban water sector over water scarcity, resource use and aging infrastructure that is at capacity or outdated has motivated a large body of research into alternative water reuse strategies (National Academies of Science, 2016). Greywater (GW), or wastewater generated from households or buildings not including toilet water, has received attention as a candidate for reuse as it has the potential to carry less organics, nutrients and pathogens than mixed wastewater (i.e. including toilet water or “blackwater”) and is therefore thought to be easier to treat for reuse purposes (Abu Ghunmi et al., 2011; Eriksson et al., 2002; Ghaitidak and Yadav, 2013; Li et al., 2009; Mayer et al., 1999). Additionally, if treated to nonpotable instead of potable standards, less resource-intensive treatment processes may be used. Combined with reducing discharge to wastewater treatment plants and offsetting potable water demand, GW recycle represents a plausible system-level approach to achieving greater water sustainability and resiliency (Ma et al., 2015; Xue et al., 2015). Indeed many developed countries have shown interest in GW recycle systems including New Zealand (Leonard et al., 2016), Australia (Australia, 2010; ChristovaBoal et al., 1996; Tapsuwan et al., 2014) and Germany (Nolde, 2000; Nolde, 2005).

A number of technologies have been used for GW treatment, from simple physical filtration systems such as membranes and sand filters to highly automated and energy-intensive systems that include biological, chemical and physical treatment mechanisms (Friedler et al., 2005; Li et al., 2009; Winward et al., 2008a). Constructed wetlands are often suggested as an economically and energetically efficient way of treating various wastewater streams (Brix, 1999; Jasper et al., 2013; Kröpfelová, 2008; Liu et al., 2015; Vymazal, 2009), though their ability to treat GW specifically has mostly been assessed on a case-by-case basis. Not only are the individual case-study systems diverse, the available data on the ability of constructed wetlands to remove or inactivate pathogens of importance for human health risk (not merely pathogen indicators) are limited. Furthermore, although some authors have found evidence that constructed wetlands as a standalone technology can meet certain existing reuse standards (Gross et al., 2007; Jenssen et al., 2005), many more have found that they cannot do so in a reliable and consistent manner (Garcia et al., 2010; Jokerst et al., 2012), particularly the more stringent standards for unrestricted water reuse (USEPA, 2012).

This paper is intended to provide a quantitative review of the case-studies that have looked at the ability of constructed wetlands to treat GW for reuse at the house, school, neighborhood or commercial building scale. Of particular concern is the ability of treated effluent to meet applicable standards or guidelines for non-potable reuse, which are mainly driven by pathogen-related human health risk (Beck et al., 2013; National Academies of Science, 2016). In order to address the limited availability of specific pathogen removal data, the ability of wetlands to meet physical or chemical criteria that directly influence pathogen removal dynamics will be reviewed. In addition, a quantitative review of common disinfection technologies appropriate for treating wetland effluent is performed with an emphasis on studies that disinfected treated GW. Thus, if it can be shown that wetlands as a single unit process can reliably produce effluent that meets physical and chemical standards including Total Suspended Solids (TSS), Biological Oxygen Demand (BOD) and turbidity, and the disinfection unit processes can treat similar waters to applicable pathogen criteria, then by extension we will argue that constructed wetlands combined with common disinfection technologies can be used as a low cost, energy efficient, reliable and safe means of recycling GW for nonpotable reuse.

**Regulatory Guidelines and Criteria**

Globally, water reuse standards are variable and governed by the intended use of the treated effluent. In general, limits are imposed on specific parameters to guard against nuisance odors, algal growth, environmental health and human health. For detailed reviews, see Li et al. (2009), Abu Ghunmi et al. (2011) or Ghaitidak and Yadav (2013).

Table 1 shows United States Environmental Protection Agency (USEPA) guidelines, California standards and Western Australia standards for restricted, unrestricted and environmental water reuse. These guidelines and standards were selected as they represent two countries at the forefront of water reuse and they include parameters that are fairly typical of other global criteria (Abu Ghunmi et al., 2011; Li et al., 2009). In the United States, standards have been proposed on the state level as a response to the geographically-driven need for water reuse, though they vary by state and by intended use of the treated effluent. California, a state that experiences chronic water supply crises and thus has a strong incentive to reuse water, is often recognized as having some of the most stringent state-level standards mostly due to the requirement for a 5 log10 reduction (LR) of poliovirus or similar virus ((CDPH), 2010). At the national level, the USEPA has issued recommended guidelines (USEPA, 2012), though official standards have yet to be adopted. Outside of the US, Australia has been somewhat of a pioneer in water reuse implementation and regulation due to the extended and widespread drought conditions of the early 2000s (Floyd et al., 2014; Wong and Brown, 2009). In all cases, the limits for organics and microbiology are the focus, with treatment of the former often a prerequisite for the effective treatment of the latter.

**Table 1**

A critical drawback of traditional standards and guidelines is that they are not risk-based and therefore lead to an unknown level of protection of human health (National Academies of Science, 2016). Recently, work based on quantitative microbial risk assessment (QMRA) for specific pathogens has been completed with corresponding recommendations for treatment requirements in the form of log10 reduction targets (LRTs) (Jahne et al., 2017; Schoen et al., 2017). This work has been largely motivated by a growing awareness that traditional indicator-based treatment goals, such as fecal bacteria concentration limits, may not reliably indicate the presence or quantity of the specific pathogens that pose an actual human health risk. Unfortunately, few studies exist that have produced reliable data on either the presence or treatability of these specific pathogens, particularly with respect to GW and GW treatment wetlands. In the following sections, wetland performance was largely evaluated in the context of the conventional concentration-based criteria, as they are what drive most regulatory and monitoring protocols, though LR performance of relevant pathogens and pathogen indicators is discussed where possible.

**Greywater Characterization**

As mentioned previously, GW is often cited as a lower strength, less polluted wastewater stream as compared to a typical mixed wastewater stream that includes toilet effluent. Greywater can also further be subdivided into what is commonly referred to as “light” and “mixed”, the former consisting of bath, shower and bathroom sink water and the latter including laundry and kitchen sink water. Numerous authors have provided detailed characterization of individual streams as well as combined streams (Eriksson et al., 2002; Jefferson et al., 2004; Rose et al., 1991){Rose, 1991, MICROBIAL QUALITY AND PERSISTENCE OF ENTERIC PATHOGENS IN GRAYWATER FROM VARIOUS HOUSEHOLD SOURCES} and still others have provided helpful compilations of the individual studies (Boyjoo et al., 2013; Friedler et al., 2011; Ghaitidak and Yadav, 2013; Li et al., 2009). Of importance for this review is both the variability in GW quality as well as the upper ranges to expect from the various sources. Accordingly, Table 2 shows the ranges reported by the previously mentioned review articles for light GW and mixed GW, mixed wastewater for comparison (Lowe et al., 2007; Metcalf, 2003), and parameter ranges for the case studies reviewed later in this article.

**Table 2**

The physical and chemical parameter ranges given in Table 2 show that, as expected, light GW is generally of lower strength than mixed GW, and mixed GW is generally of lower strength than mixed wastewater (i.e. including blackwater). The larger values for BOD and chemical oxygen demand (COD) are often attributed to heavy detergent or food waste loads associated with laundry or kitchen sources (Ghaitidak and Yadav, 2013), and can be particularly extreme if unmixed with more dilute sources, even exhibiting similar or even greater concentrations than mixed wastewater. Moreover, compared to the oxygen demand of mixed wastewater that stems in part from more readily degradable fecal material and food waste, that of GW can be due in large part to less biodegradable surfactants from soaps and detergents (ChristovaBoal et al., 1996; Sharvelle et al., 2007). Thus, not only is a biological treatment step often necessary to meet BOD standards (Nolde, 2000), it must be metabolically diverse, capable of oxidizing more recalcitrant organic compounds.

TSS concentrations in GW are often less than mixed wastewater due to the absence of feces and bathroom tissue, which represent sources of larger solids. However, GW TSS concentrations can still be much greater than regulatory standards. More importantly, numerous authors have noted an association between suspended solids and larger pathogens including bacteria, bacterial indicators and protozoa (Falabi et al., 2002; USEPA, 2000; Winward et al., 2008b, c). Thus, treatment systems that promote either the settling or filtration of solids have been shown to be effective in removing these larger pathogens (Falabi et al., 2002; Garcia et al., 2010; Gerba et al., 1999; Karim et al., 2004).

In terms of nutrients, ranges are again large. Phosphorus concentrations can be high, particularly in areas that have not adopted stringent legislation banning the use of phosphate-based detergents (Turner et al., 2013). Though not readily apparent in Table 2, past studies that have characterized individual GW streams have suggested the potential for nutrient deficiency (e.g. high C:N ratio), particularly if kitchen water is excluded (De Gisi et al., 2016; Jefferson et al., 2004; Li et al., 2009), which could reduce the efficiency of biological treatment processes. For example, Bergdolt et al. (2013) calculated the first order aerial removal rate for BOD from a FWS constructed wetland treating bathroom wash water (BOD:TN of approximately 6:1) and found it to be depressed relative to systems treating domestic or agricultural wastewaters. Of the ranges shown in Table 2 for the wetland studies reviewed, there is a potential for nutrient (nitrogen in particular) deficiency. Compared to a suggested BOD:TN ratio of 5:1 for biological processes (Metcalf et al., 1991), ranges for wetland studies reviewed here returned BOD:TN ratios of 3.3 to 49.

With respect to the bacterial pathogens identified in Table 2, again there is a general increase in strength from light GW to mixed wastewater, as expected. Also, similar to the physical and chemical parameters, it is clear that mixed GW fecal indicator concentrations often reach those of mixed wastewater, with ranges of up to 8 log (CFU/100 mL).

In addition to the large variability in bacterial pathogen data, Table 2 shows a clear lack of data regarding specific virus or protozoa counts in GW. Data are present for standard bacterial indicators (total coliform, fecal coliform), enteric-specific indicators (*Escherichia coli*, enterococci), an opportunistic bacteria associated with human skin and mucous membranes, (*Pseudomonas aeruginosa*, *Staphylococcus aureus*), a bacterium proposed as an indicator for the inactivation of enteric viruses and whose associated spores have been proposed as an indicator for parasitic protozoa (*Clostridium perfringens*) (Ottoson and Stenstrom, 2003; Payment and Franco, 1993), an aerosol-transmitted aquatic bacterium that can cause respiratory illness (*Legionella*), a gastrointestinal bacterium (*Salmonella*) and a surrogate for enteric viruses (Male-specific 2 (MS2) coliphage) (Havelaar et al., 1987). The lack of data for pathogens assessed by Schoen et al. (2017) is partly due to quantification cost and partly due to occurrence; if an infected individual is not present in the house or building being sampled, the specific pathogen will likely not be detected.

For all parameters given, the range of influent concentrations from the wetland studies that will be discussed are mostly within the ranges given for light GW, despite the fact that a mix of source waters was treated. In addition to the fact that we reported ranges, not means, this discrepancy is also likely due to proper mixing; the majority of wetland studies reviewed incorporated an initial mixing stage (e.g. mixing basin, sump, etc.) that allowed temporary spikes in contaminant loading (e.g. laundry first rinse) to be dampened. It also allowed for dilution of high strength sources (e.g. laundry and kitchen) with lower strength sources (e.g. bathroom sink).

**Greywater Treatment using Constructed Wetlands**

Wetlands are complex systems that are home to myriad abiotic and biotic processes capable of removing, degrading or transforming many compounds considered to be pollutants (Garcia et al., 2010; Jasper et al., 2013; Kadlec and Wallace, 2008; Wetzel, 2001). Numerous authors have argued that a range of processes including biological treatment and physical filtration are necessary for the effective treatment of GW (De Gisi et al., 2016; Li et al., 2009; Nolde, 2000; Nolde, 2005), processes that are simultaneously present in wetlands. However, the diverse range of processes that makes wetlands uniquely suited to a wide range of removal processes also makes them variable in their treatment performance. Additionally, widely used modeling approaches and performance databases such as the P-k-C\* model (Kadlec and Wallace, 2008) and the North American Treatment Wetland Database (NADB) used to predict performance are mostly based on systems treating domestic or agricultural wastewater, which are compositionally different from household GW (Bergdolt et al., 2013; Sharvelle et al., 2007). As such, this section is intended to review observed treatment dynamics of constructed wetlands treating GW. Specifically, we discuss the ability of constructed wetlands to remove physical/chemical parameters that influence pathogen treatability, the ability for constructed wetlands to directly remove or inactivate specific pathogens or pathogen indicators, and the ability of constructed wetlands to produce effluent of suitable quality for disinfection by commonly used technologies including chlorination and UV radiation. A total of 12 studies were reviewed, from which 37 individual system datasets were extracted. Tables 3a and 3b show the results from this review for physical/chemical and pathogen performance, respectively. Wetland systems assessed included free water surface (FWS), green roof water recycle (GROW), horizontal subsurface flow (HSSF), vertical flow (VF), recycling vertical flow (RVF) and combination systems. Studies were included only if data were provided that allowed for either the extraction or calculation of at least one parameter set from each of the following categories: i) hydraulic loading rate (HLR, daily inflow volume divided by system area) and hydraulic retention time (HRT, volumetric capacity divided by daily inflow volume), ii) physical/chemical influent and effluent data, either BOD, TSS or turbidity, and iii) influent and effluent pathogen concentrations. Where possible, adjusted HRT was used, which includes the influence of incoming GW, precipitation and evaporation. Where these data were not available, nominal HRT was used which was solely a function of reported GW loading and volumetric capacity (i.e. length \* width \* depth \* porosity).

**Table 3a and 3b**

***BOD, TSS and Turbidity***

Figure 1 shows the resulting effluent concentrations of BOD, TSS and turbidity as a function of HRT. In each plot, dotted lines represent restricted standards from Table 1 and solid lines represent unrestricted standards. For each of these three parameters, a general trend is observable regardless of system type in which greater HRTs produce higher quality effluent. Figure 1 also shows that for BOD and TSS, regardless of system type, a HRT of 3-5 days is generally necessary to achieve the less stringent restricted standards of 30 mg/L for each parameter.

**Figure 1 – a, b and c**

The ability of constructed wetlands to remove BOD has been well documented and is one of the more predictable treatment performance parameters (Kadlec and Wallace, 2008). Being a biological process, it is subject to prevailing redox conditions, the influence of temperature on metabolic rates, and the recalcitrance of the organic compounds being oxidized. In terms of redox conditions, we would expect more aerobic environments to increase the BOD removal rate as more oxygen is available to satisfy demand. Indeed, in looking at Figure 1a, we see that the VF and RVF systems generally achieve the lowest BOD effluent concentrations even at HRTs less than 5 days. The RVF systems, in particular, achieve these low effluent concentrations (less than 5 mg/L) more consistently compared to single pass VF systems, as continuous circulation likely allows for enhanced oxygenation.

Temperature has been shown to affect BOD treatment performance as well. Insofar as microbial metabolic processes in wetlands often proceed at greater rates in warmer temperatures (Reddy and DeLaune, 2008), wetlands located in colder climates may have a lesser capacity to reduce BOD concentrations, and treatment performance may be diminished in winter months. One system, a FWS located in Colorado (Jokerst et al., 2012), only achieved an average annual effluent concentration of 31.7 mg/L, despite having a HRT of 6.8 days. This was due to lower treatment efficiency in winter months, as the water temperature within the wetland averaged only 4°C. Temperature also affected the treatment performance of the second stage of this hybrid FWS + HSSF system; the data points in Figure 1 with HRTs of 14 and 20 days are the HSSF component and combination system from this study, respectively. Although the effluent from the entire system averaged 12.7 mg/L for the year, well below the 30 mg/L standard, the long detention time was not enough to counter the effects of temperature on BOD treatment performance during winter months (average effluent concentration of 45.6 mg/L for the whole system returned during winter months). An important consideration noted by Jokerst et al. (2012) however is that in climates where cold weather (and associated snow and ice) limit biological removal mechanisms during winter months, irrigation with treated effluent is often not needed, thus the need to meet at least irrigation/restricted reuse standards should be evaluated on a case by case basis.

The recalcitrance of surfactants along with the potential for nutrient deficiency in mixed GW has also been hypothesized to affect the rate of biologically mediated processes in constructed wetlands. For example, the recalcitrance of surfactants can be higher than that of the suite of organic compounds found in mixed domestic sewage or agricultural wastewater (Sharvelle et al., 2007), thus lowering the efficiency of the metabolic processes responsible for oxidizing organic matter and lowering BOD concentrations. Although we do not have data to test these mechanisms specifically, we can compare the performance data reviewed here to the North American Treatment Wetland Database (NADB, 1998) which is composed of systems that treat predominantly mixed domestic effluent, agricultural wastewater, wastewater treatment plant effluent, stormwater and industrial effluent. Figure 2a shows data from this review plotted against data from the NADB which is composed of monthly average data for 2030 month-systems. In general, all of the GW treatment wetlands reviewed performed near or better than the central tendency of the NADB. Again, we see the VF and especially the RVF systems performing the best, even outside of the space occupied by the NADB distribution. On one hand, the enhanced performance of the RVF systems is to be expected for BOD, as the systems documented in the NADB are mostly single pass, non-aerated systems. However, the tendency of the GW systems in general to perform better than the NADB systems gives evidence that is contrary to the previously suggested hypotheses; that nutrient limitation, recalcitrance of organics or both may lead to depression of treatment performance. Rather, Fgure 2a would suggest that wetland design equations for BOD based on rate constants derived from the NADB (e.g. (Kadlec and Wallace, 2008), Chapter 8) are appropriate and may even be conservative for the design of GW treatment wetlands.

**Figure 2 – Data cloud figures**

TSS is removed in constructed wetlands through a variety of mechanisms including settling, flocculation and filtration (USEPA, 2000), with different processes being more dominant in different systems. In FWS systems, low water velocities made possible by greater pore space allow for greater initial settling of larger solids (Gearheart et al., 1989), which can sometimes even occur on the order of hours (Wong et al., 2006). In HSSF and VF systems, these larger particles are also removed initially, though through more of a physical filtration or interception mechanism owing to their greater media surface area. Following removal of larger particles, filtration or adsorption of smaller and colloidal particles occurs which is directly related to the available surface area of media, vegetation or substrate (Garcia et al., 2010). For GW treatment, systems that can provide a range of removal mechanisms are most ideal as the suspended solids particle size distributions are often highly variable. For example, Ramon et al. (2004) studied bathroom sink and shower water and found that colloidal size particles were the dominant fraction in terms of number distribution with a mean particle size of 0.1 µm while much fewer, larger particles made up most of the particle volume. This variability in size distributions has been noted by other authors as well who have found volume weighted mean particle sizes of GW ranging from 100-500 µm (Frazer-Williams et al., 2008; Ramon et al., 2004; Winward et al., 2008c).

Looking at Figure 1b, TSS shows a similar trend to BOD in that effluent concentration is dependent upon HRT, with an HRT of 3-5 days being necessary to achieve the 30 mg/L standard for restricted reuse. Considerable scatter exists within each system type except for the four RVF systems, where despite HRTs of 3.5 days or less (Table 3) effluent TSS concentrations are 1-3 mg/L, below any standard given in Table 1. Comparing the GW-specific TSS dataset from this review to the equivalent dataset from the NADB in Figure 2b, we again see the GW treatment wetlands performing, in general, better than the NADB central tendency, particularly the RVF systems. As TSS removal is generally attributed to filtration processes, the continuous recirculation of the treatment volume through the RVF systems essentially means that for any “batch” of water applied to the system, its constituents are exposed multiple times to the filtration media increasing the probability of contact and adsorption. For all RVF systems reviewed, the recirculation rate tends to be approximately half the total system volume per hour, meaning that if plug flow were assumed the entire volume of the system would be recirculated (and re-exposed to the filter medium) once every two hours. Combined with the fact that the filter media in these systems are variable, including a layer of pebbles, tuff or plastic media and soil-based root zone, there are a variety of surfaces for TSS to adhere to thus increasing the probability of obtaining particle/media interactions that are conducive to effective adsorption or filtration.

Turbidity shows a similar behavior as TSS and BOD, in that a HRT of 3-5 days appears to be necessary for the majority of reduction to occur, beyond which improvements in effluent quality are slight. While no system reliably meets the 2 NTU criteria for unrestricted reuse (restricted reuse does not have a turbidity requirement), many of the systems are capable of producing effluent with turbidity levels less than 20 NTU, so long as an HRT of 3-5 days is achieved. As with BOD and TSS, we also see the RVF systems performing well, with the five RVF systems that report turbidity achieving effluent levels of less than 10 NTU. Again, this is likely attributed to the enhanced contact time with the range of media types found in these systems, particularly the lower, finer layers. For single pass systems, several authors have found that sand filtration as a polishing step is effective at removing turbidity of biologically treated effluent (Gerba et al., 1999; Li et al., 2009), likely due to the high surface area available for filtration and adsorption of smaller suspended, dissolved and colloidal organics. Although sand is not a commonly used media in the RVF systems reviewed, it may be that increased exposure time due to recirculation is an effective substitute for single-pass treatment through high surface area media.

***Microbiological Parameters***

While we have shown that the ability of GW constructed wetlands to remove BOD, TSS and turbidity is generally predictable and largely comparable to or better than the larger treatment wetland performance literature, the ability of GW constructed wetlands to reliable produce effluent of sufficient microbiological quality to meet reuse criteria is far less robust or predictable. Here we review the mechanisms by which constructed wetlands have been shown or suggested to remove pathogens or pathogen indicators and provide the results of the pathogen-related treatment performance of the GW treatment wetlands reviewed.

In general, pathogens, as they relate to water treatment and human health, are divided into three main groups due to the differences in size, occurrence, persistence and treatability: bacteria, protozoa and helminths, and viruses (USEPA, 2012). Bacteria are microscopic organisms ranging from approximately 0.2 to 10 µm in size and can be removed or inactivated in constructed wetlands through a variety of processes including sedimentation of particle-associated bacteria, physical filtration by adsorption onto vegetation, media or biofilm, UV inactivation, attack by bacteriophage and predation by micro-zooplankton (Diaz et al., 2010; Garcia et al., 2010; Gerba et al., 1999; Morato et al., 2014; Stenstrom and Carlander, 2001; Vymazal, 2005). Protozoa and helminths can be excreted in feces as spores, cysts, oocysts or eggs which make them particularly resistant to environmental stresses including temperature extremes, sunlight and desiccation. They can be larger than bacteria, ranging in size from 1 µm to over 60 µm (USEPA, 2012), making physical removal processes including sedimentation and filtration effective (Garcia et al., 2010; Karim et al., 2004). Viruses are the smallest of the three groups, ranging in size from 0.01 to 0.3 µm. Due to their small size and ability to persist in the environment, physical removal processes can be less effective compared to bacteria and protozoa, necessitating a disinfection step for high levels of removal or inactivation. Although UV inactivation is a common method of virus inactivation, viruses generally require a higher dose of UV for inactivation compared to bacteria and protozoa, which, combined with an infectivity dose that can be orders of magnitude less than bacteria and protozoa, often makes them the focus of more stringent human health risk standards (Al-Gheethi et al., 2016; Asano, 2005; USEPA, 2012).

Table 3b shows the available data from the studies reviewed for pathogen removal performance, with the “ID” column providing the link to the corresponding system from Table 3a. The last row of Table 3b shows the ranges of log reductions (LR) for each individual pathogen or pathogen indicator for the 37 individual systems. All microorganisms listed in Table 3b are bacteria; *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Legionella* are pathogens capable of causing disease in humans, while the remaining bacteria are indicator organisms, which are not themselves dangerous to human health but are used to indicate the likelihood of human health risk occurrence (USEPA, 2012). No treatment performance data were found for virus, protozoa or helminths in GW treatment wetlands.

The ranges in Table 3b show that constructed wetlands are generally capable of providing up to 2 to 5 LR for bacteria, though LRs can also regularly be less than one and even negative in isolated cases. This is generally consistent with larger treatment wetland databases, with bacterial LRs from the systems reviewed in Kadlec and Wallace (2008) showing a central tendency of 1-2 LR, though inter- and even intra-system ranges can be quite large. Also, in their respective reviews of removal processes in CWs, Vymazal (2005) and Garcia et al. (2010) found bacterial indicator removals to fall somewhat consistently in the 1-2.5 LR range. However, both cautioned against using CWs as a standalone unit process for the treatment of water with high bacterial concentrations as, despite consistent LR, effluent concentrations were best predicted by influent concentrations and often remained high (>3 log10). Though not shown, logarithmic regression analyses (all system types combined) were performed to test if HLR or HRT predicted any variation in LR for total coliform, fecal coliform or *E. coli*. Although an R2 of 0.41 was found for the prediction of total coliform by HLR, an R2 of less than 0.2 was found for all other relationships showing little ability to predict LR performance using standard system operating characteristics.

To test if system characteristics, including HRT and influent bacterial concentration, predicted effluent concentration (and therefore the ability to meet concentration-based standards), we plotted effluent concentrations against these two possible predictors for the bacterial indicators having sufficient reported data. Figure 3 shows that HRT is not a strong predictor of total coliform, fecal coliform or *E. coli* effluent concentration through influent concentration could explain some variation (R2 of 0.91, 0.26, 0.77 for total coliform, fecal coliform and *E. coli*, respectively). Based on these results, we would argue that the ability of constructed wetlands to meet indicator-based effluent standards is less a function of design and, assuming a minimum threshold of functionality is achieved (e.g. 3-5 day HRT), more of a function of the quality of water to be treated. Also, based on the large variability in effluent concentration of 8 log for total coliform and 6 log for fecal coliform and *E. coli*, the ability of GW constructed wetlands alone to produce effluent meeting even restricted reuse standards is unreliable.

**Figure 3a, b, c, d, e, f**

While the ability of water treatment technologies to meet existing reuse standards (i.e. bacterial concentration-based) is important from an existing regulatory perspective, recent work in microbial risk assessment (Jahne et al., 2016; Schoen et al., 2016) and more progressive treatment standards (e.g. CA Title 22) have begun to argue for more pathogen-specific treatment targets and require demonstration of reduction of traditionally hard to treat non-bacterial pathogens. Although, to our knowledge, no GW treatment wetland study has looked at the removal of protozoa, viruses or virus indicators, we can gain some insight if we expand our scope to mixed wastewater treatment wetlands.

Due to their larger size, the removal of protozoa has been shown to be largely a physical process, attributed to either sedimentation in FWS systems (Falabi et al., 2002; Gerba et al., 1999) or filtration in HSSF and VF systems (Redder et al., 2010). Accordingly, removal of these pathogens, the most commonly reported being *Cryptosporidium* oocysts and *Giardia* cysts, is generally quite good in constructed wetlands, particularly where sedimentation and filtration are promoted. For FWS systems, LRs of *Cryptosporidium* oocysts and *Giardia* cysts of 0.4 to 1.7 have been reported (Falabi et al., 2002; Gerba et al., 1999; Karpiscak et al., 1996). For HSSF systems, LRs of the same pathogens of 0.2-3 have been reported (Nokes et al., 2003; Quinonez-Diaz et al., 2001; Redder et al., 2010).

For virus reduction, data from mixed wastewater treatment wetlands mostly deal with a variety of bacteriophages, which are used as virus indicators. Just as with bacteria indicators, these bacteriophages (a virus that infects and replicates within a bacterium) are similar in size and structure to enteric viruses, do not infect humans, are detectable by simple, rapid and inexpensive methods, and often are more environmentally persistent than enteroviruses (Burge et al., 1981; Havelaar et al., 1993; IAWPRC, 1991; Kapuscinski and Mitchell, 1983). In order of increasing specificity, the most commonly reported bacteriophages include somatic and f-specific coliphages – types of bacteriophages that infect *E. coli*, the latter being closer in size to human enteric viruses (Cramer et al., 1976); male-specific 2 (MS2), an easily culturable type of f-specific RNA coliphage; and PRD1, a double-stranded DNA that is easily culturable, similar in size to the human rotavirus and adenovirus (Olsen et al., 1974; Rusin et al., 2000) and has been shown to persist longer in the environment than MS2 (Vinluan, 1996; Yahya et al., 1993).

Similar to bacteria, adsorption onto plant and media surfaces is a primary mechanism of virus removal in wetlands (Garcia et al., 2010; Jackson and Jackson, 2008), often occurring within the first hours of entering the wetland (Hodgson et al., 2003; Quinonez-Diaz et al., 2001). Sedimentation is generally not a significant virus reduction mechanism; qPCR analysis has shown that the majority of culturable enteroviruses are not associated with particles (Symonds et al., 2014) and wetlands that rely on pathogen removal via sedimentation have shown poor virus removal performance (Falabi et al., 2002).

In VF and HSSF systems, coliphage LRs have been shown to range from 0.2 to 2 (Falabi et al., 2002; Hench et al., 2003; Thurston et al., 2001; Torrens et al., 2009; Ulrich et al., 2005). Demonstrating the importance of media contact time, Torrens et al. (2009) observed roughly double the LR of both somatic and f-specific coliphages in VF systems with a depth of 65 cm compared to identical systems of depth 25 cm (0.7-1.3 and 0.2-0.7, respectively). They also observed larger LR of somatic coliphages compared to f-specific coliphages, supporting the notion that f-specific coliphages are a more conservative and appropriate indicator. Using seeded MS2, Chendorain et al. (1998) found an overall LR of 1.6 in a FWS system with a 9 day HRT and found removal rates to be bimodal with the majority of reduction occurring in the first 3m of the 30m long wetland. Gersberg et al. (1987) used seeded MS2 alongside seeded poliovirus in a HSSF system operated at a HRT of 5.5 days. They found overall LR of 2.7 for MS2, compared to 3.0 of poliovirus, supporting the conservative nature of the indicator. They also found greater removal of indigenous f-specific coliphages in vegetated vs unvegetated cells, with observed LRs of 2.0 and 1.3, respectively. Using indigenous MS2 as well as seeded PRD1 in floating macrophyte FWS systems, Karim et al. (2004) calculated decay rates for each indicator in both the water column and sediment. They found that wetland sediments prolong the survival of viral indicators and that PRD1 is a more conservative indicator, with water column and sediment decay rates of 0.397 d-1 and 0.107 d-1 for MS2 and 0.198 d-1 and 0.054 d-1 for PRD1. Lastly, Vidales-Contreras and colleagues have performed a number of tracer experiments with seeded PRD1 and bromine that largely corroborate the findings discussed above. They have shown that PRD1 persists for longer than MS2 in HSSF wetlands and is thus a more conservative indicator (Vidales et al., 2003), that overall reduction rates are greater than inactivation rates (decay rates of -1.17-d vs. -0.16-d) suggesting that physical removal via initial adsorption is a more important than virus inactivation in HSSF systems (Vidales et al., 2003), and that plant and media surface area are critical to efficient virus reduction, observing greater reduction of PRD1 in HSSF than FWS systems (Vidales-Contreras et al., 2006), reporting a PRD1 LR of 2 in a 6 year old HSSF system operated at a HRT of 5.5 days. Taking into account the conservativeness of PRD1 that has been demonstrated, this would suggest that for a properly designed HSSF system operated at a HRT of 3-5 days, 1-2 LR of virus can be achieved.

To reiterate, we do not intend to argue that constructed wetlands as a single unit process can reliably meet microbiological reuse standards, just as there is no expectation that traditional primary, secondary or even tertiary wastewater treatment unit processes can meet microbiological standards, as evidenced by the need for final disinfection unit processes at wastewater treatment plants. However, we have shown that so long as constructed wetlands achieve an HRT of 3-5 days, restricted criteria for BOD and TSS of 30 mg/L can be achieved and turbidity will generally be below 20 NTU. Moreover, RVF systems can reliably achieve BOD, TSS and turbidity levels of less than 5 mg/L, 5 mg/L and 10 NTU, respectively. With respect to pathogens, wetlands can be expected to provide approximately 1-2 LR for bacteria, protozoa and viruses, though the reliability of these removals is somewhat questionable. Thus, we now turn our attention to viable disinfection unit processes that can be combined with constructed wetlands to reliably achieve the microbiological reductions required.

**Greywater Disinfection Processes**

Similar to the review performed for GW constructed wetlands, we searched the literature for studies evaluating the effectiveness of common disinfection technologies applied to treated GW. Disinfection technologies reviewed included chlorination, ozone and ultraviolet radiation (UV). Although we found only two cases that disinfected wetland effluent directly (El Hamouri et al., 2007; Winward et al., 2008c), we kept our scope limited to GW, with studies that evaluated raw and treated GW including treatment by fine filtration, coarse filtration, cartridge filtration, sand filter (SF), rotating biological contactor (RBC) membrane batch reactor (MBR) and horizontal subsurface flow constructed wetland (HSSF). A total of 9 papers were found from which 85 individual trials were extracted.

Tables 4a and 4b show the results of the literature review, with Table 4a providing relevant experimental details including authors, type of disinfection, dosage, type of water being treated, and quality of water being treated. Table 4b, linked to Table 4a through the ID column, shows the disinfection results for the available pathogens or pathogen indicators reported. In all, data were found for bacterial indicators (total coliform, fecal coliform, *E. coli* and *Enterococci*), a bacteriophage virus indicator (MS2 coliphage), an enteric bacterium (*Salmonella enterica*) and two opportunistic bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*)*.*

**Tables 4a and 4b**

Of the water quality parameters in Table 4a, turbidity was the most frequently reported. In the context of disinfection, turbidity can be considered a general indicator of water quality that may influence the effectiveness of disinfection technologies. For chlorine and ozone, turbidity can be an indirect measurement of the presence of organics, which can both quench the reactive species of the applied chemicals and, in the case of particle-associated pathogens, protect the pathogens from disinfection (Benami et al., 2016; Dietrich et al., 2007; Hunt and Marinas, 1999; Janex et al., 2000; Lechevallier et al., 1981; Xu et al., 2002). For UV, turbidity is a measurement of constituents that can attenuate the radiation making it less effective and, similar to ozone and chlorine, can shield particle-associated pathogens from disinfection (Benami et al., 2016; Templeton et al., 2005; Winward et al., 2008c). Thus, producing effluent of low turbidity is important for maintaining disinfection effectiveness.

If we exclude the trials from Ekeren et al. (2016), which intentionally tested water of high organic carbon content, all disinfection trials treated water with turbidity values less than 10 NTU. If we compare these trials to the effluent produced by wetlands in Table 3a, we see that not all wetlands were able to produce water with these low turbidity values. However, as previously discussed, the RVF systems were able to reliably achieve turbidity levels of less than 10 NTU. This is not to say that effluents of greater turbidity cannot be disinfected but, as we will discuss below, the effectiveness of the disinfection technologies may be reduced, requiring either higher chemical dosages or greater radiation levels.

Figures 4 and 5 show the results of the reviewed disinfection trials for chlorination and UV. Although we included ozone trials in Table 4, we will only discuss in detail chlorine and UV as disinfection options as i) chlorine and UV have been more widely tested for GW applications (Winward et al., 2008b, c), ii) ozone tends not to perform as well as chlorine and UV (Ekeren et al., 2016) and iii) life cycle costs for ozone disinfection systems are greater than chlorine and UV (Beck et al., 2013).

***Chlorine Disinfection Trials***

Figure 4 shows the results for chlorine disinfection trials, which all used sodium hypochlorite specifically. Figures 4a and 4b show effluent concentrations as a function of chlorine residual concentration multiplied by contact time (CT). Figure 4a shows bacterial indicators relevant to Table 1 criteria and standards (as well as the 200 CFU/100mL fecal coliform guideline for reference), while Figure 4b shows pathogenic bacteria and MS2 coliphage. In general, most parameters for which effluent concentrations were reported showed full reduction (i.e. effluent concentration either reported as zero or inactivated) at dosages greater than 90 mg/L-min, the lowest prescribed CT from Table 1. Total coliform trials that did not result in full inactivation were reported by Winward et al. (2008b), in which observed tailing at CTs of 60-150 mg/L-min was attributed to ‘robust particle sheilding’ – TSS and turbidity of the tested water were 29 mg/L and 20 NTU, respectively. This was despite an initially high chlorine dosage of 80 mg/L. In contrast, Ekeren et al. (2016), who specifically tested high organic waters (Turbidity 26-37 NTU), found full inactivation of *E. coli* and *S. enterica* at CTs of 180-474 mg/L-min. Although they applied greater CTs, Ekeren et al. (2016) attributed the observed disinfection efficiency to a phenomenon observed by others (Dietrich et al., 2003; Winward et al., 2008b) in which water with greater organic content allows for greater initial chlorine dosages while not exceeding residual limits (a residual greater than 4 mg/L will damage metal fixtures), and greater initial chlorine dosages increase chlorine particle penetration to inactivate particle-associated microorganisms. Fecal coliform trials that did not result in full inactivation were reported by Friedler et al. (2011), with average fecal coliform effluent concentrations of 2.1 and 2.0 CFU/100 mL resulting from a contact time CT of 15 and 30 mg/L-min, respectively. It should be noted however that these results are after a contact time of 0.5 hours; after a contact time of 6 hours in which residual chlorine remained between 0.5 and 1.0 mg/l, average effluent fecal coliform concentrations were reduced to less than 1 for both dosages.

**Figures 4a, b, c, d**

Figures 4c and 4d show the total LRs achieved as a function of chlorine CT. Before we continue, we note that LR data for *E. coli* in Figure 4c, as well as all *S. enterica*, all MS2 coliphage and the *P. aeruginosa* data point at 474 mg/L-min in Figure 4d are the results of artificial seeding. This was done in order to better explore the full disinfection capabilities of the approaches tested, without being constrained by influent concentration. Figure 4c shows that for bacterial indicators, LRs are variable at CTs less than 100 mg/L-min and potentially limited by influent concentration. At CTs up to around 450 mg/L-min, we see LR of *E. coli* of about 6.5 from Ekeren et al. (2016). LRs for total coliform and *Enterococci* at 450 mg/L-min are limited by influent concentration, which we can see if we compare to the parallel trials from these studies that achieved full inactivation at 68 mg/L-min.

Figure 4d shows LR data for pathogenic bacteria and MS2 coliphage. Ekeren et al. (2016) showed that LR of *S. enterica* greater than 7 could be achieved at a CT greater than 180 mg/L-min, and LR of *P. aeruginosa* of greater than 7 could be achieved at a CT of 474 mg/L-min. Friedler et al. (2011) showed that lower CTs of 15-30 mg/L-min resulted in LRs of the opportunistic bacteria *S. aureus* and *P. aeruginosa* of 1-2, noting that higher initial chlorine doses were more effective at inactivation of *S. aureus* than *P. aeruginosa*. Other studies supported this, finding *P. aeruginosa* to generally be the bacterium most resistant to chlorination (Blanky et al., 2015). Friedler et al. (2011) also showed that given a low initial chlorine dose (less than 5 mg/L), *P. aeruginosa* tended to persist after 6 hours of storage despite maintenance of a residual concentration of 0.5-1.0 mg/L.

Data for MS2 coliphage inactivation come from Beck et al. (2013) and Ekeren et al. (2016), who tested relatively low organic and high organic GW, respectively. Whereas Ekeren et al. (2016), somewhat counterintuitively, generally achieved greater bacterial inactivation (see discussion above) they were only able to demonstrate LR of MS2 coliphage of 3.8 at a CT of 474 mg/L-min compared to LR of 5-7 at CTs of 200-450 mg/L-min demonstrated by Beck et al. (2013). This suggests that in terms of chlorine disinfection of viruses, removal of organics is critical in meeting standards such as the 5 LR required by California Title 22 ((CDPH), 2010).

***UV Disinfection Trials***

Figure 5 shows the results of the UV disinfection trials. Similar to Figures 4a and 4b, Figures 5a and 5b show effluent concentrations plotted against UV dosage. For dosages less than 100 mJ/cm2, effluent concentrations are variable, though all fecal coliform concentrations fall below the 200 CFU/100mL guideline for restricted and environmental reuse from Table 1. Above 100 mJ/cm2, the only bacterial indicator that did not show full inactivation was total coliform from an experiment by Winward et al. (2008c). In this particular trial, they used untreated GW from bathroom sinks and showers and found that particle shielding created a tailing effect, particularly for total coliform. While *E. coli* and *Enterococci* were fully inactivated at a dosage of 277 mJ/cm2, total coliform persisted at a mean concentration of 1.0 log10MPN/100mL. The authors even increased the dose to 1107 mJ/cm2 and still observed a mean concentration of 0.9 log10MPN/100mL (data not shown), giving evidence to the strength of the particle shielding effect against UV inactivation.

**Figures 5a, b, c, d**

Figures 5c and 5d show the total LRs achieved as a function of UV dose. Again we note that some LR data for *E. coli* in Figure 5c (5.5 LR at 21-28 mJ/cm2), as well as all *S. enterica*, all MS2 coliphage and the 7.1 LR for *P. aeruginosa* in Figure 5d are the results of artificial seeding. Of the non-seeded bacterial indicators, we see a LR of approximately 2-4 around a UV dosage of 100 mJ/cm2, noting that some of these LRs may be limited by influent concentration. For the seeded *E. coli* trials from Ekeren et al (2016) in which 5.5 LR (full inactivation) was achieved in high organic GW despite a dosage of just 21-28 mJ/cm2, we see that UV has the potential to achieve high initial LRs for this bacterial indicator even if particle shielding may be a factor, a conclusion also reached by Winward et al. (2008c) for *E. coli* and *Enterococci*.

Figure 5d shows a similar pattern for bacterial pathogens and MS2 coliphage, in which large LRs are achievable at dosages less than 100 mJ/cm2. LRs for *S. enterica* of 7.4-8 at dosages of 21-28 mJ/cm2 were achieved by Ekeren et al. (2016), while a full inactivation LR of 1.9 (non-seeded) for *S. aureus* was achieved by Friedler et al. (2011) at just 19 mJ/cm2. Similar to chlorine trials, *P. aeruginosa* proved somewhat variable. Ekeren et al. (2016) were able to achieve an LR of 7.1 at 26 mJ/cm2, while Friedler et al. (2011) required at least 39 mJ/cm2 to achieve the full inactivation LR of 1.9 (non-seeded). More to this point, in a treatment train composed of RBC and UV units designed to treat GW for toilet flushing, Friedler and Gilboa (2010) reported no statistical difference between average concentrations of *P. aeruginosa* from UV disinfected and undisinfected effluent. Although a considerable fraction of samples taken from the UV disinfection trials were below the detection limit for *P. aeruginosa* (not so for undisinfected samples), regrowth of the opportunistic pathogen masked these effects, resulting in a calculated negative average removal.

For MS2 coliphage, Beck et al. (2013) reported LRs of 3.9-5.9 at 100 mJ/cm2, achieving the California Title 22 standard of 5 log removal but not reliably so. Even at the low organic levels reported in these trials (turbidity from 1.4-6 NTU), tailing was suggested as the mechanism prohibiting full MS2 inactivation (influent concentrations of 5.6-8.2 log). However, as noted by the authors, MS2 is significantly more resistant to UV disinfection than poliovirus (Meng and Gerba, 1996; Shin et al., 2005), meaning that the LRs achieved at 100 mJ/cm2 would likely meet the California criteria for treatment. Trials conducted at 26 and 30 mJ/cm2 by Ekeren et al. (2016) and Beck et al. (2013) support the need for higher dosages for viruses, reporting MS2 LRs (not fully inactivated) of 2.7 and 1.2-1.8, respectively.

**Conclusions**

In all, we have shown that evidence exists to support the assertion that constructed wetlands, combined with appropriate disinfection unit processes, can treat GW and provide effluent appropriate for certain reuse applications. If designed with a HRT of 3-5 days, single-pass constructed wetlands can generally meet restricted reuse chemical/physical standards and, with respect to BOD and TSS, tend to perform as well as, if not better than, we would expect using traditional design equations and rate constants. If recirculation is added, as in the case of the RVF systems, effluent can be reliably produced in which BOD, TSS and turbidity are below 5 mg/L, 3 mg/L, and 10 NTU, respectively, which meets USEPA guidelines for restricted and environmental reuse and all Western Australia physical/chemical criteria. We have also shown that wetlands can generally provide 1-2 LR of bacteria, protozoa and viruses, though used as a standalone unit process cannot reliably meet microbiological effluent standards.

In our review of GW disinfection experiments, we have shown that based on the available data, if organics are sufficiently removed from GW, chlorine CTs of 100 mg/L-min or UV dosages of 100 mJ/cm2 are appropriate for meeting all USEPA guidelines and all Western Australia guidelines, whereas higher CTs or dosages are likely required to meet California Title 22 criteria. In terms of pathogen LRs, chlorine CTs of 200-400 mg/L-min and UV dosages of 100-300 appear necessary to provide full inactivation.

While similar disinfection performance can be achieved with both chlorine and UV, we lastly call attention to a highly relevant study performed by Quanrud et al. (2004) who compared the formation potential of carcinogenic trihalomethanes (THM, a common disinfection byproduct) from wetland-derived organic matter and wastewater treatment effluent organic matter. They found that wetland-derived organic matter has a higher aromaticity, lower biodegradability, and higher chlorine reactivity than typical wastewater treatment plant effluent, resulting in greater THM formation potential following wetland treatment. Thus, combined with the fact that UV does not require dosage and storage units for chemicals and may be cheaper than equivalent chlorination systems (Beck et al., 2013), UV seems particularly suited as a disinfection unit process to be combined with smaller scale, GW constructed wetlands.

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