INNOQUA is demonstrating how nature-based solutions can treat wastewater to a standard at which it can be safely discharged back to the environment or used for irrigation purposes. This technical bulletin examines disinfection techniques to reduce pathogen loads in treated wastewater, before it is re-used for irrigation or other purposes.

INTRODUCTION – THE NEED FOR DISINFECTION

Wastewater not only contains chemical hazards that can lead to water pollution and eutrophication, it also contains biological hazards such as enteric bacteria, viruses and protozoan cysts that are associated with human diseases (USEPA, 2003). Table 1 shows some of the infectious microorganisms that can be present in raw domestic wastewater, and the diseases that can result from infection.

Table 1 Infectious microorganisms potentially present in raw domestic wastewater. Adapted from USEPA, 2003

Type	Organism	Disease caused
Bacteria	*Escherichia coli*	Gastroenteritis
	Leptospira spp.	Leptospirosis
	Vibrio cholerae	Cholera
Protozoa	*Cryptosporidium parvum*	Cryptosporidiosis
	Giardia lamblia	Giardiasis
Viruses	Hepatitis A virus	Infectious hepatitis
	Rotavirus	Gastroenteritis

Typical wastewater treatment comprises preliminary and primary stages incorporating mechanical screening and physical settlement, followed by secondary biological stages. Primary treatment uses gravity to settle out larger particulate material, while secondary treatment relies on microorganisms to remove the un-settleable and soluble contaminants via biological predation, assimilation and oxidation (Tchobanoglous, Burton, & Stensel, 2004). Following a final settlement or clarification stage, the treated wastewater is often discharged into receiving water bodies or (sometimes) used for agricultural purposes. Neither primary nor secondary treatment are specifically designed to remove biological hazards[1], which means that the partially treated wastewater almost certainly is a source of pathogens (Table 2).

Although regulatory consents to discharge wastewater into receiving water bodies (such as lakes, rivers and the sea) do not normally include pathogen limits, there are regulatory limits for the quality of those receiving bodies – particularly when used for recreational purposes such as bathing. Examples for the USA (USEPA, 2012) and EU (OJEU, 2006) are presented in Table 3. Various authorities have also developed guidance and standards for pathogen levels in different wastewater uses – including irrigation of edible crops. Table 4 summaries the irrigation water standards as recommended by the WHO, USEPA and EU.

[1] An exception being the use of Membrane Bioreactors (MBR), in which the membranes physically exclude suspended solids and microorganisms from the treated wastewater.

Table 2 Faecal indicator organism concentration of wastewater that has undergone different treatments. Adapted from Kay et al., 2008

Type	Treatment Level	Mean Total Coliform population, cfu/100 ml	Mean Faecal Coliform population, cfu/100 ml	Mean Enterococci poopulation, cfu/100 ml
Crude Sewage	None	3.9×10^7	1.7×10^7	1.9×10^6
Primary Settled Sewage	Primary settlement	3.8×10^7	1.8×10^7	2.4×10^6
Septic Tank Effluent	Mainly primary settlement	2.5×10^7	7.2×10^6	9.3×10^5
Activated Sludge Effluent	Primary settlement and secondary biological treatment	7.8×10^5	2.8×10^5	2.4×10^4
Trickling Filter Effluent	Primary settlement and secondary biological treatment	1.4×10^6	4.3×10^5	4.1×10^4

Table 3 US Recreational Water Quality Criteria (RWQC) (USEPA, 2012) and EU Bathing Water Directive (BWD) criteria (OJEU, 2006)

Regulations	Water Type	Grade	Intestinal enterococci (cfu/100 ml)	*Escherichia coli* (cfu/100 ml)
BWD	Inland	Excellent[1]	200	500
		Good[1]	400	1000
	Coastal	Excellent[1]	100	250
		Good[1]	200	500
RWQC	Marine and Fresh	Illness Rate 32/1,000[2]	110	320
		Illness Rate 36/1,000[2]	130	410

1: 95th percentile
2: 90th percentile

Table 4 Pathogen limits applied to treated wastewater used for crop irrigation (Alcalde-Sanz & Gawlik, 2017; USEPA, 2012; WHO, 2016)

Limits	Wastewater use	Source
≤1,000 *E. coli* per 100ml	Root crops	WHO
≤10,000 *E. coli* per 100ml	Leaf crops	
≤100,000 *E. coli* per 100ml	Drip irrigation of high-growing crops	
No detectable faecal coliforms / 100ml	Surface or spray irrigation of crops intended for human consumption without prior processing	USEPA
≤200 faecal coliforms / 100ml	Surface irrigation of crops intended for human consumption following commercial processing	

Limits	Wastewater use	Source
≤10 *E. coli* per 100ml (or below detection limit)	Any irrigation method for all food crops, including those intended for human consumption without prior processing	EU*
≤100 *E. coli* per 100ml	Any irrigation method for crops intended for human consumption following processing – or for crops intended for consumption raw, where the edible portion is not in direct contact with the irrigation water	
≤1,000 *E. coli* per 100ml	Drip irrigation of crops intended for consumption raw, where the edible portion is not in direct contact with the irrigation water; Drip irrigation of crops intended for human consumption following processing	

*Proposed

Although treated wastewater is likely to be significantly diluted when it discharges into a bathing water body, a substantial population of pathogens could still be present, giving rise to health and safety concerns. Likewise, the use of inadequately treated wastewater for irrigation can give rise to significant human exposure to faecal pathogens, potentially resulting in serious illness. In both cases disinfection – which is the process for inactivating and destroying pathogenic organisms – is needed to prevent the spread of waterborne diseases.

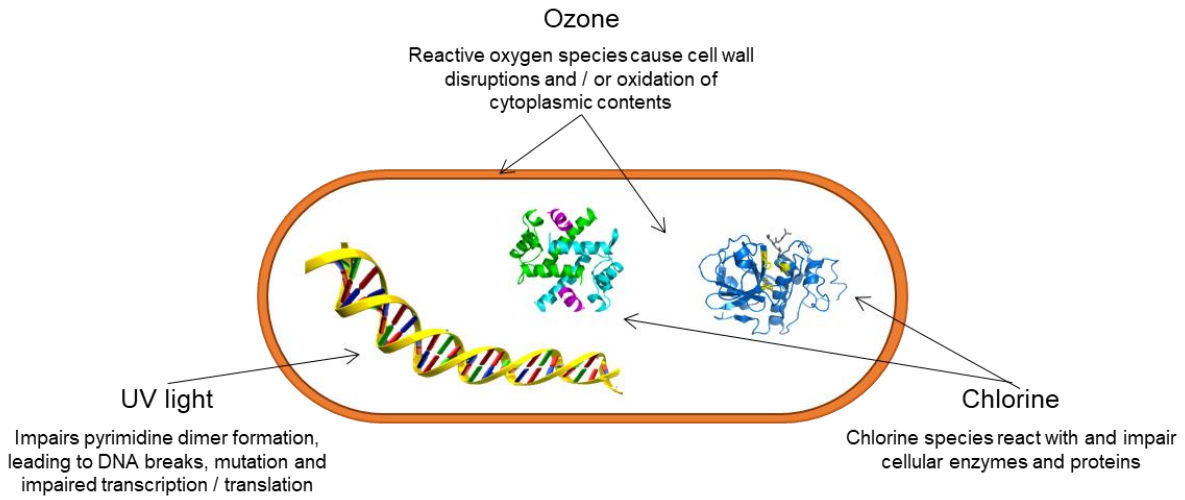
DISINFECTION METHODS

Disinfection uses chemical and/or physical measures to inactivate or destroy pathogens through the following five principal mechanisms (Tchobanoglous et al., 2004):

1. Damage the cell wall;
2. Alter cell permeability;
3. Alter the colloidal nature of the protoplasm;
4. Alter the DNA and RNA of the organism; and
5. Inhibit enzyme activity.

Common disinfection methods include chlorination, ozonation and ultra-violet (UV) disinfection (USEPA, 2003, 2011) – their modes of action are outlined in Figure 1.

Figure 1 Modes of action for common disinfection approaches. Adapted from: Russell, Furr, & Maillard (1997) and Bhilwadikar et al., (2019)



For all of these methods to be effective, suspended solids, turbidity and other interfering properties normally need to be reduced to below target thresholds. For this reason, disinfection treatment is usually located at the end of the treatment chain (USEPA, 1999b, 1999a, 1999c, 2011).

CHLORINATION

Chlorination is a generic term for disinfection processes that use chlorine (Cl) and chlorine derivatives, such as NaClO, $Ca(ClO)_2$ and ClO_2 (USEPA, 2011). Elemental chlorine, the hypochlorite anion (ClO^-) and ClO_2 are strong oxidants. They can oxidise the cellular material of the target organism, modify cell wall permeability and precipitate proteins – altering and inactivating enzymes to achieve pathogen inactivation (Tchobanoglous et al., 2004).

Chlorination has historically been conducted by dosing Cl_2 gas into water, but the health and safety implications of handling such a toxic compound mean that the hypochlorite salt, NaClO or $Ca(ClO)_2$, is now favoured. This is safer and easier to handle, and is free from the risk of Cl_2 gas leakage (USEPA, 1999a, 2011). Commercially, sodium hypochlorite is supplied as an aqueous solution – but it can also be produced onsite via electrolysis of diluted high purity sodium chloride solution (which also raises health and safety concerns, since a by-product of this electrolysis is hydrogen gas). The ClO^- ion and its associated acid, the hypochlorous acid (HClO), are both effective disinfectants – although HClO is a much stronger oxidant, and hence a much stronger disinfectant. This means that hypochlorite disinfection is most effective under neutral to acid conditions (USEPA, 2011).

Chlorination is effective at removing bacteria, viruses, and bacterial spores – but does not readily inactivate protozoa, especially *Cryptosporidium* spp. (USEPA, 2003, 2011). In addition, chlorine or chlorine related residues in the treated wastewater may have toxic effects on aquatic organisms in the receiving water body. This is why reductants such as sulphur dioxide, sodium sulphite and sodium bisulphite are dosed into chlorine-treated wastewater – to react with the residual oxidative disinfectant to form the non-harmful chloride ion (Tchobanoglous et al., 2004; USEPA, 2003, 2011). Overall kill of *E. coli* with chlorination can range from 2.0 to 6.0 $\log_{10}$ (Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, & Australian Health Ministers Conference, 2006).

OZONATION

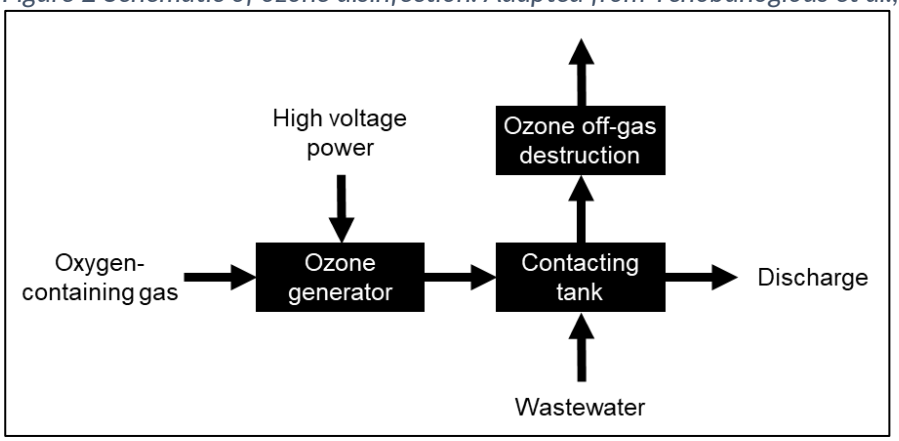
When oxygen molecules (O_2) are dissociated by a suitable energy source, then oxygen atoms can be formed. If an oxygen atom collides with an oxygen molecule, then ozone (O_3) is generated. In nature, lightning during a thunderstorm can produce ozone. When ozone is dosed into water, the following reactions can occur (Tchobanoglous et al., 2004):

1) $O_3 + H_2O \rightarrow HO_3^+ + OH^-$
2) $HO_3^+ + OH^- \rightarrow 2HO_2$
3) $O_3 + HO_2 \rightarrow HO + 2O_2$
4) $HO + HO_2 \rightarrow H_2O + O_2$

HO_2 and HO are free radicals with potent oxidising abilities and are considered the active disinfectant in ozonation processes. They can directly oxidise or destroy cell walls – causing leakage of cellular constituents – as well as damage and depolymerise nucleic acids (Tchobanoglous et al., 2004).

Ozone is unstable and will decompose shortly after generation, which is why it has to be produced on the site where it will be used. It is commonly produced by passing an oxygen-containing gas through a dielectric discharge held at between 6 and 20 kV. The feed gas can be either air or high-purity oxygen (Tchobanoglous et al., 2004; USEPA, 2011). A basic schematic of ozone disinfection is shown in Figure 2.

Figure 2 Schematic of ozone disinfection. Adapted from Tchobanoglous et al., 2004



Since ozone is also an irritatant and toxic gas, any residual ozone in the off-gas must be destroyed to prevent its discharge into the atmosphere. Since the destruction product is oxygen, the treated discharge gas is recycled for further ozone generation in some applications (Tchobanoglous et al., 2004).

Ozone is an effective bactericide and viricide, but is generally more expensive to implement than chlorination or UV treatment due to reqirements for a high voltage power supply, use of corrosion-resistant equipment and efficient gas / water contact systems (USEPA, 1999b). Overall kill of *E. coli* with ozonation can range from 2.0 to 6.0 $\log_{10}$ (Natural Resource Management Ministerial Council et al., 2006).

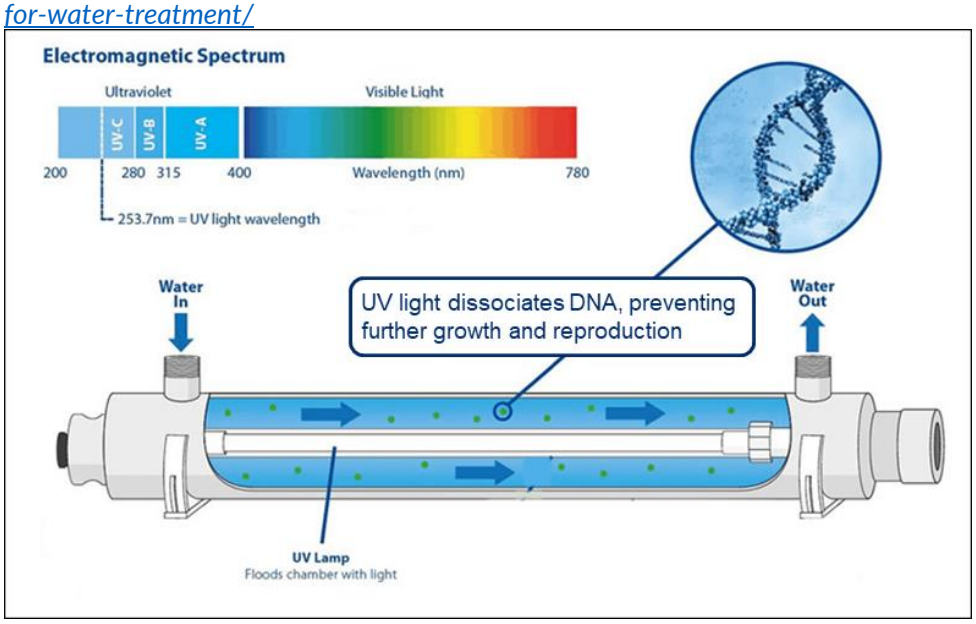
ULTRAVIOLET LIGHT

Ultraviolet light is a form of electromagnetic radiation with wavelengths from 10nm to 400nm (Tchobanoglous et al., 2004; USEPA, 2011). The optimum wavelengths for effective inactivation of microorganisms are in the range of 220 to 320nm, which largely co-incides with the UV-C spectrum (200-280 nm) (Tchobanoglous et al., 2004; USEPA, 1999c, 2011). UV radiation damages cells at the genetic level, preventing microorganisms from growing or reproducing (Tchobanoglous et al., 2004). DNA has the highest UV absorbance at a wavelength of ~260 nm (Tchobanoglous et al., 2004) – although commonly used low-pressure mercury vapour lamps emit monochromatic light at a wavelength of 253.7 nm (USEPA, 1999c).

Unlike ozonation and chlorination, which are chemical treatments, UV disinfection is a physical treatment. It requires no chemical preparation prior to disinfection, nor storage and handling of hazardous materials (USEPA, 1999c). As shown in Figure 3, UV disinfection takes place as wastewater flows through a unit in which the UV lamp is isolated within a highly (UV) transparent quartz sleeve. The 'contact' time is often less than a minute, which is much shorter than ozonation or chlorination (USEPA, 1999c). In addition, UV disinfection leaves no toxic residues in the treated wastewater, and requires no further disinfectant destruction steps. The simplicity and safety of the process make UV disinfection particularly 'user-friendly' (USEPA, 1999c).

However, for UV disinfection to be effective, the incoming wastewater must have a low concentration of total suspended solids (TSS), and the quartz sleeve must be regularly cleaned to ensure optimum UV penetration through the water. It is worth noting that UV is not as effective as chlorination and ozonation at inactivating viruses and bacterial spores – but is effective at inactivating bacteria like *E. coli* and protozoa such as *Cryptosporidium* spp. (USEPA, 1999c, 2011). UV kill of *E. coli* can range from 2.0 to >4.0 $\log_{10}$ (Natural Resource Management Ministerial Council et al., 2006).

Figure 3 UV disinfection schematic. From: https://www.alfaauv.com/blog/all-about-uv-disinfection-systems-for-water-treatment/



DISINFECTION AND THE INNOQUA PROJECT

As outlined in previous Technical Bulletins, the focus of the INNOQUA project has been development of nature-based wastewater treatment solutions suitable for on-site applications. In some potential markets the treated wastewater will be used for irrigation of edible crops, and it is therefore essential that a disinfection step is applied. Assuming that the treated wastewater contains *E. coli* populations of between 10^4 and $10^6 \log_{10}$ (based on treatment efficacies outlined in Table 2), then reductions of between 2 and $4\log_{10}$ would be required to bring populations down to a target of 10^2 (or 100 *E. coli* per 100ml). This population would be considered suitable for the treated wastewater to be used to irrigate processed crops in the USA and EU (Table 4). This degree of disinfection is not possible with conventional nature-based systems such as coconut or peat filters, or reedbeds – which typically deliver reductions of $2\log_{10}$ (Premier Tech Aqua, 2020; Tricel, 2020; Yorkshire Ecological Solutions, 2020).

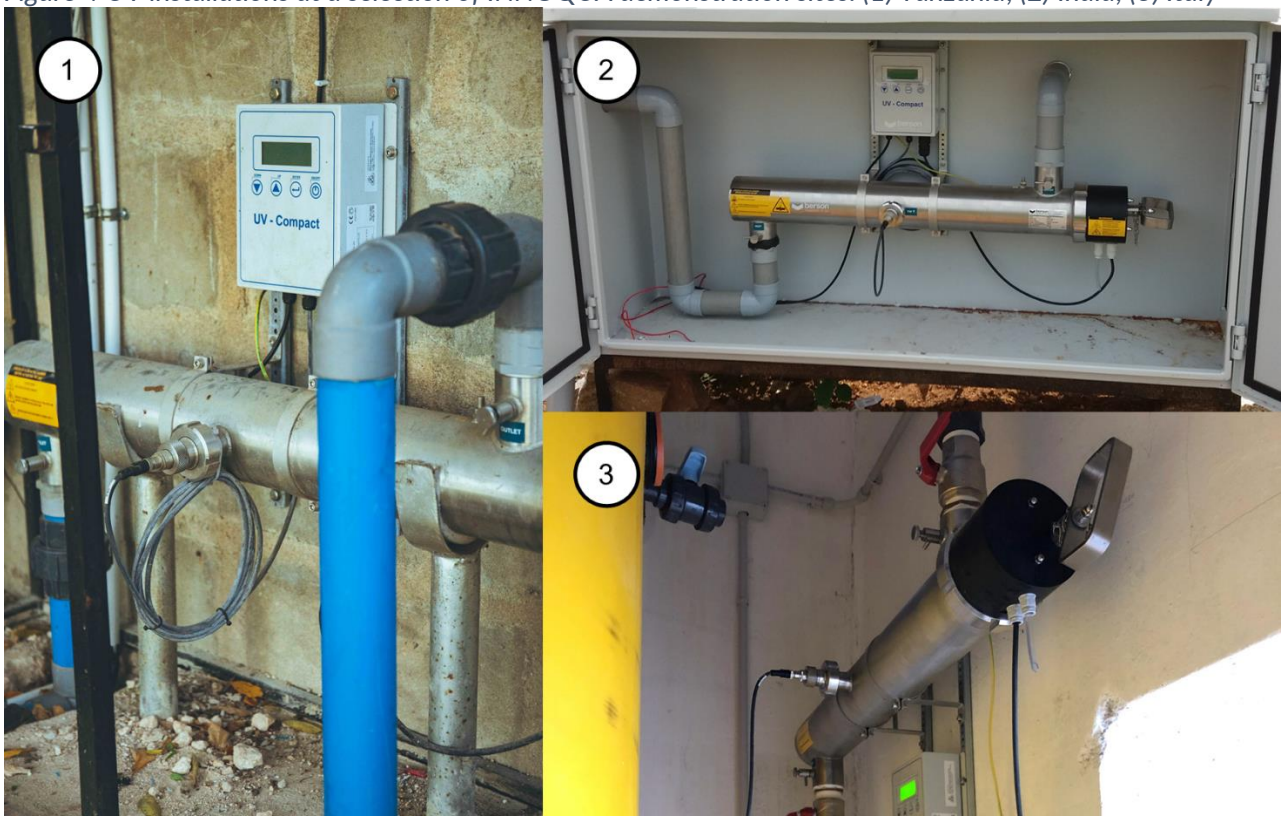
Although any of the common disinfection systems described in this bulletin would be capable of delivering the required treatment, UV was selected for the INNOQUA project as it is cost-effective, simple and reliable. UV disinfection units have been installed at several INNOQUA demonstration sites to test their efficacy at reducing pathogen loads in effluent from the different nature-based primary and secondary treatment systems (Table 5 and Figure 4). The use of UV in such applications is challenging, since flows are intermittent and of low volume – and wastewater clarity can be variable. Performance data will be explored in future Technical Bulletins.

Table 5 Information on the demonstration sites equipped with UV disinfection

Location	Source of wastewater	Preliminary treatment	Nominal daily flow (litres)
Italy	Domestic dwelling	Lumbrifilter	500
India	Domestic dwellings	Lumbrifilter and Daphniafilter	1,500
Peru*	Educational institution	Lumbrifilter and Daphniafilter	1,000
Turkey*	Domestic dwellings	Lumbrifilter and Daphniafilter	3,000
Tanzania*	Domestic dwellings	Lumbrifilter and Daphniafilter	1,500

*Reuse of the treated wastewater is taking place

Figure 4 UV installations at a selection of INNOQUA demonstration sites: (1) Tanzania; (2) India; (3) Italy



A series of open days and training events are planned for each of these sites[2]. If you would like to take part, arrange a visit – or simply know more about the local installation – then please contact the relevant site manager:

Country	Site manager	Contact details
Italy	Pietro De Cinque	pietro.decinque@de5.it
India	Tatjana Schellenberg	schellenberg@borda.org
Peru	Joshelyn Paredes-Zavala	joshelyn.pz@gmail.com
Turkey	Serkan Naneci	serkan.naneci@ekodenge.com
Tanzania	Evelyn Herrera Lopera	herrera@borda.org

In the next technical bulletin, we will explore performance data from the European demonstration sites and their ability to meet European wastewater treatment standards. Further details of the INNOQUA project can be found at www.innoqua-project.eu.

[2] These events will be physical and/or virtual, depending on prevailing guidance related to COVID-19

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