Pathogens and indicators in wastewater matrices

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As climate change and increasing population sizes continue to place stress on water resources, communities are increasingly looking to recycled water as a supplementary water source, whether for drinking water, domestic irrigation, industrial or agricultural use. Protecting public health by ensuring the safety of water supplies is a key concern for the water industry and health authorities.

Guidelines for the safe use of recycled water require monitoring for the removal of key enteric pathogens but these are reliant on traditional indicators such as *Escherichia coli (E. coli)*, coliforms and faecal coliforms to demonstrate the microbiological quality of the water. However, as with potable water, it is impractical and uneconomical to screen recycled water for every possible enteric pathogen. To reduce the costs of monitoring wastewater, a preferred option would be to use an indicator organism that correlated with the presence of a pathogen or class of pathogens [1] (also termed an index organism). However, finding such an organism is unlikely since it would require an exclusive association between the pathogen and indicator organism. Indicators such as *E. coli,* while valuable in the context of ensuring the safety of potable water supplies, are of less value in domestic wastewater applications because this matrix is faecally contaminated by default, so faecal indicators will always be present while pathogens may be absent.

The ideal indicator

The ideal indicator mimics the behaviour and characteristics of a pathogen but is itself easier and faster to isolate, culture or identify, is non-pathogenic and is a cheaper alternative to direct detection of the pathogen. The indicator should be present when the human pathogen is present and absent when the pathogen is absent, have similar environmental requirements, pattern of die off and susceptibility to disinfection. The presence or absence of the indicator makes its selection difficult as pathogens are not part of the normal microbial flora of the human system; they are only present in and excreted by infected individuals, with infection often being seasonal and related to prevalence within the community.

The continued operation of wastewater treatment plants requires constant monitoring of key parameters. The behaviour of different types of pathogens becomes problematic when selecting an indicator because it is unlikely that a single indicator will be representative of all pathogenic bacteria, viruses, protozoans and helminths, requiring indicator selection to be tailored for the pathogens and treatment process of interest. Traditionally, indicators have been used to suggest the presence of pathogens [2], although there is no direct correlation between numbers of any particular indicator and enteric pathogens [3].

Alternative indicators

Rather than selecting an indicator that is ideal for an individual pathogen, the opportunity exists to select an indicator that is representative of the process efficacy [1]. Process indicators are an organism or group of organisms that demonstrate the efficiency of a process, such as total heterotrophic bacteria or total coliforms for chlorine disinfection. Model or index organisms are a group or species that behave in a similar manner to the pathogen of interest. This relies on the model organism such as *E. coli* having similar survival in the environment in response to disinfectants such as *Salmonella.* The presence of the model organism in a treatment plant can provide an index for the presence of the specific pathogen [1].

Enteric pathogens in wastewater

As mentioned, enteric pathogen presence in wastewater is dependent on the level of community infection. The types and numbers of pathogens that enter wastewater are likely to be seasonal and, as such, not all pathogens will be detectable throughout the year. Seasonality is generalised and can vary depending on climatic conditions. This inconsistency and variability makes detection difficult and direct detection of pathogens from any water source tends to be time-consuming and expensive. As such, research to find suitable indicator microorganisms has been attempted by numerous groups around the world.

Bacterial pathogens and indicators

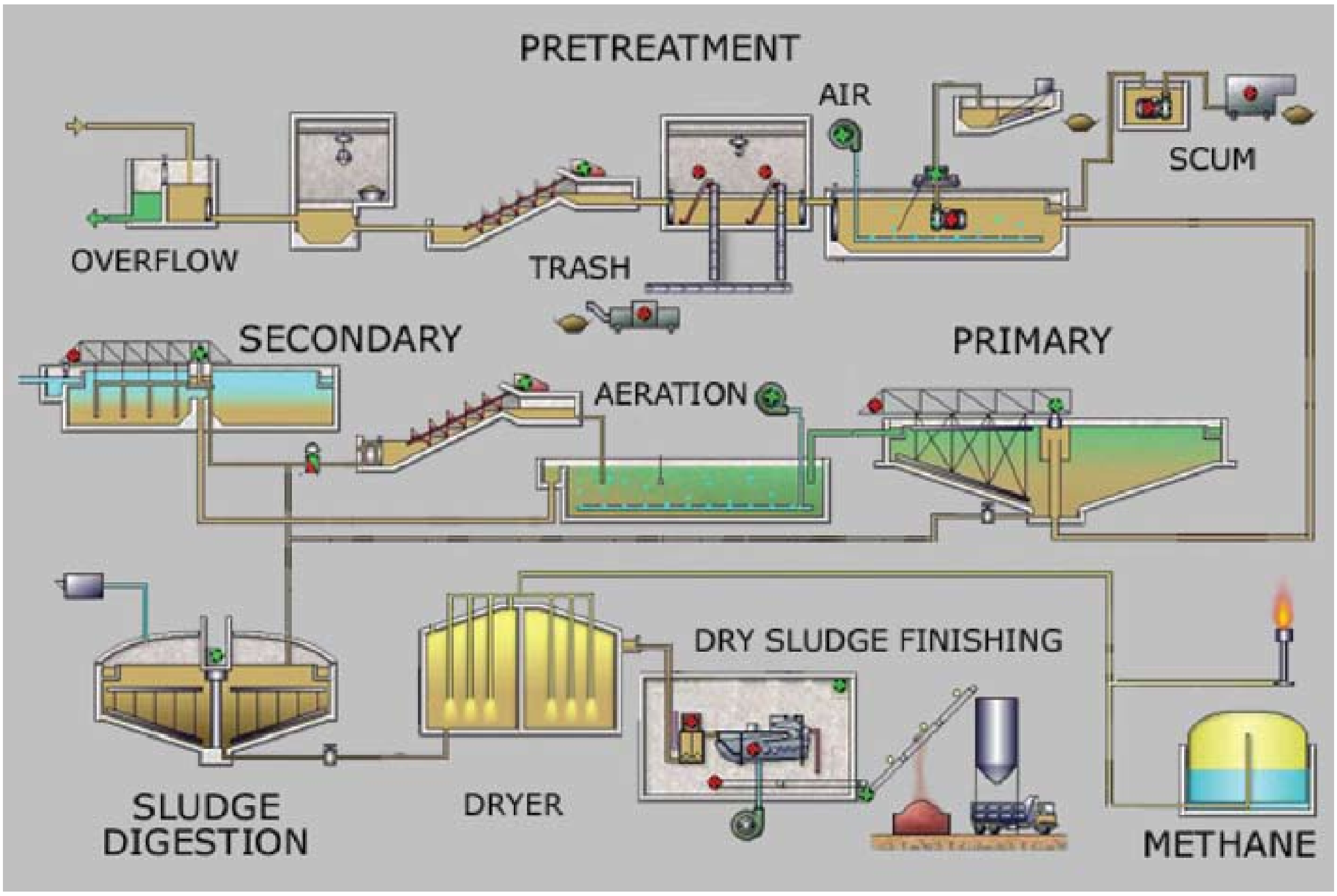
Bacterial pathogens are a major cause of gastroenteritis worldwide, with the leading cause of food-borne diseases from *Campylobacter, Salmonella* and *Shigella* [4]. The established methods for the detection of bacterial pathogens in wastewater is based on culture using artificial media, incorporation of selective agents or treatment to reduce background contaminants. Often, additional tests are required for confirmation of identity. The culture-based methods determine whether the cell is able to grow (in artificial conditions) but do not determine whether infection in a host is possible.

Campylobacter, Salmonella and *Shigella* are highly susceptible to standard disinfection processes, being more sensitive than *E. coli* to chlorine [5]. Therefore, this renders them less of an issue for the water industry provided treatment conditions are optimal [5]. In developed countries, the potential for issues only arises during system failure or upset, which can be due to heavy rains, breakdown or inappropriate monitoring.

Bacterial indicators include coliforms, enterococci (similar removal rates to coliforms [6]), *Bifidobacteria* and *Bacteroides fragilis.* All are non-pathogenic and present in high numbers in the human gut and faeces, but very little is known of the behaviour of *Bifidobacteria* and *B. fragilis* in wastewater treatment processes.

E. coli is considered to be an important bacterium to the water industry, both as a cause of water-borne outbreaks by *E. coli* 0157:H7 and as an indicator organism for the detection of faecal contamination. *E. coli* 0157:H7 has been reported in 31 outbreaks in the US between 1982-2002, accounting for 9% of all outbreaks by this pathogen [7]. Most facilities use faecal coliforms or total coliforms as an indicator, but neither group of organisms correlate with pathogenic bacteria removals (except *Salmonella)* [8].

Other bacteria of interest to the water industry include *Aeromonas hydrophila* (now listed on the United States Environmental Protection Agency Candidate Contaminant List (USEPA CCL)), *Klebsiella* species, *Pseudomonas aeruginosa* (potential for



Wastewater treatment plant, reprinted with permission from Josefpm, *Wikipedia.*

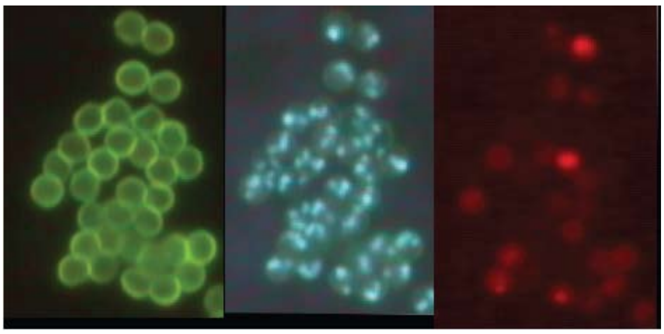
mucoid strains to be resistant to oxidant-based disinfection [9], *Mycobacterium* spp (listed on the USEPA CCL as an emerging pathogen and highly resistant to disinfection [10], and *Vibrio* species (although only in developing countries).

Clostridium perfringens offers a greater challenge because the spores are robust, survive longer, and are more heat- and chlorine-resistant than other bacteria in wastewater [11]. Harwood *et al.* [6] tested for *C. perfringens* at each point in the wastewater treatment process and determined that it was present in 93% of influent samples, 86% of biological treatment samples, 79% of filter effluent samples and 61% of disinfected effluent samples.

Protozoan pathogens and indicators

Protozoan parasites are numerous in wastewater, including *Cryptosporidium, Giardia, Entamoeba* and Microsporidia, which are of particular interest to the water industry. Methods are expensive and time-consuming, involving concentration from large volumes, purification using immunomagnetic separation and labelling with fluorescent antibody for enumeration under fluorescence microscopy. *Cryptosporidium* is highly resistant to chlorine-based disinfectants, has been implicated in a number of gastroenteritis outbreaks around the world, most notably Milwaukee, USA (1993) [12] and therefore has become highly important to the water industry. *Giardia,* although present at higher numbers than *Cryptosporidium,* has greater susceptibility to disinfection with chlorine and is therefore less problematic under effective operating parameters at wastewater treatment plants. Microsporidia is listed on the USEPA CCL as an emerging pathogen that can cause opportunistic infections. Although limited reports on water-borne outbreaks exist [13], Microsporidia has been detected in wastewater effluents [14]. Sensitivity to chlorine has been disputed, although John *et al.* [15] claim it is as sensitive to chlorine as *Giardia.*

Possible indicators for protozoa suggested in the literature include aerobic spores, anaerobic spores and particle profiling (particle size distribution). Spores have a greater resistance to chlorine than vegetative cells and as such can more reliably



Cryptosporidium **and** ***Giardia*** **observed under fluorescence microscopy and DIC.**

represent the disinfection of protozoa. Anaerobic spores such as sulphite reducing clostridia have been suggested as a surrogate for *Cryptosporidium* in wastewater, although numbers do not correlate with the pathogen [6]. Particle profiling has been developed as a useful tool for microbial detection in untreated raw wastewater where direct agricultural use is in place [16], with correlation between particle removal and the removal of faecal coliforms and *Salmonella* spp observed. This has so far been untested for protozoa and further data are required to validate the reported correlations.

Viral pathogens and indicators

Viral pathogens are a major cause of gastroenteritis worldwide. The established methods for the detection of viral pathogens in wastewater are based on concentration of virus particles followed by cell culture for culturable viruses and polymerase chain reaction (PCR) or reverse transcription PCR (RT-PCR) for non-culturable viruses. Culture-based methods are able to determine infection within an animal host cell, while the molecular methods such as PCR and RT-PCR are only able to determine virus presence (and potentially numbers) or absence, not infectivity.

Culturable viruses important in wastewater include many of the enteroviruses, a limited range of the adenoviruses and reoviruses (rotavirus). Non-culturable viruses include norovirus, rotavirus, human calicivirus, Hepatitis A virus, Hepatitis E virus and polyomavirus. Human infective viruses are unable to replicate in the environment as they require a suitable host. Viruses such as polyomavirus and reovirus can cause asymptomatic infection in childhood, with a high level of seroconversion in the community, but are not generally considered pathogens. Viruses in general are highly sensitive to disinfection with chlorine and as such are treatable within the wastewater treatment process.

A range of viral indicators, including bacteriophage, enteric virus genomes, poliovirus vaccine strain (now discontinued), polyomavirus and reovirus, have been suggested and tested through wastewater treatment processes. Bacteriophage offer the easiest option for enumeration as this is an agar plate based assay and is complete within 24 hours. However, Harwood *et al.* [6] found no correlation between coliphage and enteric virus removal by wastewater treatment processes (in particular filtration and disinfection). Alternatively, enteric virus genomes, although relying on recovery from effluents prior to PCR, offer a faster result because there is no culture step involved. Correlation between cultured virus and enteric virus genomes has not been demonstrated [17,18], and may potentially overestimate the health risk.

Helminth pathogens and indicators

Helminths have the highest prevalence in tropical and subtropical regions and areas with inadequate sanitation, usually in developing countries, but also occur in rural areas of the south-eastern United States [19]. Detection of helminth ova from wastewater involves either centrifugation or sedimentation, followed by flotation and examination by microscopy. Due to the size of helminth eggs, such as *Ascaris lumbricoides,* the majority are removed through sedimentation processes in wastewater treatment and thus become more problematic in biosolids [20]. Particle profiling has been reported as a useful indicator for the removal of helminths from wastewater, with a high correlation of R^2=0.98 observed between numbers of helminth ova and the volume of particles of 20-80 microns [16].

Conclusion

The selection of individual microorganisms as indicators for the presence and removal of pathogens is a difficult task. Due to the seasonality of pathogens circulating in the community, the selection of an appropriate indicator that behaves in the same manner as the pathogen is hindered because it will not mimic the pathogen presence and absence within the wastewater stream. As an alternative, the indicators can be used as conservative markers of pathogen removal, treatment efficiency or indicative of pathogen behaviour using the process indicator, faecal indicator and model/index organisms guide set out by Ashbolt *et al.* [1]. The need to improve detection of pathogens or improved indicators is important to water recycling in conjunction with the risk management approach adopted in the Australian Guidelines for Water Recycling [21].

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