Pathogen survival in recycled water

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Water shortages affect more than 2 billion people worldwide in over 40 countries, with 1.1 billion people living without sufficient drinking water [1]. Captured stormwater and treated wastewater can be used for supplementing non-potable water supplies. However presence of enteric pathogens in the reclaimed water can lead to potential health hazards [2].

Pathogens can be actively removed during residence in environmental systems such as rivers, reservoirs. Their survival is influenced by a range of factors in the receiving environment. Enteric viruses, followed by protozoan pathogens, are of concern in recycled water due to their relatively low infectious dose and resistance to disinfection processes. A better understanding of the major factors influencing the decay of pathogens in the environment will greatly assist risk-based regulatory frameworks that are required to minimise health risks from the use of recycled water.

The intended use of reclaimed water influences the decision the level of treatment required. The final pathogen type and numbers in the reclaimed water are determined by the type of water reclamation process used [3]. Secondary wastewater treatment processes such as coagulation, flocculation and sedimentation generally result in 2-5 log reduction in pathogen numbers [4]. However, infectious viruses and protozoan pathogens can survive secondary treatment and in some cases are present in the tertiary treated effluent [2, 5]. Advanced treatment technologies such as reverse osmosis and advanced oxidation appear as viable options, in particular for indirect potable re-use application. The combination of these treatment methods can result in more than 6 log reduction in pathogen numbers but is expensive to run [4].

It is generally accepted that pathogens lose viability in water and other environments over time [6-8]. A range of factors has been implicated in the inactivation of pathogens in reclaimed water including temperature [9], dissolved oxygen [10], organic carbon concentration [6], pathogen types [7] and autochthonous microorganisms [6]. This article presents data from three different water recycling projects where the effectiveness of natural processes to reduce pathogen numbers were studied.

The scenarios

Scenario 1: irrigation of sporting ovals with recycled water

The first scenario was a recycling scheme to provide water for irrigation of sporting ovals. The question to be answered was if there was a chance of bacterial pathogens persisting on the grass, thus posing an increased risk of skin infection from athletes using the ovals. It was also thought that knowledge of the degree of persistence of pathogens on the oval surface could assist in improving the management of the water recycling scheme.

The recycling system used secondary treated effluent from a local sewage treatment plant in Perth which had undergone rapid sand filtration and chlorination [7]. The ovals were irrigated late at night to minimise the potential of human contact and allow a minimal drying time prior to potential use of the ovals. To determine the actual decay times of selected bacterial pathogens on the ovals, survival experiments were undertaken, one during summer and one in winter. The experiments were done by irrigating selected areas of the grass around the sporting complex with treated effluent seeded with pathogens [7]. The sites varied by the amount of sunlight and shade. Grass surface and thatch samples were collected hourly and processed to determine the number of seeded pathogens remaining.

Scenario 2: managed aquifer recharge (MAR) of treated effluent

In the second scenario, the treated effluent was used for a managed aquifer recharge (MAR) scheme where treated water was recharged to the underlying aquifer via infiltration galleries [11]. The recharged water was recovered 50m down gradient for use

for green space irrigation. As with the first scenario, the potential presence of pathogens could pose a health risk so the ability of the aquifer to remove pathogens was assessed to enable a risk assessment to be undertaken. The survival experiment was done in a monitoring well located at 7m down gradient from the infiltration galleries. This well was drilled to a depth of 10.60m and was shown, through chemical analysis of the groundwater, that it was in the plume of the recharged water, thus ideally located for the pathogen inactivation study.

The inactivation experiments were done using nylon diffusion chambers (7-14mL capacity) fitted with 100K MWCO filters on both ends (Figure 1). The pathogens were placed inside the chambers, with groundwater taken from the MAR site. The diffusion chambers were designed to allow groundwater to pass through the chambers but to prevent the movement of the pathogens into the aquifer. A number of bacterial, viral and protozoan pathogens and indicator microorganisms were used in the diffusion chambers. Selected chambers were collected at predetermined times and the number of detectable microorganisms in the chambers were determined.

Scenario 3: impact of wetlands to treat urban stormwater

In the third scenario, stormwater was collected and treated in a wetland prior to injection into an aquifer (300m) under an aquifer storage transfer recovery scheme in Salisbury, South Australia. The aim of the project was to determine if urban stormwater could be captured and transformed into potable water using the treatment capacity of the wetland and aquifer. A major factor influencing this conversion to potable water was the ability of the wetland and aquifer to remove any microbial pathogens that might have been present; a survival experiment was undertaken in the wetland during the winter season when most stormwater was available for capture. A pathogen decay experiment was undertaken using diffusion chambers similar to those described above and again tested using a range of bacterial, viral and protozoan pathogens and indicator organisms.

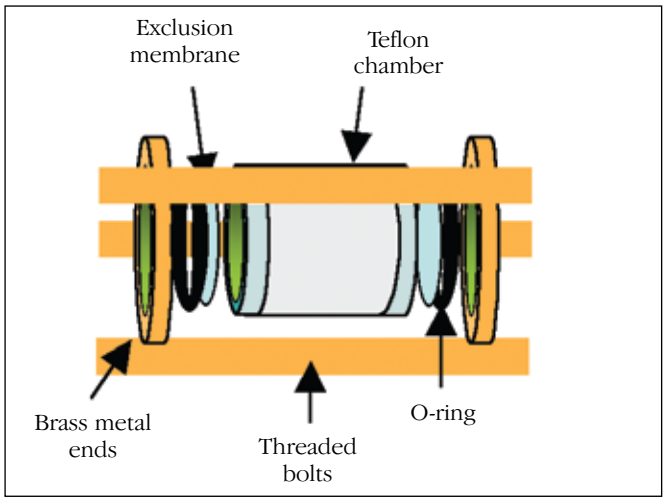

Figure 1. Schematic design of diffusion chamber.

Observations on pathogen decay

Our research has shown that pathogen inactivation occurred due to environmental processes in all three of the scenarios tested. The results indicate that different factors are most likely responsible for causing this decay in the different re-use schemes. Decay of the pathogens was faster on the grass surface irrigated with effluent compared with groundwater and wetland (Figure 2 and Table 1).

The pathogen inactivation on grass surfaces showed that inactivation was faster under sunlight during the summer compared with winter with 1 $\log_{10}$ reduction (T_{90}) varying from 3-12 hours (Table 1). Rapid inactivation of the bacteria on the grass surface irrigated with treated effluent was expected during the summer due to high ambient temperature and intensity of sun light. Slower inactivation during winter was possibly due to the combination of low temperature, high moisture and low solar radiation intensity compared with summer.

Inactivation of pathogens on the grass surface irrigated with effluent is primarily influenced by moisture content, sunlight and temperature, whereas in groundwater and wetland water chemistry, activity of indigenous microorganisms and temperature were found to have a dominant role. Unlike the impact of sunlight on grass surfaces, sunlight had no influence on pathogen survival in the groundwater and the wetlands (the wetlands are covered with shade cloth to deter birds). Thus other factors have a

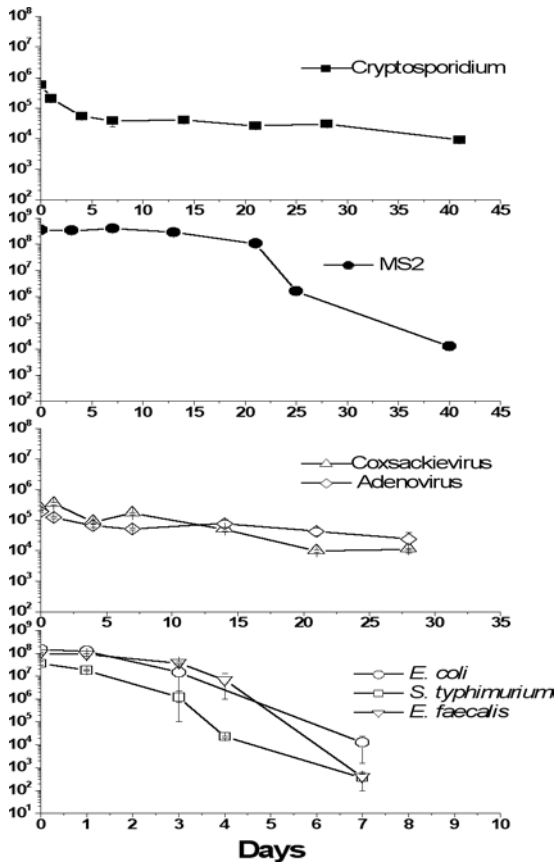

Figure 2. Inactivation of pathogens and indicator microorganism in groundwater during MAR with secondary treated effluent.

Table 1. One $\log_{10}$ (T_{90}) inactivation time of enteric microorganisms under three different water recycling processes.

Microorganism	Effluent on grass surface (hours) *Summer*	*Winter*	Secondary effluent (days) *Groundwater*	Stormwater (days) *Wetland*
E. coli	3.3	8.4	1	5
S. typhimurium	2.5	6.6	1	5
S. aureus	3.7	11.7	–	–
E. faecalis	4.2	7.7	1	6
MS2	14.3	12.5	14	–
Adenovirus	–	–	28	33
Coxsackievirus	–	–	18	–
Cryptosporidium	–	–	31	80

greater influence. The observed pathogen inactivation times were faster in the groundwater than in the surface wetlands. These differences are most likely due to the higher temperature of groundwater (22°C) than wetland (9°C). It has been established that autochthonous microorganisms contribute significantly to pathogen decay in aquatic systems such as aquifers [6]. It is also known that temperature has a secondary influence on the activity of these autochthonous microbes, thus explaining the differences between the inactivation rates observed in the groundwater and wetland areas [6, 12].

Pathogen type has also been noted to be important, with bacteria much more efficiently removed compared to the viruses and protozoa. In the schemes presented here, Cryptosporidium oocysts were the most resistant to decay. Thus it is important to also have a good understanding of the likelihood of different pathogen types to be present in any water source being considered for recycling.

Concluding remarks

In general, our studies have confirmed that environmental systems can be used to assist in the treatment of recycled water to achieve the removal of microbial pathogens. A range of different environmental factors can have an influence on the decay of microbial pathogens. The type of environmental factor that has the greatest influence depends on the environment where the recycled water is used or stored and local ambient conditions, in particular seasonal differences.

The type of pathogen that can be present in the recycled water is also very important. Our studies have shown that the greatest risks associated with pathogens in recycled water are the enteric viruses protozoan due to their resistance to environmental pressures and their low infectious dose. Results obtained to date indicate that inactivation of Cryptosporidium oocysts appears to be primarily temperature-driven and at higher ambient temperatures they may not be expected to survive long.

Research is continuing to further elucidate the role of different environmental processes have on the decay of a range of microbial pathogens. The results to date, however, have shown that environmental processes can result in a significant reduction in pathogen numbers and should be considered as effective barriers under the multiple barrier approach for risk mitigation.

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Jatinder Sidhu is a research scientist at CSIRO. His research interests include understanding the fate and behaviour of microbial pathogens in aquatic environments and biosolids.

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