145

Viability of nematode eggs in high rate algal ponds: the effect of physico-chemical conditions

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Abstract The viability of *Parascaris equorum* eggs was studied in two experimental pilot-scale high-rate algal ponds (HRAPs) working in parallel with 4 and 10 days hydraulic retention time respectively. Semi-permeable bags of cellulose (15000 daltons pore size) were used to study the effect of physico-chemical conditions on the survival of these helminth eggs. Three thousand eggs were used in each bag. Replicates of these bags were submerged for 4 and 10 days in the HRAPs and egg viability was compared with that in control bags submerged in sterile water. After 4 days exposure, 60% reduction in viability was achieved, reaching 90% after 10 days, much higher than the 16% and 25% found in the control bags for 4 and 10 days respectively. Ionic conditions of the HRAP may have been responsible for up to 50–60% of the egg mortality, suggesting that mortality due to the ionic environment could be more important than physical retention and other potential removal factors.

Keywords High rate algal pond; nematode eggs; osmotic conditions; *Parascaris equorum*; viability

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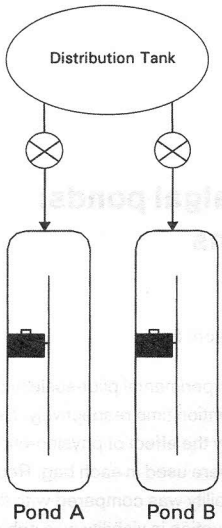
Introduction

Evidence on disease transmission associated with raw wastewater reuse points strongly to the helminths as the number one problem with only limited transmission of bacterial and viral disease (Shuval, 1991). The very low doses required to produce infection (Boutin, 1982), their cosmopolitan distribution and their long persistence in the environment (Feachem *et al.*, 1983) make helminths the main problem in wastewater reuse, especially in rural areas. International guidelines strongly recommend the use of low-cost highly efficient pathogen removal systems for wastewater treatment and waste stabilization ponds are the most efficient for the removal of helminth eggs (Schwardzbrod *et al.*, 1989, Shuval, 1991). High rate algal ponds have basically the same removal mechanisms as stabilization ponds for nutrient removal (Oswald *et al.*, 1988) and faecal bacterial indicators (García and Bécares, 1997), but the mechanisms underlying the removal of helminth eggs in these systems are still not clear. Sedimentation and environmental factors such as temperature may be the most important mechanisms also in HRAP. El Hamouri *et al.* (1994) showed that, for the same retention time, HRAP ponds were much more efficient than anaerobic ponds in the removal of helminth eggs, which suggests that other factors beside sedimentation could also be acting. In this study we used dialysis bags to investigate how ionic conditions in the HRAP could affect the viability of the nematode eggs, while excluding other removal factors such as sedimentation or predation.

Materials and method

The study was carried out during March 1998 in León, northwest Spain (latitude: 42°30′ N). Two identical HRAP pilot plants (see details in Figure 1) were designed according to Oswald (1963). Plants were continuously fed with wastewater from a village of 2000 inhabitants. Hydraulic retention times were 4 days for Pond A and 10 days for Pond B respectively; depth: 30 cm; length: 235 cm; width: 70 cm; surface area: 1.54 m^2; circulation velocity: 20 cm/s.

Parascaris equorum eggs were used in the experiment due to their high resistance to environmental exposure, their availability and a simple method for viability analysis



	Pond A	Pond B
Temperature*	8.6	9.3
DO (ppm)*	14.2	13.7
pH*	9.7	9.3
Conductivity ((S/cm)*	436	323
Ammonia (mg/l)	2.7	2.9
Orthophosphate (mg/l)	0.4	0.4

* Values at midday

Figure 1 Plan view of the pilot-scale high rate ponds and mean physico-chemical characteristics of the reactors

(Crompton, 1989). Nematodes were directly obtained from slaughterhouse animals, and the free eggs were dissected from the uterus of female worms. The eggs were washed and concentrated following Stien's (1989) protocol to obtain about 6000 viable eggs per ml. Eight cellulose semipermeable bags (15000 daltons pore size) were filled with 50 ml sterile water containing 3000 eggs. Four bags were submerged in each HRAP and removed after 4 and 10 days. The osmotic exchange of the bags had been previously checked with an osmolarimeter and no differences were found inside and outside of the bags. When removed from the bags, the eggs were settled and incubated at 25°C for 4 weeks in Petri dishes with agar to allow the eggs to develop. Eight control cellulose bags with eggs were also maintained in the lab at 4°C for 4 and 10 days and after incubation at 25°C viability was compared with the HRAP bags. Using the methodology of Caseres *et al.* (1987) the viability of the eggs and changes in their morphology were tested weekly during the four week incubation period using a microscope. The physicochemical variables of the reactors were analysed daily during the experiment.

Results and discussion

Average values of temperature, dissolved oxygen, pH, conductivity, ammonia and phosphates for each of the reactors are presented in Figure 1.

The viability of the eggs after 4 and 10 days exposure in the HRAPs and in the control bags are shown in Table 1. With regard to the environmental conditions of the control bags, our data suggest that cold and wet conditions have little effect on the mortality of the eggs. Storey (1987) showed that mortality for tapeworm eggs was the same at 4°C as at room temperature (9–18°C). This suggests that environmental conditions of the control bags are a good indicator of the natural mortality of the eggs with time.

Table 1 shows that mortality in the control bags slightly increased from 4 (16%) to 10 (25%) days exposure to lab conditions; this mortality is much lower than that observed in the HRAP bags. Conditions in the HRAP were responsible for a mortality increase of 44 percentage points after 4 days exposure and 64 percentage points after 10 days exposure. Non-parametric analysis of variance showed significant differences in the viability between control and HRAP and also between 4 and 10 days exposure of the bags in both the lab and HRAP environments.

Table 1 *Parascaris equorum* viability in HRAP after 4 and 10 days exposure

	4 days exposure		Control 4 days		10 days exposure		Control 10 days	
	Viable	Not viable	Viable	Not viable	Viable	Not viable	Viable	Not viable
Average	40%	60%	84%	16%	11%	89%	75%	25%
Standard dev.	1.08	1.08	1.26	1.26	1.38	1.38	1.15	1.15

Semi-permeable membranes only allow the exchange of small ions and water between the reactor and the eggs. Clarke and Perry (1980) have shown that the membrane of *Ascaris* eggs is also semipermeable during the first stage of their development; this is a possible explanation for the toxic effect of the HRAP water on the eggs. Other studies (Ghiglietti, 1996) propose ammonia as potentially toxic for *Ascaris*. In our case this parameter could be important, but only during the night. Other factors potentially responsible for egg inactivation could be pH (Carberry *et al.*, 1989), values of which reached 10 units, and solar radiation (Horák, 1994). Other potential effects on nematode mortality such as predation by zooplankton, microbial attack, or toxicity due to algal excretions (Zulkifli, 1992) can be discounted due to the experimental design. Sedimentation has commonly been cited as the main removal mechanism for helminth egg mortality, especially in waste stabilization ponds (Ayres *et al.*, 1992), with some evidence that this is also important in HRAP (El Hamouri *et al.*, 1994). Nevertheless the present results suggest that the effect of the physicochemical factors should be considered at least as important as physical retention.

Conclusions

This experiment has evaluated only the effect of ions and general osmotic conditions on the viability of nematode eggs in HRAP. This effect was found to be responsible for 50–60% of mortality, depending on exposure time. Thus mortality due to the ionic environment of HRAP could be least as important as physical retention and other potential removal factors.

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