Evaluation of *Scenedesmus quadricauda* as a surrogate of *Cryptosporidium* oocysts removal in direct filtration

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Abstract A comparative study on the evaluation of a *Cryptosporidium* removal surrogate was conducted using *Scenedesmus quadricauda*, green algae. Bench-scale direct filtration experiments were carried out at various initial concentrations: 500–5,000 oocysts/ml for *C. parvum* oocysts and 500–10,000 cells/ml for *S. quadricauda*. From the results, algal cell or *Cryptosporidium* oocyst counts of the filtrates (C) increased in proportion to their initial concentrations (C_0). However, no significant differences in C/C_0 profiles were observed over the examined range of the initial concentration, which implied that the removal efficiencies for *S. quadricauda* cells and *C. parvum* oocysts were not related to the initial concentrations. Examination of the deposition in the sand filter showed that a large part of *S. quadricauda* cells or *C. parvum* oocyst counts were captured in the upper layer of the sand filter, and the deposition rates were gradually reduced along the filter depth. Total cell or oocysts counts deposited in the sand filter increased commensurate with the initial concentration for both microorganisms. The ratio of the deposited cell or oocyst counts to the deposited amounts of flocs showed quite similar values between *S. quadricauda* and *C. parvum*, which meant that these two microorganisms were alike in their removal behavior. From these similar characteristics of removal, *S. quadricauda* was thought to be a reasonable and reliable surrogate of *C. parvum* oocysts removal in sand filtration.

Keywords Algae; *Cryptosporidium*; microbial removal; sand filtration; *Scenedesmus*; surrogate

Introduction

Contamination of raw and finished waters in drinking water supply and sewage by *Cryptosporidium parvum* oocysts has been increasingly reported in many countries (Roach *et al*., 1993; LeChevallier and Norton, 1995; Hsu *et al*., 1999; Duncanson *et al*., 2000; Robertson *et al*., 2000; Hashimoto *et al*., 2001). The occurrence of *Cryptosporidium* at a high level in water sources and poor or suboptimal operation of a water treatment plant are supposed to be the main reasons for the contamination of drinking water and, consequently, to cause an outbreak of cryptosporidiosis. It is known as a waterborne disease causing gastrointestinal illness and being fatal to immunodeficienct individuals. Many outbreaks of cryptosporidiosis were reported to be responsible for public water supplies (Hayes *et al*., 1989; MacKenzie *et al*., 1994). Having a high resistance to chlorine that is commonly used as disinfectant in water treatment processes, and a considerable viability under various environmental conditions, *C. parvum* oocysts can survive in a distribution system once they infiltrate into a water supply system (Korich *et al*., 1990; Robertson *et al*., 1992; Fayer *et al*., 1998; Hirata *et al*., 2000). This fact indicates that thorough monitoring of *C. parvum* oocysts and proper operation of treatment facilities should be routinely carried out in water treatment plants and the assessment of their removal efficiency also should be checked regularly for preventing such a mass transmission of cryptosporidiosis through the water supply.

However, direct use of this parasite for the removal performance study may pose a potential health risk and the detection procedure takes considerable times, costs and skill.

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Therefore, a reliable and non-hazardous surrogate for the *Cryptosporidium* removal is highly necessary to conduct the tests in water treatment plants. Several surrogates such as polystyrene sphere, turbidity and algae have been suggested and examined in recent for the purpose (LeChevallier and Norton, 1992; Li *et al*., 1997; Akiba *et al*., 2001).

In our previous work on a survey of algal removal based on five-year data obtained from eight water treatment plants that have adopted coagulation, sedimentation and rapid sand filtration processes, we found that the removal rate and behaviour of green algae was very similar to those of *C. parvum* oocysts. The survey also found that *Scenedesmus quadricauda* was the dominant species of green algae in the drinking water sources. From the point that this species of algae has been known to have its non-hazardous properties, to be easy to culture and determine, we suggested *Scenedesmus quadricauda* as a suitable surrogate for *C. parvum* oocyst removal. In this study, direct filtration experiments were conducted using *C. parvum* oocysts and *S. quadricauda* cells at different initial concentrations in order to investigate their filtration behaviour, and the availability of using this species of algae as a surrogate of *C. parvum* oocysts removal in sand filtration was evaluated from the results.

Materials and methods

Preparation and enumeration of *S. quadricauda* and *C. parvum* oocysts

A pure culture of *S. quadricauda* (NIES-96) was obtained from the GEF (Global Environmental Forum) and cultured in a liquid media (M11: $NaNO_3$ 100 mg/l, K_2HPO_4 10 mg/l, $MgSO_4 \cdot 7H_20$ 75 mg/l, $CaCl_2 \cdot 2H_2O$ 40 mg/l, Na_2CO_3 30 mg/l, Fe_2CO_3 1 mg/l and $Na_2EDTA \cdot 2H_2O$ 1 mg/l). The algal culture of incubation period for 3–4 weeks was used for the experiments. The commercial *C. parvum* oocysts (Kanto Chemistry, #74002) were used in the experiments. Although the nominal concentration was 1×10^7 oocysts per 5 ml (or a vial), the concentration of oocysts were always measured and confirmed prior to use. Algal cell or oocyst counts were determined with a microscope (Olympus, BX-60) at a magnification of 200× and 400×. For *C. parvum* oocysts, acid-fast staining (Ziehl Neelsen staining) was applied and the counting was carried out using both normal and fluorescent view of microscopy.

Direct filtration tests

The direct filtration tests were conducted using the experimental set-up shown in Figure 1 under the operating conditions shown in Table 1. The initial of *C. parvum* oocysts were

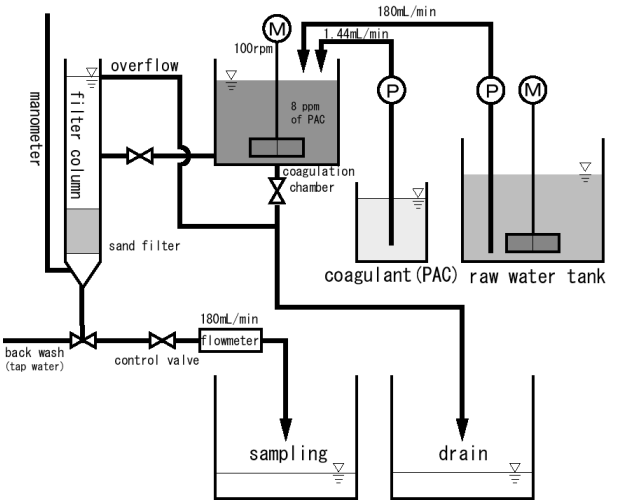

Figure 1 Schematic of the experimental set-up

Table 1 Operation conditions

coagulation	mixing speed (rpm)	100
	chamber volume (l)	1.5
	retention time (min)	8.3
	PAC concentration (ppm)	8
filtration	column diameter (mm)	30
	filter depth (mm)	100
	flow rate (ml/min)	180
filter media (sand)	median diameter (mm)	0.6
	uniformity	1.4
	void rate	0.3

500, 1,000, 2,000 and 5,000 concentrations oocysts/ml, and those of *S. quadricauda* were 500, 1,000, 5,000 and 10,000 cells/ml. Raw water was prepared for each initial concentration of both *S. quadricauda* and *C. parvum* oocysts. Kaolin stock solution was added to raw water in order to set the initial turbidity to about 10 units. The alkalinity of raw water was made up to 45 mg/l as $CaCO_3$ using a $NaHCO_3$ stock solution. 0.1 N of NaOH and HCl solutions were used for pH adjustment of raw water so that its pH was around 7.0. Poly aluminium chloride (PAC; 10% as Al_2O_3), used as a coagulant, was fed into the coagulation chamber (1.5 l) at a dosage of 8 ppm.

The raw water mixed with PAC was introduced to a filtration column and filtrated with a constant flow rate of 180 ml/min. Filtrate samples were taken at scheduled time intervals for 150 minutes from an outlet of the filtration column. The temperature of raw water ranged between 20°C and 22°C. Turbidity of the filtrates was measured with a turbidimeter (Mitsubishi Chemical, SET-PT-706D) right after sampling. 10 mL of each filtrate was taken for counting, which was centrifuged using a centrifuge at $1{,}500 \times g$ for 10 minutes, and concentrated by 20 times with aspiration of the supernatants. Then, 20 µl of each concentrate was applied to the microscopy for counting.

After the filtration tests were finished, the sand filter was divided into five portions of every 2 cm height, and each portion was placed into a beaker. Then, 500 ml of distilled water was added to each beaker, and the mixture was stirred thoroughly using a magnetic stirrer in order to suspend the flocs attached to the sand into bulk. 300 ml of each suspension was filtered with a GF/B filter, and the filters were dried at 105°C for 2 hours and then weighed. The algal cell or oocyst counts in the suspensions were also measured.

Results and discussion

Filtration behaviour of *S. quadricauda* and *C. parvum* oocysts

The ratios of the filtrate concentrations to the effluent ones (C/C_0) were displayed in Figure 2a and 2b for *S. quadricauda* and *C. parvum* oocysts, respectively. The C/C_0 profiles showed nearly the same pattern of filtration, regardless of the differences in the influent concentration. Typical filtration cycle of ripening, stable filtration and breakthrough was observed during the operation time of 150 minutes: filter ripening appeared for the first 5 minutes from the start; stable filtration for about 20 minutes; and then breakthrough began to occur. During the breakthrough period, the filtrate concentrations increased linearly at almost same rates for all influent concentrations, and the final levels of the filtrates reached 23–33% for *S. quadricauda* and 23–27% for *C. parvum* oocysts at the end of the filtration running, compared to the influent levels. These results implied that the filtration behaviour or efficacy of *C. parvum* oocysts and *S. quadricauda* in direct filtration process was independent of the influent concentration. In other words, water treatment plants with a high level of *C. parvum* oocysts in their water sources would have a high probability of detection of this parasite in their treated effluent proportionally to the influent level.

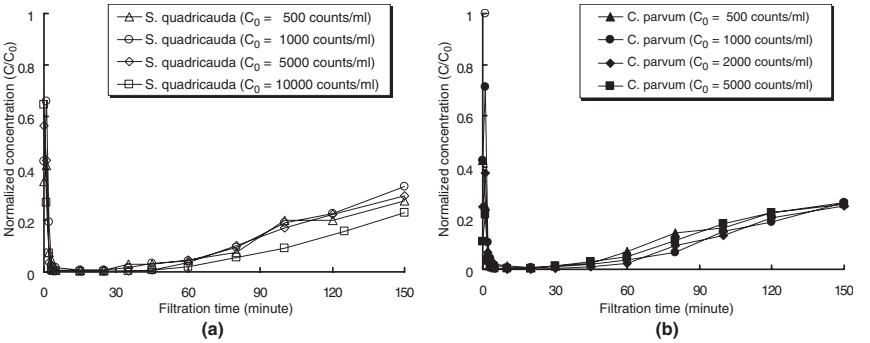

Figure 2 The effluent concentration profiles (C/C_0) of: (a) *S. quadricauda*, and (b) *C. parvum* oocysts for various initial levels

The comparison between the C/C_0 profiles of *S. quadricauda* and *C. parvum* oocysts found a similarity in their filtration behaviour, which indicated that this algae could be a potential surrogate of *Cryptosporidium* removal. This corresponded well with the previous result that some species of green algae had shown the removal characteristics similar to those of *C. parvum* oocysts in direct filtration tests (Akiba *et al.*, 2001). Log_{10} removals and the filtrate concentrations of *S. quadricauda* and *C. parvum* oocysts were illustrated in Figure 3a and 3b, respectively. Direct comparison of them for each influent concentration made the similarity between the two microorganisms look much clearer.

During the stable period, log_{10} removal of *S. quadricauda* were 2.1, 2.2 and 2.6 units, and those of *C. parvum* oocysts were 2.2, 2.3 and 2.6 units for each influent concentration of 500, 1,000, and 5,000 cell or oocyst counts/ml as shown in Figure 3a. A fairly similar removal efficacy was observed between *S. quadricaud*a and *C. parvum* oocysts. Although no significant differences in the log_{10} removals were observed during the ripening and breakthrough periods for any influent level, a slight increase appeared with the increase in the influent level during the stable filtration period. On the other hand, log_{10} removals of turbidity were observed at the range of 1.63–2.21 units, and showed little correlation with the influent levels. Although turbidity has been used as an index of *C. parvum* oocysts removal, physical or chemical characteristics of inorganic matters like kaolin are very different from those of organisms like algae, which causes the difference in the collision and filtration efficiencies due to their surface properties (Huang *et al.*, 1999). Those results were consistent with the fact that *S. quadricauda* algae, was more preferable to turbidity as a surrogate of *Cryptosporidium* removal in this study. During the breakthrough period, a leakage of fine flocs from the sand filter was observed. The number of the flocs in the fil-

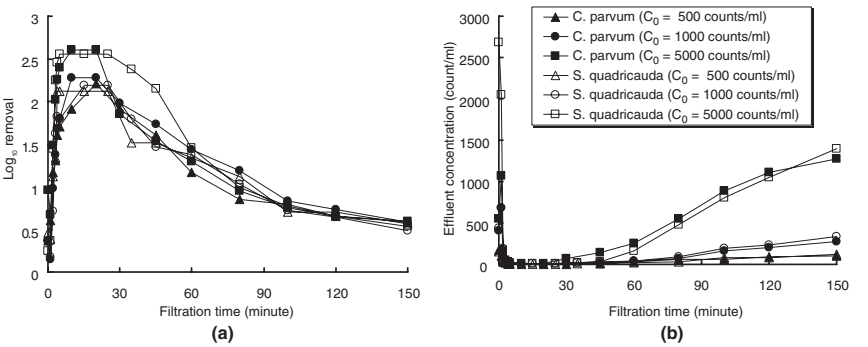

Figure 3 (a) The removal rates and (b) effluent levels of *S. quadricauda* and *Cryptosporidium* for the initial levels of 500, 1,000 and 5,000 counts per ml

trates appeared to increase with the filtration times, which seemed to result in the increase in turbidity and the cell or oocyst counts. The occurrence of the fine flocs in the filtrates may have been due to the come-off of the flocs deposited in the sand filter by fluid drag force. The increases in the head-loss of 0.55–0.65 cm/min were observed over the filtration time of 150 minutes, and supposed to cause the increase in the fluid flow through the void spaces of the filter, resulting in the exfoliation of the flocs deposited in the filter layer from the filter media.

The cell and oocyst counts of *S. quadricauda* and *C. parvum* oocysts in the filtrates were displayed for each initial level in Figure 3b. The comparison of the counts between the two microorganisms indicated that the filtration efficiencies of *S. quadricauda* were almost similar to that of *C. parvum* oocysts over the examined range of the initial levels. A comparison of the filtrate counts at the same filtration times between *S. quadricauda* and *C. parvum* demonstrated a high correlation with R^2 of 0.95. On the other hand, a correlation with R^2 of 0.87 was observed between *C. parvum* and turbidity concentration in the filtrates. This implies that the use of *S. quadricauda* as a surrogate of *Cryptosporidium* removal is quite reliable, compared to turbidity. The similarity in the filtrate profiles was clearly displayed during breakthrough period. The results from Figure 3a and 3b suggest that *S. quadricauda* would be quite reliable as a surrogate for the removal of *C. parvum* oocysts, considering filtration behaviour and removal efficiency.

The cell/oocyst counts and suspended solids deposited in the sand filter

Figure 4a and 4b illustrate the counts of *S. quadricauda* and *C. parvum* oocysts deposited in the unit volume (one cubic centimeter) of the sand filter, respectively. The highest depositions were observed on the upper layer of the filter, and appeared to be gradually reduced along the depth of filters. This decrease in deposition with the filter depth is thought to be due to the grain size distribution of the sand and the change of physical and chemical characteristics of the flocs in the filter layer. Ebie and Doi (1998) demonstrated that the stratification of the filter media, larger grain on the deeper layer, occurred after the back-washing applied, and also that the zeta potential of the filter media and the flocs decreased as the depth of the sand filter went deeper. The effect of grain size on effluent oocyst concentrations in sand filtration system was well described by Logan *et al.* (2001), which fine-media sand column showed higher removal efficiency than coarse-media sand column. It can be assumed, therefore, that as the depth of the filter goes deeper, the physical straining effect of the filter decreases due to the stratification of the sand, and the repulsion forces between the flocs and the filter media increase due to the decrease in the zeta potential of the flocs and the sand. As a result, the attachment efficiency between the flocs and the filter media gets lower with the increase in the depth of the filter and the deposition rates also decrease.

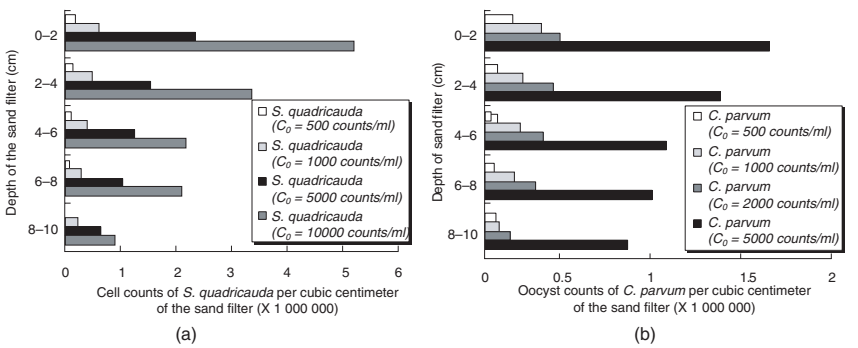

Figure 4 The counts of: (a) *S. quadricauda* and (b) *C. parvum oocysts* deposited in the unit volume (1 cm^3) of the sand filter (total filter volume is about 70 cm^3)

Table 2 shows the cell/oocyst and the mounts of suspended solids deposited in the sand filter layer for each initial level after the filtration experiments finished. A linear increase in the counts deposited in the filter was found with the increase in the initial levels for both *S. quadricauda* and *C. parvum* oocysts. These linear correlations reflected well the previous results that the removal efficiencies were almost independent of the initial levels shown in Figure 2. Similarity in the counts deposited in the sand filter between *S. quadricauda* and *C. parvum* oocysts was observed and this also means the filtration behaviour of *S. quadricauda* is similar to that of *C. parvum* oocysts. Consequently, an estimation of removal performance of conventional treatment system for *C. parvum* oocysts may be possible with the use of *S. quadricauda* as a surrogate from these linear relationships between the initial levels and the deposited counts, and the similarity in filtration behaviours between *S. quadricauda* and *C. parvum* oocysts.

Figure 5 describes the cell/oocyst counts divided by the amounts of suspended solid captured in the sand filter layer according to the influent concentrations. The counts trapped in the unit amounts of suspended solids increased almost linearly, as the influent concentrations increased. This means that the treatment efficiency was hardly affected by the initial levels, and that the density of cell or oocyst counts was proportional to the influent concentrations, which is supposed to result in increasing the floc size and enhancing the coagulation efficiency as suggested above. As shown in Figure 5, *S. quadricauda* and *C. parvum* oocysts showed fairly same values for each other at each influent concentration. These results were consistent with those observed in Figure 3b, and it was verified obviously that there exist similarities in the filtration characteristics and the removal behaviours between *S. quadricauda* and *C. parvum* oocysts.

Table 2 The count of algal cell or oocyst and the amount of suspended solids deposited in the sand filter layer for each initial level

	S. quadricauda				*C. parvum*			
Influent concentration (counts/ml)	500	1,000	5,000	10,000	500	1,000	2,000	5,000
Cell/oocyst count (10^6)	7.37	26.7	94.3	190	5.83	13.5	22.3	81.0
Suspended solids (mg)	220	227	220	269	184	192	176	193

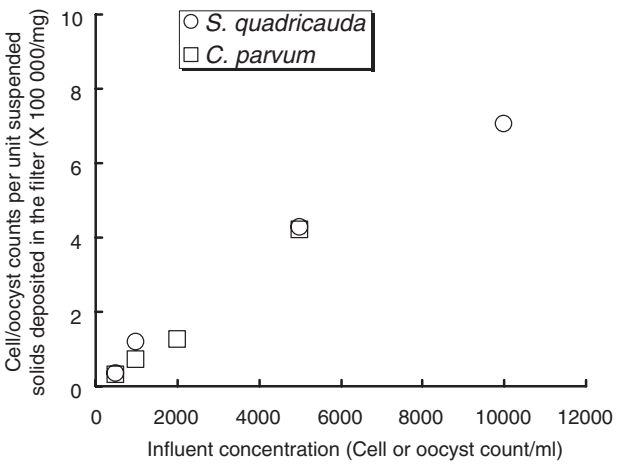

Figure 5 Relationship between the initial levels and the cell/oocyst counts per unit suspended solids deposited in the filter

Conclusions

This study investigated the filtration characteristics of *S. quadricauda* and *C. parvum* oocysts with conducting bench-scale direct filtration experiment under the condition of various influent concentrations of both microorganisms. The effect of the influent concentration on the final quality was estimated, and the comparison of *S. quadricaud*a to *C. parvum* oocysts was examined. The conclusions obtained from this study were summarised as follows.

- The filtrate concentrations of *S. quadricauda* and *C. parvum* oocysts depended highly on the influent concentrations, while the removal rates appeared to be independent in the range of 2.1–2.6 $\log_{10}$.
- The cell or oocyst counts of both microorganisms captured in the sand filter increased in proportion to the influent concentrations with almost the same deposition rates.
- Filtration behaviour and removal efficiency of *S. quadricauda* was quite similar to that of *C. parvum* oocysts with a high correlation ($R^2 = 0.95$).

The results suggest that the use of *Scenedemus quadricauda* could be a reliable and reasonable way of estimating the *C. parvum* oocysts removal efficiency in the water treatment processes including coagulation and filtration. Although this study took into account only the variation of the influent concentration, the other initial conditions like background turbidity or the operation conditions like filtration rate should be considered as variable parameters for further evaluation of the surrogate for *Cryptosporidium* removal.

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