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Algae as surrogate indices for the removal of *Cryptosporidium* oocysts by direct filtration

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Abstract To evaluate the appropriateness of using algae as surrogate indices for the removal of *Cryptosporidium parvum* oocysts in conventional water treatment by rapid sand filtration, investigations on algal removal at eight water treatment plants and laboratory experiments using three species of algae and *C. parvum* oocysts were conducted. From the 5 years data collected from eight water treatment plants, the algal removal showed 0.63 ~ 1.83 log in coagulation and 1.66 ~ 4.17 log in sand filtration including coagulation. In jar tests, zeta potentials of flocs at an ALT ratio of 0.05 were –8.5 mV, –8.5 mV, –7.0 mV and –10.5 mV, and the removal rates at pH 7 were 2.05 log, 1.15 log, 1.51 log and 1.49 log for *Microcystis viridis, Microcystis aeruginosa, Selenastrum capricornutum* and *C. parvum* oocysts, respectively. In direct filtration tests, the removal rates of algae and *C. parvum* oocysts, except for *M. aeruginosa,* were around 3-log during the filtration time of 15–45 minutes when the best removal occurred. *S. capricornutum,* out of the three species of algae, showed almost the same coagulation characteristics as *C. parvum* oocysts and also behaved in a filtration pattern similar to *C. parvum.* From these results, algae were considered useful surrogate indices for the removal of *C. parvum* oocysts, and *S. capricornutum* was thought to be an appropriate one in rapid sand filtration.

Keywords Algae; coagulation; *Cryptosporidium*; filtration; surrogate index

Introduction

Many outbreaks of cryptosporidiosis and giardiosis have been reported in the last few decades. Water is considered to be a major medium for the outbreak of pathogenic infections, as a result of contamination of either raw or treated water. *Cryptosporidium* oocysts and *Giardia* cysts have been routinely found in sewage, and identified as important causes of waterborne disease mostly causing a gastrointestinal illness (Rose, 1988). Both pathogens have been reported to be able to survive during routine wastewater treatment and penetrate into the effluent. And they are much more resistant to disinfection by a variety of disinfectants than conventional bacterial indicators due to their existence in a form of oocyst or cyst in the environment. Filtration, a commonly employed water treatment process, is the most practical treatment technology that can be used for *C. parvum* oocysts removal. Currently, however, there exist no accurate and precise methods for determining *C. parvum* oocysts removal rates in filtration. Direct use of *C. parvum* oocysts in a study for the evaluation of filter performance would pose a potential health risk. Therefore, reliable and non-hazardous surrogates are necessary. Algae commonly found in drinking water sources, like lakes and rivers, are known to have physical properties similar to *C. parvum* oocysts. Zeta potential of algae has been reported to be in a range of –20 mV to –30 mV at neutral pH, and this showed almost same value of *C. parvum* oocysts of –20 mV (Drozd and Schwartzbrod, 1996) and –25 mV (Ongerth and Pecoraro, 1995). More advantageous are the facts that algae are non-hazardous and easy to determine by a simple microscopic method. In this study, we conducted a field survey of collecting and analyzing the data obtained from eight water treatment plants in Japan where monitoring of algae counts had

been routinely carried on. And the coagulation and filtration characteristics between algae and *C. parvum* oocysts were investigated through the laboratory experiments in order to evaluate the reliability of using algae as surrogate indices for the removal of *C. parvum* oocysts. Jar tests for coagulation characteristics and direct rapid sand filtration tests for filtration characteristics were conducted on a laboratory scale in the experiment.

Materials and methods

Algae and Cryptosporidium

Microcystis viridis, Microcystis aeruginosa and *Selenastrum capricornutum* were selected for the experiment. The culture strains of the algae were received from the GEF (Global Environmental Forum) and grown in appropriate culture media (MA for *M. viridis*, CB for *M. aeruginosa* and CS for *S. capricornutum*) at a temperature of 20°C, under a light-dark cycle of 12:12 hours. Algal cultures after incubation for about 4 weeks, and cell count, were used for the experiment at an appropriate concentration. The commercial inactivated *Cryptosporidium parvum* oocysts (Kanto Chemistry, #74002) were also used at the appropriate concentration in the laboratory experiment. The concentration was 10^7 oocysts per 5 mL (one bottle). Oocysts were counted prior to use.

Field survey

Eight water treatment plants were selected for the field survey. All of them employ conventional treatment processes of coagulation, sedimentation and rapid sand filtration, and conduct algal cell counting on raw, settled and filtrated waters for cyanophyta (blue-green algae), chlorophyta (green algae), diatomeae (diatom) and some other minor species of algae. Algal count data for 5 years from April 1992 to March 1997 were collected and used in this study.

Laboratory experiments

Jar tests. Test water was made up to turbidity of 10 units by adding kaolin solution to distilled water. And then algal cells or oocysts were added to 500 mL of test water and made to a final concentration of 1,000 cells/oocysts, respectively. Jar tests were performed with a jar tester (Sugiyamagen, T-6 S) at a pH range of 3 to 10 and ALT ratio of 0 to 0.1 with rapid mixing at 100 rpm for 5 minutes, and at 40 rpm for 25 minutes, followed by sedimentation for 30 minutes. Supernatants were taken after rapid mixing for measuring zeta potential. Zeta potentials of algal cells and *C. parvum* oocysts were measured by a zeta potentiometer (Microtech Nichion, ZEECOM). Supernatants taken after sedimentation were concentrated by 100 times using a centrifuge at 1,500 × g for 10 minutes, and 0.05 mL of the concentrated samples were used for counting cells or oocysts. Counting was conducted using a microscope (Olympus, BX-60) at a magnification of × 100~× 400.

Direct filtration tests. The direct filtration tests were conducted under operation conditions (Table 1) with the experimental setup shown in Figure 1.

Raw waters were made for each algae and *Cryptosporidium*. Kaolin stock solution was added in order to make the initial turbidity of raw water about 10 units. The alkalinity of raw water was made up to 40 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. Initial counts of the algal cells or *C. parvum* oocysts in raw water were 1,000 cells or oocysts/mL. PAC (poly-aluminium chloride) was used as a coagulant in direct filtration tests. PAC (10% as Al_2O_3) was fed into the mixing chamber (1.5L) at a dosage of 8 ppm. The raw water after coagulation with PAC was introduced to a filtration column. The flow rate was maintained at 180 mL/min with outlet valve control. Filtrate samples were taken at

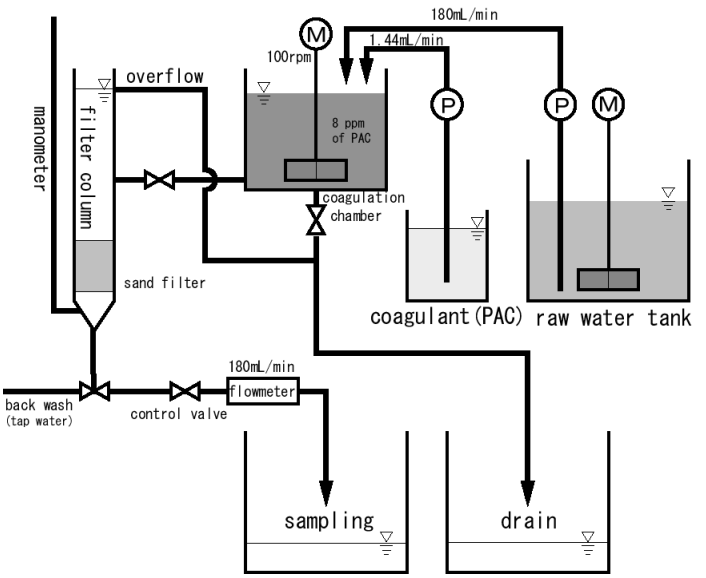

Figure 1 Schematic of the experimental set up

Table 1 Operation conditions

coagulation	mixing speed	100 rpm
	chamber volume	1.5 L
	retention time	8.3 minutes
	PAC concentration	8 ppm
filtration	column diameter	30 mm
	filter depth	100 mm
	flow rate	180 mL/min
filter media (sand)	diameter	0.6 mm
	uniformity	0.31.4
	void rate of filter	0.3

scheduled time intervals (every 1 minute from 0 to 5 minutes, then every 10 minutes till 180 minutes) from an outlet of the filtration column. Turbidity of filtrates was measured with a turbidimeter (Mitsubishi Chemical, SET-PT-706D). 10 mL of filtrates were concentrated by 10 times using a centrifugate at 1,500 × g for 10 minutes. And then algal cells or oocysts were counted using a microscope at a magnification of × 100~ × 400. Algal cells or oocysts in a mixing chamber were also counted. After a filtration test finished, the sand filter layer was divided into five portions every 2 cm height, and each portion was taken in a beaker. Then, 500 mL distilled water was added to each beaker, and the mixture was stirred thoroughly using a magnetic stirrer. After that, 300 mL of each mixture, excluding sand, was filtered with a GF/B filter, and the filter was dried at 105°C for 2 hours and weighed. The algal cell or oocyst counts in the mixture were also measured.

Results and discussion

Field survey

The major group in the raw water was diatomeae occupying 87% among the three groups, while chlorophyta occupied 11% and cyanophyta 2%. With the data obtained from eight selected water treatment plants (from A to H), the removal rates of the three groups of algae were calculated by coagulation/sedimentation only and both coagulation/sedimentation and filtration. Average removal rates of each algal group as a result of such calculation are summarized in Table 2 and Figure 2.

Table 2 Summary of the average log removal rates at eight water treatment plants

W.T.P.	Removal by coagulation/sedimentation			Removal by coagulation/sedimentation and filtration		
	Cyanophyta	Diatomeae	Chlorophyta	Cyanophyta	Diatomeae	Chlorophyta
A	1.33	1.27	1.38	2.76	3.68	3.69
B	1.52	1.69	1.83	3.12	3.74	3.68
C	1.59	1.61	1.82	3.14	4.17	3.76
D	0.63	1.33	0.96	2.07	3.13	1.87
E	N.A.*	1.68	1.11	N.A.*	2.98	2.04
F	0.82	1.41	1.09	1.66	3.25	2.28
G	0.98	1.69	1.27	1.67	3.30	2.14
H	0.84	1.67	1.43	1.88	3.52	2.60

*N.A. stands for data not available

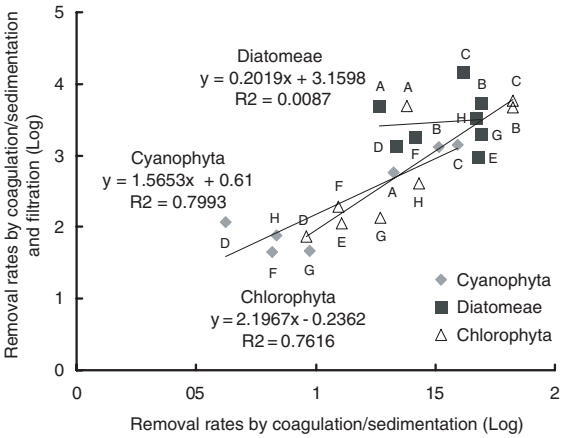

Figure 2 The removal rates of algal groups in conventional water treatment

Log removal rates in coagulation/sedimentation were 0.63 to 1.59 for cyanophyta, 1.27 to 1.69 for diatomeae, and 0.96 to 1.83 for chlorophyta. A difference of about 0.9 log was found in the maximum and minimum removals of cyanophyta and chlorophyta groups, while the removal of diatomeae was relatively uniform. Average log removal rates in coagulation/sedimentation for cyanophyta, diatomeae and chlorophyta were 1.10, 1.55 and 1.36, respectively. This showed that a certain extent of differences in coagulation properties existed between the three algal groups. Average of total algal removal rate in coagulation/sedimentation was 1.35 log, which means that we can achieve around 96% of algal removal by the coagulation process. Log removal rates in coagulation/sedimentation and filtration were 1.66 to 3.14 for cyanophyta, 2.98 to 4.17 for diatomeae, and 1.87 to 3.76 for chlorophyta. And average log removal rates in coagulation/sedimentation and filtration for cyanophyta, diatomeae and chlorophyta were 2.33 log, 3.47 log and 2.76 log, respectively. As for the above results in coagulation/sedimentation, slight differences in removal rates between the three groups were observed. And the diatomeae group also showed better removal among the three groups. The major species of diatomeae found in the raw water was *Nitzchia* spp. and its average removal rate was 3.03 log. This species has a long shape of body (width of 3 to 5 μm, length of 15 to 70 μm) and generally grows in eutrophicated water. Its surface properties and shape, being supposed to be easier to coagulate and to filtrate, were thought to be one of the reasons why diatomeae showed the higher removal rate among the groups. On the other hand, the major species of cyanophyta was *Phormidium* spp. and that of chlorophyta was *Scenedesmus* spp. and their average removal rates were 2.41 log and 2.64 log, respectively.

Figure 2 displayed the relationship between removal rate by coagulation/sedimentation and removal rate by coagulation/sedimentation followed by filtration. Obvious correlations were seen in cyanophyta and chlorophyta, which showed the total performance of algal removal depended on the coagulation efficiency in the conventional treatment process. Average removal rate of total algae in coagultaion/sedimentation and filtration was 2.88 log (about 99.9%). It has been reported that the average log removal of *Cryptosporidium* showed 2.25 (in a full-scale plant) and 2.98 (in a pilot plant) in conventional treatment and between 2.79 (in a full-scale plant) and 2.97 (in a pilot plant) in direct filtration (Nieminski and Ongerth, 1995). Comparing the survey results to the reported ones, it was clear that the range of algal removal was similar to that of *Cryptosporidium*. This fact explained that algae could be supposed an appropriate surrogate index for the removal of *Cryptosporidium*. In addition, among the three groups, chlorophyta showed a removal range so similar to *Cryptosporidium* that this group of algae deserved to be investigated as a surrogate index of *Cryptosporidium* removal in conventional water treatment.

Jar tests

Optimum ALT ratio of 0.04 to 0.05 was obtained from jar tests that were examined at an ALT ratio from 0 to 0.1 for *M. viridis*, *M. aeruginosa*, *S. capricornutum* and *C. parvum*. Zeta potentials of the algae and *C. parvum* lay in the proper range of coagulation, which is known as –10 to 10 mV. The results of the jar tests were shown in Table 3.

Although the experiments were conducted under the same conditions, slight differences in the removal rates were observed among the algae and *C. parvum*. The obtained algal removals were 1.15 log to 2.05 log as shown in Table 3. Compared with *C. parvum* oocysts removal of 1.49 log, the removal of *M. viridis* removal was higher by the order of 0.56 log, that of *M. aeruginosa* was lower by 0.34 log and that of *S. capricornutum* was almost the same with a difference of 0.02 log. These results described that there exist some species of algae showing behavior in coagulation characteristics similar to that of *C. parvum* oocysts, and suggested the possibility of using algae as surrogate indices for the removal of *C. parvum* oocysts. No strong relationship was, however, seen between the zeta potentials and the removal rates. Even though the surface electric property like zeta potential is considered an important parameter to evaluate the coagulation efficiency, size, morphology and structure of a particle could also effect the formation or strength of the flocs, which is thought to be the reason why not so strong a relationship between zeta potential and removal rate by coagulation was observed in the jar tests. And this suggested that the coagulation characteristics of particles should be examined in order to evaluate the removal characteristics of the particles more precisely as well as the surface electrical properties.

Direct filtration tests

Direct filtration tests were conducted for three species of algae and *C. parvum*. The variations of turbidity and cell/oocyst counts in the filtrate were examined. And un-flocculated cell/oocyst counts in the mixing chamber were also measured. The results of the filtration tests were displays in Figure 3 and Figure 4. Maximum removal rates of turbidity and

Table 3 Coagulation characteristics of the algae and *C. parvum* oocysts

	Z.P. at ALT ratio of 0.05	Removal rate
M. viridis	–8.5 mV	2.05 log
M. aeruginosa	–8.5 mV	1.15 log
S. capricornutum	–7.0 mV	1.51 log
C. parvum	–10.5 mV	1.49 log

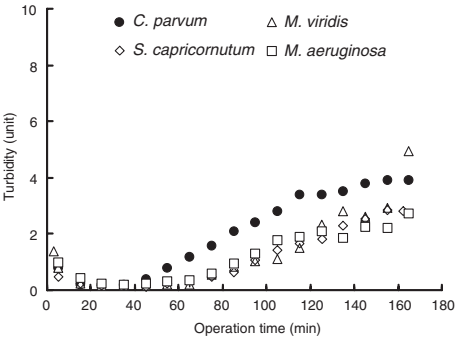

Figure 3 Variation of turbidity in each effluent

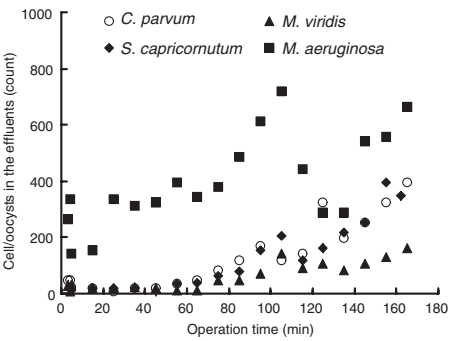

Figure 4 Variation of cell/oocyst counts in each effluent

cell/oocyst counts were observed during a filtration time of 15 to 45 minutes. The breakthrough, however, started to occur from around 60 minutes of the filtration time. Figure 3 showed the variation of turbidity in the effluents for the three species of algae and *C. parvum*. Although the effluent turbidity for *C. parvum* appeared somewhat higher than that for algae, the minimum effluent turbidities were around 0.1 unit for each algae and *C. parvum* at the elapsed time of 35 minutes. And no differences in filtrate turbidity between each species of algae were observed during operation. The treatment efficiencies seemed to be not so different for those algae and *C. parvum* in the experiment. This means that turbidity doesn't show eminent removal characteristics of particles in direct filtration.

In Figure 4, however, different behaviors of the particles in the effluent cell/oocyst count were observed. The removal rates of cell/oocyst counts were low for *M. aeruginosa*, high for *M. viridis* and almost the same for *S. capricornutum*, compared to *C. parvum* oocysts. And all the particles, except for *M. aeruginosa*, were removed by around 3-log during the period of their best removals. Comparing Figure 3 to Figure 4 displayed no significant relationship between turbidity removal and particle removal. Although monitoring and controlling turbidity in water treatment plants has been thought to be one of the appropriate ways to check the performance of the treatment and controlling the *Cryptosporidium* at present, monitoring particle counts considering the properties also should be taken into account for further investigation of the performance of water treatment processes.

The counts of cell/oocyst that didn't form the flocs in the mixing chamber were shown in Table 4. From the results, the counts of *M. aeruginosa* were almost two times those of the others, which explained the poor removal performance of *M. aeruginosa*. Comparison of Table 2 and Table 4 to Figure 4 found a strong correlation between coagulation characteristics and filtration characteristics. It was obvious that the removal by direct filtration depended on the efficiency of coagulation, which corresponded to the survey results shown in Figure 2. Among the algae, *S. capricornutum* showed such similar coagulation and filtration characteristics to *C. parvum* oocysts that it was supposed to be an appropriate surrogate for the removal of *C. parvum* oocysts in direct filtration process.

Table 4 Un-flocculated counts in the mixing chamber

	Un-flocculated counts (counts/ml)
M. viridis	180
M. aeruginosa	420
S. capricornutum	220
C. parvum	240

The amount of suspended solid and the cell/oocysts counts captured in the sand filter by each 2 cm of the filter depth was described in Figure 5. Higher removal was observed at the upper layer of the sand filter, and the amount of suspended solid and the cell/oocyst counts were gradually decreased with the depth of the filter. No considerable differences were seen between the algae and *C. parvum* oocysts. On the other hand, cell/oocyst counts showed differences from particle to particle. And these results described very well that the particle count of algae and *C. parvum* oocysts was a more meaningful parameter than turbidity as shown in Figure 3 and Figure 4.

Table 5 summarized the total amount of suspended solid and the cell/oocyst counts captured in the sand filter. The cell/oocyst counts per milligram of suspended solid were also described. Almost the same amounts of suspended solid were observed for *C. parvum* oocysts and *S. capricornutum*. And no significant differences were seen between the algae and *C. parvum* oocysts. The cell/oocyst counts of *M. viridis* and *S. capricornutum* were near to that of *C. parvum* oocysts, while that of *M. aeruginosa* was almost half. It was well expressed that the coagulation efficiency directly affected the performance of the sand filter to reject the flocculated particles. The less the particles were flocculated, the larger number of particles passed through the filter layer, which resulted in the deterioration of the filtrate, as shown in Figure 4. The cell/oocyst counts per suspended solid captured in the sand filter means the removal efficiency of the particles in this system.

Except for *M. aeruginosa*, the remaining three showed nearly the same values, which suggests that some of algae have similar behavior to *C. parvum* oocysts in the direct filtration process. And of the three algae, *S. capricornutum* showed quite similar coagulation and filtration characteristics to *C. parvum* oocysts. From the results in this research, using algae as surrogate indices for the removal of *Cryptosporidium* is supposed to be possible. And proper use of algae like *S. capricornutum* might be a useful measure to evaluate the performance of water treatment plants for the removal of *C. parvum* oocysts.

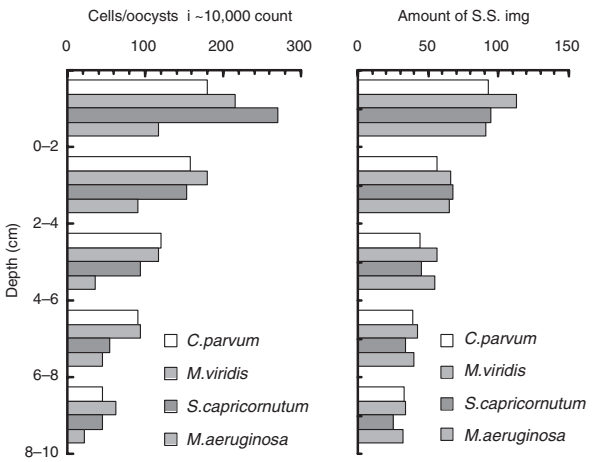

Figure 5 Amount of suspended solid and cell/oocyst counts captured in the sand filter

Table 5 Amount of suspended solid and counts of each algae and *C. parvum* captured in the sand filter

	Suspended solid (mg)	Cells or oocyst (counts)	Cell or oocysts per suspended solid (counts/mg)
C. parvum	265	5.9×10^6	2.2×10^4
M. viridis	311	6.7×10^6	2.2×10^4
S. capricornutum	266	6.2×10^6	2.3×10^4
M. aeruginosa	283	3.1×10^6	1.1×10^4

Conclusions

From the results of the survey research, the average algal removal was 2.88 log. It was very near to the reported *Cryptosporidium* removal in conventional treatment plants. This showed that algae would be removed to nearly the same extent as *Cryptosporidium* in the conventional treatment process. And from the results of both the survey research and the laboratory experiments, it was clear that the coagulation characteristics of the particle considerably affected the efficiency of algal removal. Therefore, the coagulation characteristics of the particles concerned should be investigated thoroughly for the estimation of surrogates for *Cryptosporidium* removal in the conventional treatment process. Among the three species of algae considered in this study, *S. capricornutum* showed the coagulation and filtration characteristics similar to *C. parvum* oocysts. So this species of algae was supposed to be a proper surrogate for the removal of *C. parvum* oocysts in the direct filtration process. Consequently, algae is thought to be an appropriate surrogate index for *Cryptosporidium* removal, and monitoring algal counts would be one of the useful measures for the routine monitoring of the *Cryptosporidium* removal performance of water treatment plants.

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