Effects of chlorine and ozone on algal cell properties and removal of algae by coagulation

Jeanine D. Plummer and James K. Edzwald

ABSTRACT

The effects of ozone and chlorine on algae were examined with respect to cell surface characteristics, lysis and coagulation ability. Two algae were studied: a green alga (*Scenedesmus quadricauda*) and a diatom (*Cyclotella* sp.). Cell properties were characterized using scanning electron micrographs, particle size distributions and electrophoretic mobility measurements. Jar tests were used to evaluate the coagulation of the algal suspensions with a polyaluminium chloride (PACl). The results showed that changes in the characteristics of the algal cells from ozone or chlorine yielded an improvement in removal of *Scenedesmus* through a combination of lysis and improved coagulation ability with PACl. *Cyclotella* removal was not enhanced by preoxidation. Additionally, preoxidation increased the organic carbon concentration of the settled water, which could lead to increased tastes and odours and production of disinfection by-products.

Key words | algae, chlorine, coagulation, lysis, ozone

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INTRODUCTION

Algae in drinking water supplies can cause a number of problems. Prior research has shown that algae are negatively charged bio-particles that contribute to the coagulant demand of water supplies (Bernhardt & Clasen 1991), and also that extracellular organic matter (EOM) can adsorb to particles causing a stabilizing effect (Bernhardt *et al.* 1985). Algae can negatively impact filtration. Larger algae can form a surface mat on filters while small, motile algae can penetrate filters leading to shortened filter runs and increased use of backwash water (Bernhardt 1984; Bernhardt *et al.* 1986). Tastes and odours arise from metabolic products of algae as well as the decay of dead cells (Mallevialle and Suffet 1987). Cyanobacteria (blue-green algae) have the added problem of toxins, which have caused the death of both birds and animals that have drunk from water sources with cyanobacterial blooms (Yoo *et al.* 1995). Lastly, algae and their extracellular products are precursors for disinfection by-products, including trihalomethanes (Hoehn *et al.* 1980; Wachter & Andelman 1984; Graham *et al.* 1998; Plummer & Edzwald 2001), haloacetic acids (Plummer & Edzwald 2001) and haloacetonitriles (Oliver 1983).

Treatability studies have shown that algal cells must be destabilized before removal; however, shape, morphology and motility make removal of algae more difficult than removal of inorganic particles (Bernhardt & Clasen 1991; Steynberg *et al.* 1996). In addition, extracellular organic matter from algae may act as a coagulant aid or may hinder coagulation depending on the species of algae and EOM concentration (Bernhardt *et al.* 1985).

Preoxidation of waters containing algae can significantly impact coagulation ability. Preoxidation with potassium permanganate can improve algal removal in direct filtration by immobilizing motile algae and causing the release of biopolymers that act as coagulant aids (Petruševski *et al.* 1996). At intermediate doses of $KMnO_4$ (3 mg l^{-1}), algal removal may also occur due to cell lysis, which results in the release of intracellular components and may increase coagulant demand (Knappe *et al.* 1998). Pilot plant (Petruševski *et al.* 1994) and full-scale (Richard

Table 1 | Cell and organic concentrations and oxidant dosages for experiments

					Oxidant dose ratios*		
Algae	**Cell conc. (cells ml^{-1})**	**TOC (mg l^{-1})**	**DOC (mg l^{-1})**	**Oxidant dose* (mg l^{-1})**	**Oxidant to cells (mg per 10^3 cells)**	**Oxidant to TOC (mg mg^{-1})**	**Oxidant to DOC (mg mg^{-1})**
Scenedesmus	100,000	25.5	0.75	1	0.01	0.04	1.33
				3	0.03	0.12	4.00
				8	0.08	0.31	10.7
	20,000	4.44	0.22	1	0.05	0.23	4.55
				3	0.15	0.67	13.6
Cyclotella	20,000	1.07	0.33	1	0.05	0.93	3.03
				3	0.15	2.80	9.09

*Ozone and chlorine.

& Dalga 1993) work has shown improvements in particle and algal removal with preoxidation. However, preozonation effects on EOM have been found to be species dependent (Hoyer *et al.* 1987; Chandrakanth *et al.* 1996). For example, Hoyer *et al.* (1987) found coagulation was improved for low ozone doses applied to EOM extracted from *Fragilaria* and *Pseudanabaena,* but not for *Dictyosphaerium*.

The goal of this research was to evaluate preoxidation effects on algae and the subsequent coagulation of waters containing algae. Preoxidants studied included chlorine and ozone. Variables included algal type, algal concentration and oxidant concentration. The specific research objectives were: (1) to examine ozone and chlorine effects on algal cell properties, and (2) to examine ozone and chlorine effects on coagulation and separation of algae.

MATERIALS AND METHODS

Experimental design

The experimental design involved two phases. Cell properties were examined in Phase I. Ozone and chlorine were applied separately to synthetic water samples of each algal species. Table 1 shows the conditions used in this research. The effects of these oxidants on cell properties were characterized using scanning electron micrographs (SEMs) for changes in morphology and cellular structure, and particle size distributions for monitoring cell lysis. Cell mobility before and after oxidation was determined by EPM measurements.

Phase II experiments were designed to evaluate the effect of oxidants on the coagulation and separation of algae and algal-derived organic matter. A synthetic water was prepared with a calcium concentration of 30 mg l^{-1} as $CaCO_3$ and alkalinity of 100 mg l^{-1} as $CaCO_3$. The water was then spiked with the algal suspension to achieve the desired cell concentration (approximately 20,000 or 100,000 cells ml^{-1}). The samples were pretreated with chlorine or ozone in batch mode at pH 7 (see Table 1 for dosages). Controls were run without preoxidation. The control, ozonated and chlorinated suspensions were then used in jar test experiments.

Jar tests were carried out in 500-ml beakers. Coagulant was added under rapid mixing conditions (100 rpm for 30 sec) followed by flocculation at 25 rpm for 45 min and settling for 60 min. After the settling period, a 150-ml

sample was taken 5 cm below the surface of each beaker using a 100-ml wide bore pipette. The treated samples were analysed for total and dissolved organic carbon (TOC and DOC), turbidity and particle counts.

A polyaluminium chloride (PACl) coagulant was used for the coagulation experiments. The PACl was a high basicity coagulant (70% basicity) with 16.5% Al_2O_3, and a specific gravity of 1.35 (Holland Co., Adams, Massachusetts). The coagulation experiments were performed at pH 6.5–6.8. For these pH conditions, $Al_{13}O_4(OH)_{24}^{+7}$ is a dominant coagulation species accomplishing particle destabilization primarily by charge neutralization (Van Benschoten and Edzwald 1990; Pernitsky 2001). This allowed evaluation of algal cell removal via particle counting since precipitation of $Al(OH)_3(s)$ particles was assumed not a coagulation mechanism. As precipitation could occur for alum, this coagulant was not chosen for study.

This study was conducted at algal concentrations of 20,000 cells ml^{-1} and 100,000 cells ml^{-1}. These cell concentrations were selected for experimental reasons—reasonable concentrations to measure pre-oxidation effects and to conduct coagulation, flocculation and settling experiments. These are representative concentrations for many drinking water reservoirs under mesotrophic and eutrophic conditions, and even for short periods for oligotrophic reservoirs when algal blooms can reach 10^3 cells ml^{-1} or greater.

Algal culturing

Two different algal types that occur in drinking waters were studied. Axenic cultures of *Scenedesmus quadricauda* (green alga, UTEX Collection #76) and *Cyclotella* sp. (diatom, UTEX Collection #1269) were obtained from the Culture Collection of Algae at the University of Texas at Austin (Starr & Zeikus 1993). *Scenedesmus* was cultured in the late log growth phase in a continuously mixed, 15-l chemostat. The chemostat was supplied with a constant inflow of synthetic, sterilized algal growth media (Guillard & Lorenzen 1972). A gravity overflow port maintained a constant volume. The chemostat was housed in a 25°C water bath, supplied with filter sterilized air, and provided with 3770 lux (350 foot-candles) of illumination on a 16 hour light, 8 hour dark cycle. Steady state conditions were monitored by particle counts (MetOne WGS 260 grab sampler, LB – 1010 sensor, MetOne, Inc., Grants Pass, Oregon) on a daily basis as evidenced by little or no change in growth with respect to time. Particle count results were verified by microscopic counts which showed the particle counter to produce accurate counts and sizing. *Scenedesmus* grows predominantly in colonies of four cells. 'Cell' counts for *Scenedesmus* in this research are the number of colonies and 'cell' size is the size of this four-cell colony. The cell concentration in the chemostat was maintained at 500,000 to 600,000 cells ml^{-1}, and cell size ranged from 5 to 20 μm (mean size of 10 μm).

Cyclotella was grown in batch mode in 500-ml Erlenmeyer flasks containing 250 ml of the algal growth media. Cultures received 2150 lux (200 foot-candles) of light on a 12 hour light cycle, and were maintained at 17°C. Growth was monitored daily by particle counting. Stock cultures were prepared weekly by aseptic transfer of a known number of algal cells from a culture in the log growth phase to a freshly sterilized flask with growth media. Cultures were harvested and used in experiments in the log growth phase. These cultures had a cell concentration of 250,000 to 350,000 cells ml^{-1} and individual cell size range of 4 to 8 μm (mean size of 6 μm).

Analytical methods

Ozone was generated with a Welsbach T-408 ozone system (Welsbach Ozone Systems, a division of Polymetrics, Inc., San Jose, California). Ozonation of the samples was conducted in batch mode because of the ease of controlling the dosage. For ozone dosing, the appropriate volume of ozone stock was added to the sample of interest in a headspace free, ozone-demand free reactor to achieve the desired dosage of ozone. The sample was mixed continuously for 30 min after which there was no residual ozone concentration.

Scanning electron micrographs of the algal samples (100,000 cells ml^{-1}) were prepared by the Central Microscope Facility at the University of Massachusetts. Thirty millilitre samples were first concentrated by

settling, fixed and washed in a buffer solution. The samples were then deposited on cover glasses, dehydrated with successively higher concentrations of ethanol and dried by critical point drying. Dried samples were mounted on aluminium stubs and sputter coated with gold-palladium. The specimens were observed and photographed in a JEOL JSM-5400 scanning electron microscope at 5 or 10 kV. A minimum of 10 fields (approximately 50 cells per field) were examined at 750 × magnification. Cells were also examined and photographed at 5000 × magnification. Full details of the procedure are provided in Plummer (1999).

The electrophoretic mobility (EPM) of the algae was measured with a Zeta-Meter System 3.0 + (Zeta-Meter, Inc., Staunton, Virginia) which measures the EPM using the principles of electrophoresis. All other analytical methods (TOC, DOC, turbidity, particle counting) were conducted in accordance with *Standard Methods* (1998).

RESULTS AND DISCUSSION

Cell properties

Cell morphology

Scanning electron micrographs (SEMs) of the algae before and after oxidation demonstrate the effect of the oxidants on cell structure and cell wall features. These effects are useful in evaluating oxidant effects on the cells and potential effects on coagulation and removal. Figure 1 shows SEMs for *Scenedesmus* at a concentration of 100,000 cells ml^{-1} before and after oxidation with ozone or chlorine. Without pretreatment, a colony of four cells is enclosed within a loosely fitting reticulate layer. This layer is suspended by tubular propping spikelets and rosettes (Staehelin & Pickett-Heaps 1975). Terminal spines are readily apparent. With a low ozone dose (1 mg l^{-1}), the reticulate layer appears more loosely folded, but there is no evidence of lysing. Severe alterations were observed after an ozone dose of 3 mg l^{-1}. The reticulate layer is detached from the spikelets, and both the spikelets and trilaminar sheath (which encloses each individual cell) are readily visible. A small fraction of the cells were lysed at this intermediate ozone dose. At high ozone doses (8 mg l^{-1}) extensive damage occurred including detachment of the reticulate layer and spikelets, and perforation of the cell wall.

The results for preoxidation of *Scenedesmus* with chlorine were similar to the ozone results. A low chlorine dose (1 mg l^{-1}) imparts damage to the reticulate layer, but did not significantly affect the individual cells. A 3 mg l^{-1} chlorine dose resulted in severe reticulate layer damage. With a dose of 8 mg l^{-1}, damage to the cells was as extensive as observed for ozone, with evidence of cell lysing.

Figure 2 shows SEMs for *Cyclotella* cells (100,000 cells ml^{-1}) without pretreatment, and after treatment with either ozone or chlorine. With no pretreatment, the siliceous cell wall and radial symmetry of the cells is clear. The cell wall, or frustule, consists of two lid-like valves, one fitting inside the other. The overlapping area of the valves is connected by bands that constitute the girdle, and the cells are intact. With a low ozone dose (1 mg l^{-1}), the majority of the cells show no apparent damage. For a few cells, minor damage to the girdle was observed. An ozone dose of 3 mg l^{-1} did not damage a significant number of cells. Again, some cells showed warping or penetration of the girdle; however, perforation of the cell wall was not observed. The effect of chlorine was similar to ozone. A low chlorine dose (1 mg l^{-1}) produced only minor damage. However, at a dose of 3 mg l^{-1} chlorine, lysing was observed for a small number of cells.

The findings for *Scenedesmus* are in agreement with alterations observed by Sukenik *et al.* (1987), who found increased convolutions of the reticulate layer of *Scenedesmus* after ozonation or chlorination. Edzwald and Paralkar (1992) found cell surface alterations from ozone at doses as low as 1 mg l^{-1}. At 3 mg l^{-1}, *Scenedesmus* had reticulate layer damage and loss of terminal spines. Of the two algae species tested, *Cyclotella* was the most resistant to damage from oxidation. Further confirmation of the results in this study comes from Petruševski *et al.* (1994), who found that ozone applied at 1.8 mg l^{-1} partly removed the organic coating on valve elements of cylindrical diatoms and roughened the surface of the cells.

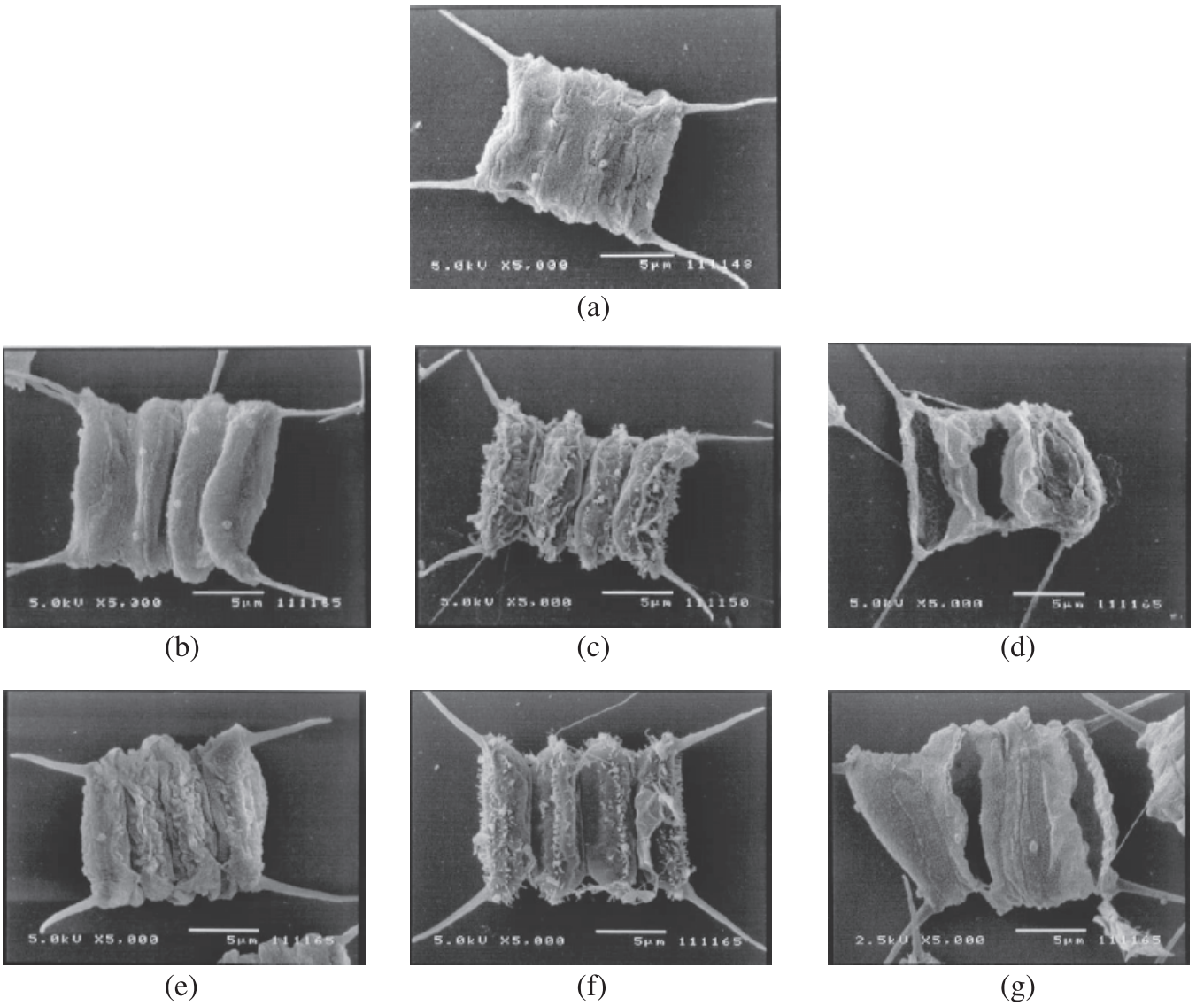

Figure 1 | Scanning electron micrographs of *Scenedesmus quadricauda* (100,000 cells ml^{-1}) before and after oxidation. (a) No oxidant, (b) 1 mg l^{-1} ozone, (c) 3 mg l^{-1} ozone, (d) 8 mg l^{-1} ozone, (e) 1 mg l^{-1} chlorine, (e) 3 mg l^{-1} chlorine, (f) 8 mg l^{-1} chlorine. Scale bar 5 µm.

In this study, an algal concentration of 100,000 cells ml^{-1} was used with oxidant doses ranging from 1 to 8 mg l^{-1}. In a prior study by Sukenik *et al.* (1987), ozone doses of 2.6 to 8.1 mg l^{-1} and chlorine doses of 2 to 20 mg l^{-1} were applied to algal suspensions of 2×10^6 cells ml^{-1}. This produced an oxidant to cell ratio of 0.001 to 0.01 mg per 10^3 cells, compared to 0.01 to 0.08 mg per 10^3 cells in this study. The impact of the oxidants on cellular structure could depend on the oxidant to cell ratio. However, it is also important to note that samples used here were pure cultures and thus there was no oxidant demand from organic matter other than the cells and EOM. Sukenik *et al.* (1987) found increasing damage to the cells with increasing dose (as was found in this study). However, at the highest dose tested, the damage was limited to changes in wart organization, rosette appearance and the reticulate layer. Lysing was not observed. In this study, the higher oxidant doses produced lysing. Therefore, the oxidant to

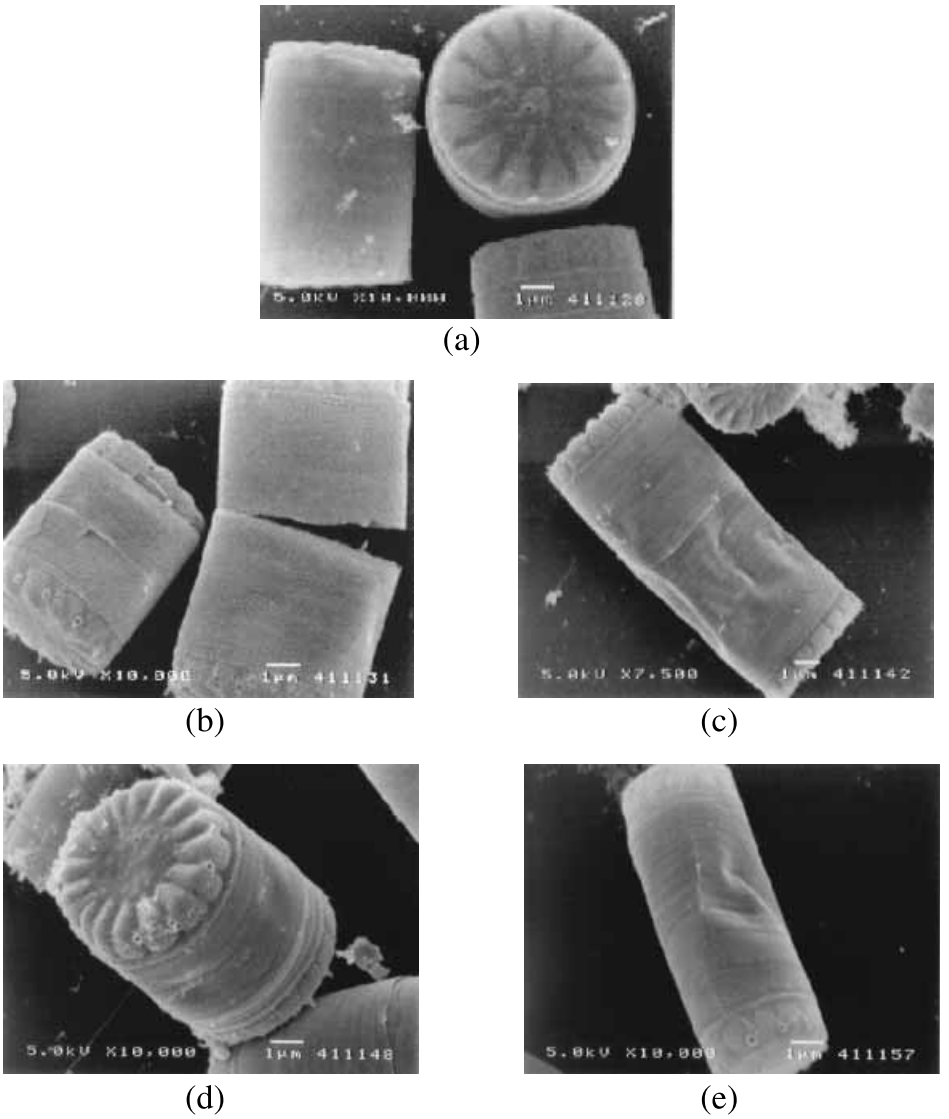

Figure 2 | Scanning electron micrographs of *Cyclotella* sp. (100,000 cells ml^{-1}) before and after oxidation. (a) No oxidant, (b) 1 mg l^{-1} ozone, (c) 3 mg l^{-1} ozone, (d) 1 mg l^{-1} chlorine, (e) 3 mg l^{-1} chlorine. Scale bar 1 μm.

cell ratio rather than just the oxidant dose appears to have significance in the extent of damage to the cells.

In summary, scanning electron micrographs for *Scenedesmus* at a concentration of 100,000 cells ml^{-1} showed that low ozone or chlorine doses (0.01 to 0.03 mg per 10^3 cells) affected cell morphology but did not result in extensive lysing of the cells. At the highest oxidant dose examined (0.08 mg per 10^3 cells), lysing of cells was observed. For *Cyclotella,* which was only examined at 0.01 and 0.03 mg per 10^3 cells, extensive alteration of cells was not observed. Comparing the two algal species, *Cyclotella* showed less cell wall alteration than *Scenedesmus* at the same oxidant dose. From a practical viewpoint of drinking water treatment, low oxidant dose to cell ratios would have an effect on cell morphology, but would not result in significant lysing of cells. Higher oxidant dose to cell ratios could also cause lysis and the release of intracellular organic matter for more susceptible species of algae and

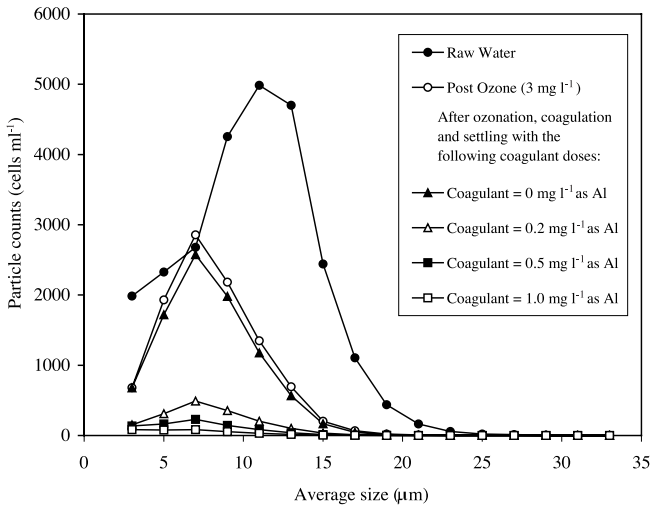

Figure 3 | Particle size distribution of *Scenedesmus* suspension: Raw water; after 3 mg l^{-1} ozone (0.15 mg per 10^3 cells); and after ozonation, coagulation and settling with coagulant doses as noted (starting concentration: 20,000 cells ml^{-1}, pH 6.5–6.8).

this organic matter may contain DBP precursors, toxins or taste and odour compounds.

Particle size distributions of algae

An examination of particle size distributions (PSDs) of the algae provides further insight into the cellular changes upon oxidation. Figure 3 shows the PSD for a 20,000 cells ml^{-1} suspension of *Scenedesmus* before oxidant addition; after 3 mg l^{-1} ozone (0.15 mg per 10^3 cells); and after ozonation, coagulation and settling with varying coagulant doses. After ozonation only (no flocculation or settling), the total particle (cell) count in the 2–34 μm size range was reduced by 60% and the particle sizes were shifted to smaller sizes. Other experimental data (not shown here) for an ozone dose of 1 mg l^{-1} (0.05 mg per 10^3 cells) showed that total cell numbers were reduced by only 8%. This is in agreement with the SEMs and again indicates that the ozone dose to cell number ratio is significant. After ozonation, coagulation and settling, particle numbers were greatly reduced and the PSDs showed a peak at 7 μm (see Figure 3). Particle counts after chlorination with either 1 or 3 mg l^{-1} chlorine for a suspension of 20,000 cells ml^{-1} (results not shown) were decreased by approximately 10%. Again, the size distribution was shifted to smaller sizes.

For *Cyclotella* at a concentration of 20,000 cells ml^{-1} (results not shown), the shift in particle size after ozonation with either 1 or 3 mg l^{-1} ozone (0.05–0.15 mg per 10^3 cells) was so great that the particle counter was unable to fully capture the size distribution of particles as the lower limit of the counter was 2 μm. Although the total particle count in the 2–18 μm size range was greatly reduced, actual reductions in particle number can only be estimated. With chlorination, particle numbers were not appreciably altered, though the cell size was reduced.

The reduction in algal cell number after ozonation is in agreement with Richard & Dalga (1993), who found a 40% and 73% reduction in algal numbers after ozonation with 0.8 and 1.6 mg l^{-1} ozone, respectively. Sukenik *et al.* (1987) found that chlorine, ozone and chlorine dioxide all caused a reduction in cell viability and the number concentration of cells. For example, ozone reduced culture optical density at 420 nm (a surrogate for cell concentration) by 10% at a dose of 2.6 mg l^{-1} (0.0013 mg per 10^3 cells). The particle size distributions shown here support the conclusion that cell destruction occurred for *Scenedesmus* when the ratio of the ozone dose to algal concentration was high (i.e. 3 mg l^{-1} ozone applied to a 20,000 cells ml^{-1} suspension, yielding a dose of 0.15 mg per 10^3 cells).

Electrophoretic mobility

The EPM of the cells was measured before and after oxidation (20,000 cells ml^{-1} algae oxidized with 1 or 3 mg l^{-1} ozone or chlorine). Figure 4 shows the change in EPM values upon oxidation for each algal species. Without treatment, the *Scenedesmus* cells were negatively charged with an EPM of -1.53 $(\mu m\ s^{-1})/(Vcm^{-1})$. Ozone at 1 mg l^{-1} (0.05 mg per 10^3 cells) reduced the EPM by 10.5%, while 3 mg l^{-1} ozone (0.15 mg per 10^3 cells) reduced the EPM by 15.7%. Similar reductions were found for preoxidation with chlorine. The EPM of *Cyclotella* without treatment was -2.38 $(\mu m\ s^{-1})/(Vcm^{-1})$. Preoxidation with either chlorine or ozone had little or no effect on the EPM.

The reduction in mobility for *Scenedesmus* cells by ozone or chlorine may be due in part to changes in the

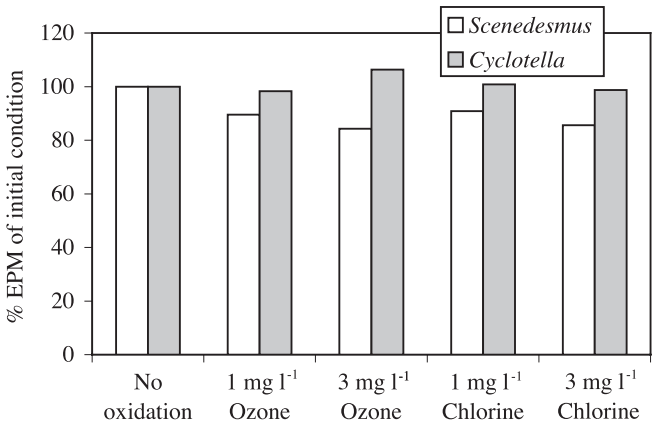

Figure 4 | Algal cell EPM before and after oxidation (20,000 cells ml^{-1}).

exterior portions of the cell wall, which were observed in the SEMs. Conversely, *Cyclotella* appeared much less affected by oxidation in the SEMs, and the mobility of the cells was not appreciably changed.

Coagulation of algae and EOM

Coagulation results

The coagulation of an algal suspension with polyaluminium chloride was examined using bench scale jar tests. Figure 5 shows the effect of coagulant dose and preozonation on coagulation for a *Scenedesmus* suspension of 20,000 cells ml^{-1}. Without preozonation, the highest coagulant dose tested (1.0 mg l^{-1}) resulted in an 83% reduction in particle counts. As there were no other particles than algae in the water and the PACl accomplishes coagulation primarily by charge neutralization, particle counts directly correspond to algal cell removal. Preozonation resulted in a lower settled water turbidity and particle counts at all coagulant doses tested. With 1.2 mg l^{-1} preozone (0.058 mg per 10^3 cells) and no coagulant added, the particle count after the flocculation and settling period was reduced from 21,300 cells ml^{-1} to 8,900 cells ml^{-1}, indicating 58% removal by lysis or self-flocculation and settling. Preozonation at 1.2 mg l^{-1} followed by coagulation with 0.2 mg l^{-1} coagulant reduced particle counts by 94%, while 1.0 mg l^{-1} coagulant reduced particle counts by 99%. The turbidity ranged from 0.5 to 0.13 NTU over the range of coagulant doses

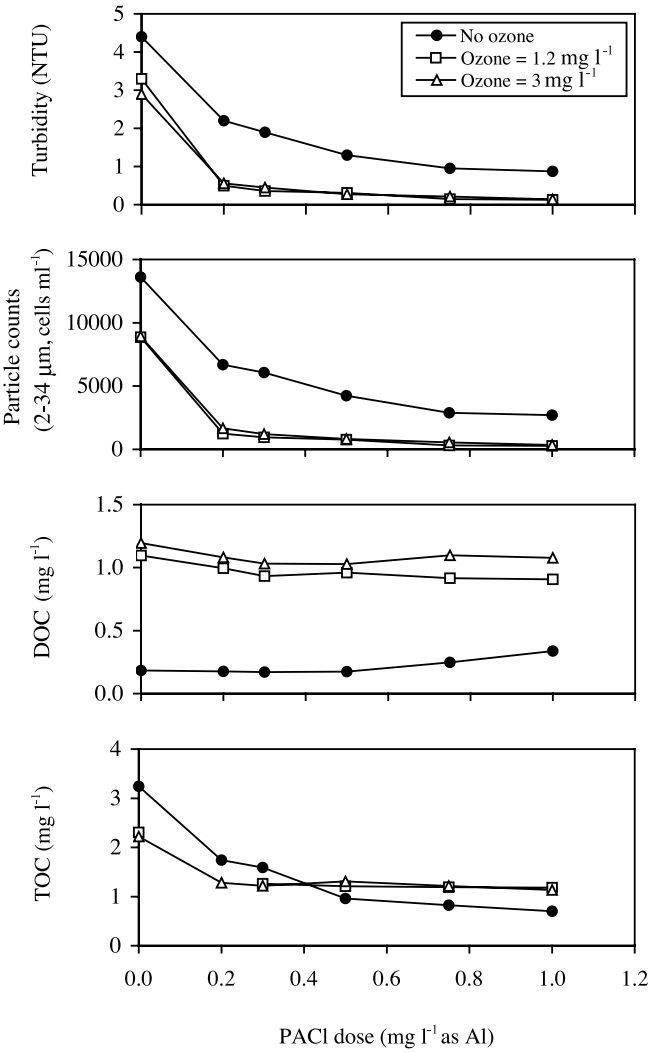

Figure 5 | Effect of ozone dose and coagulant dose on sedimentation performance and removal of *Scenedesmus quadricauda* (20,000 cells ml^{-1}, pH 6.5–6.8).

tested. With preozonation applied at 3 mg l^{-1} (0.15 mg per 10^3 cells), particle counts were further reduced and the turbidity was decreased to 0.08 NTU at the highest coagulant dose tested (1.0 mg l^{-1}). However, preozonation increased the DOC concentration from 0.2 mg l^{-1} to over 1 mg l^{-1}, presumably due to both lysis and increased liberation of adsorbed extracellular material. This additional DOC was difficult to remove by coagulation with PACl. As a consequence, the settled water TOC and DOC concentrations increased when preozonation was

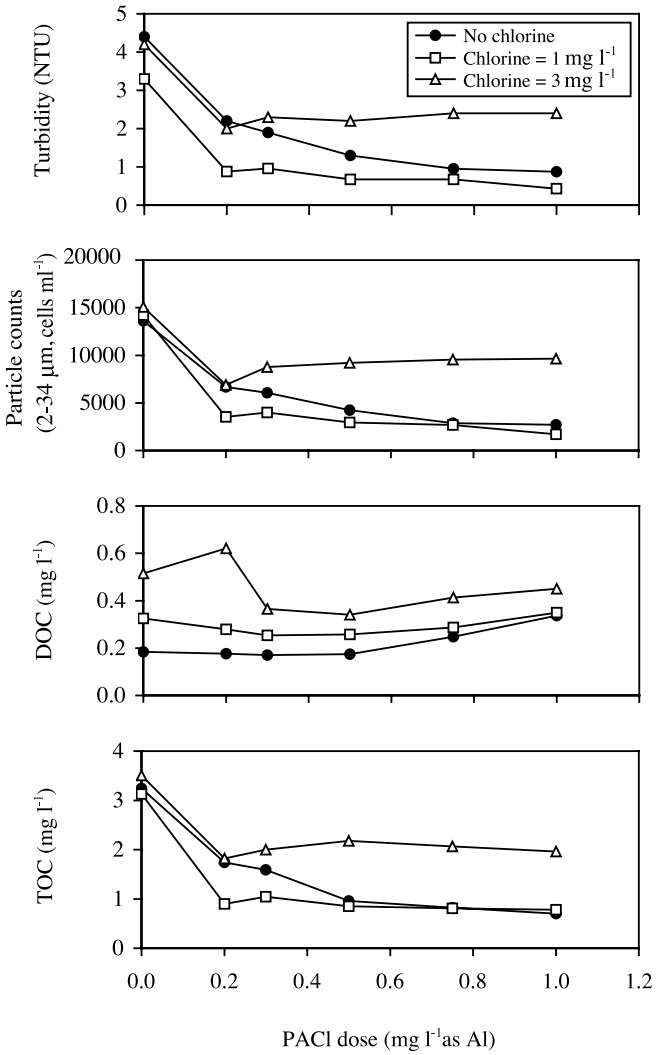

Figure 6 | Effect of chlorine dose and coagulant dose on sedimentation performance and removal of *Scenedesmus quadricauda* (20,000 cells ml^{-1}, pH 6.5–6.8).

applied compared with the control case. Other experiments with 100,000 cells ml^{-1} at low ozone to cell ratios of 0.01–0.03 mg per 10^3 cells showed only an 8% reduction in cell counts without coagulation. These results agree with those previously presented indicating a small degree of cell lysis at these ozone dosages.

Figure 6 shows the effects of coagulant dose and prechlorination on coagulation of 20,000 cells ml^{-1} *S. quadricauda*. Without prechlorination, particle counts were reduced to 2,900 cells ml^{-1} with 0.75 mg l^{-1} as Al, while doses above this value did not further improve particle removal. With 1 mg l^{-1} prechlorine (0.05 mg per 10^3 cells) and no coagulant added, the particle count after the flocculation and settling period was reduced from 15,900 cells ml^{-1} to 14,200 cells ml^{-1} (10% reduction) indicating only minor removal by lysis or self-flocculation and settling. With this prechlorination dose (1 mg l^{-1}), effective coagulation was achieved with a coagulant dose of only 0.25 mg l^{-1}. Regardless of the coagulant dose used, this low chlorine dose decreased the settled water turbidity and particle counts, and also reduced the TOC concentration at low coagulant doses. As with preozonation, prechlorination increased the DOC concentration. Increasing the chlorine dose to 3 mg l^{-1} led to a deterioration of the settled water quality with increased particle counts and organic carbon concentrations.

For *Cyclotella,* initial jar tests conducted with the algae in the log growth phase used a 45 min flocculation time and 60 min settling time (as was used for *Scenedesmus* jar tests). Under these conditions, good removal of algae (settled water particle counts less than 500 cells ml^{-1} and turbidity near 0.10 NTU) was achieved for both the untreated and preoxidized suspensions. Therefore, it was difficult to discern whether preoxidation was harmful, beneficial or had no effect on coagulation. Additional jar tests were conducted with shorter flocculation and sedimentation times (15 and 30 min, respectively).

Figure 7 shows the experimental results for *Cyclotella* with shortened flocculation and sedimentation times, with and without ozone. Without pretreatment, a coagulant dose of 0.5 mg l^{-1} as Al resulted in a settled water particle count of 2,040 cells ml^{-1} (87% algal removal) and a turbidity of 0.46 NTU. It is important to note that with the longer flocculation and settling times, this coagulant dose yielded 260 cells ml^{-1} and a turbidity of 0.08 NTU. After oxidation with 1 mg l^{-1} ozone (0.05 mg per 10^3 cells), the settled water particle count was greatly increased to 7,030 cells ml^{-1} (61% algal removal). DOC and TOC concentrations also increased with preozonation. Similar results were observed when the *Cyclotella* suspensions were pretreated with chlorine (Figure 8). Removal of particles after prechlorination was poor and organic carbon concentrations increased.

Considering all experimental conditions tested, pretreatment with ozone or chlorine at 1 mg l^{-1} for 20,000

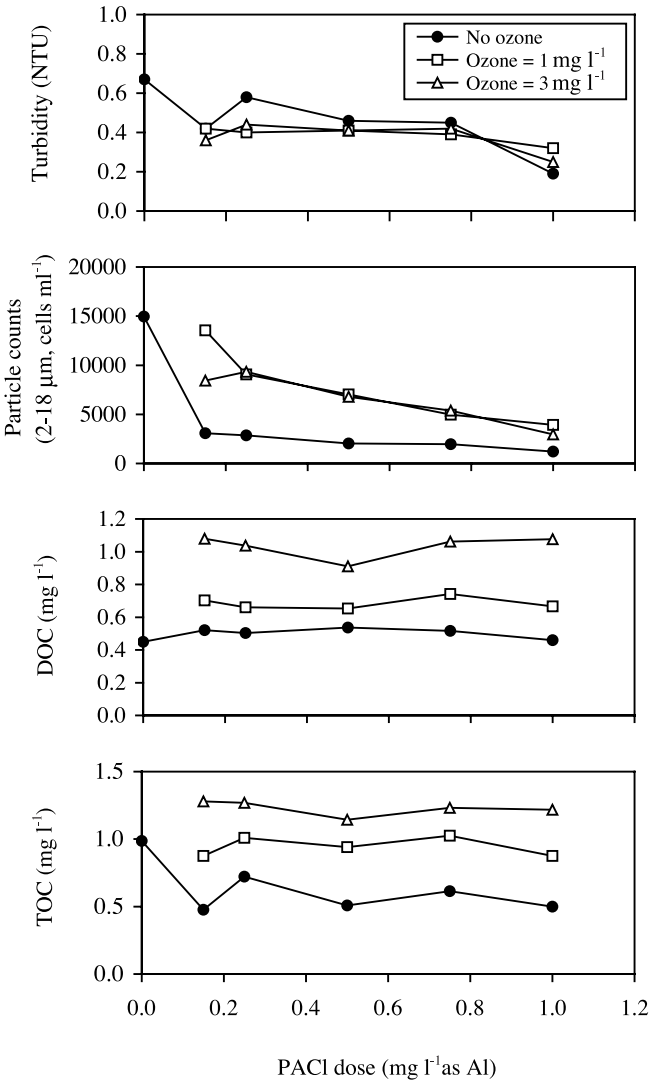

Figure 7 | Effect of ozone dose and coagulant dose on sedimentation performance and removal of *Cyclotella* (20,000 cells ml^{-1}, pH 6.5–6.8).

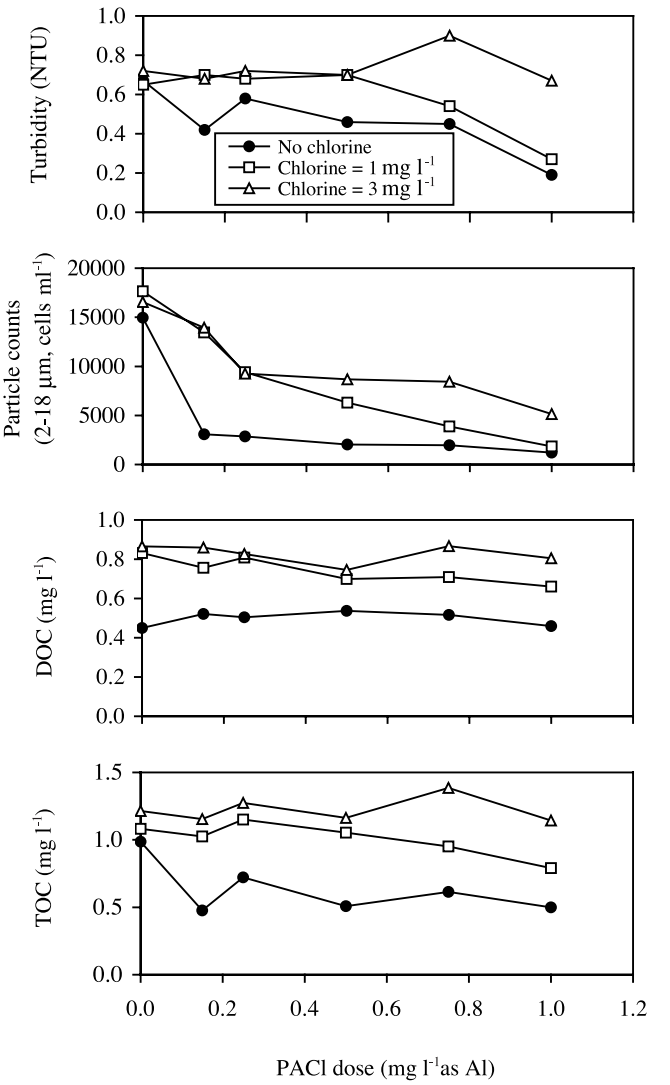

Figure 8 | Effect of chlorine dose and coagulant dose on sedimentation performance and removal of *Cyclotella* (20,000 cells ml^{-1}, pH 6.5–6.8).

cells ml^{-1} was beneficial for the removal of *Scenedesmus* cells. Removal by lysis was evident when no coagulant was added. With coagulant, effective coagulation was observed at lower coagulant doses, and lower settled water turbidity and particle counts were measured. For *Cyclotella,* no benefit was observed when preoxidation was applied. However, for both algae, DOC increased after oxidation and resulted in higher settled water DOC concentrations.

Discussion of coagulation results

It has been shown here that preozone enhanced the removal of *Scenedesmus* by coagulation and settling. This was not found for *Cyclotella.* Others have found that sometimes preozone improves algae and particle removal. Sukenik *et al.* (1987) found a decrease in alum dose needed to flocculate ozonated algae and attributed this result to the disturbance to the reticulate layer, which presumably caused a decrease in colloidal stability. Similar results were found in this study based on the SEM and EPM data.

However, extensive evidence was also found for lysis of *Scenedesmus*, especially when the cell concentration was low compared with the oxidant dose. *Cyclotella* showed less cell wall alteration after oxidation, and consequently did not experience the same improvements in flocculation after oxidation. Petruševski *et al.* (1994) further argued that oxidation roughened the surface of cells and improved the attachment of metal coagulant precipitates. Results at the pilot scale have also demonstrated the benefits of preozonation. Montiel & Welté (1998) measured algal removals at a pilot plant with flotation and filtration in which the raw water had algal counts up to 70,000 algae ml^{-1} (predominantly green algae and diatoms). They observed an improvement in algal removal from 75% to 93% when preozonation (1 mg l^{-1}) was added to the treatment plant. Lastly, the detrimental effect of chlorine at high doses is in agreement with coagulation results from Sukenik *et al.* (1987). When 2 mg l^{-1} chlorine was applied to algal suspensions, algae removal was similar to the control case. However, increasing the chlorine dose to 10 or 20 mg l^{-1} (for a suspension of 2×10^6 cells ml^{-1}) resulted in poorer coagulation of the algae.

With regard to the dissolved organic carbon concentration increase after oxidation, this phenomenon has also been observed by others. Sukenik *et al.* (1987) measured DOC increases of approximately 1.2 times the original DOC after application of either ozone or chlorine. Widrig *et al.* (1996) showed that very high doses of coagulant were necessary to remove algal-derived DOC.

CONCLUSIONS

Ozonation or chlorination of an algal suspension had a significant impact on the character of the suspension, including changes in cell surface characteristics, cell viability and electrophoretic mobility. For *Scenedesmus*, oxidation caused lysis of some cells, altered the cell wall of remaining cells, and decreased the electrophoretic mobility. These changes also caused an increase in the DOC of the suspension, presumably due to increased liberation of extracellular organic matter as well as lysis. *Cyclotella* was also affected by oxidation. Although the DOC increased, the SEMs showed little alteration to the cell surface, and the EPM of the cells was not appreciably changed.

When preoxidation was applied to *Scenedesmus*, turbidity and particle counts were reduced after coagulation with PACl. This reduction in cell numbers was due to lysis of some cells and also improved coagulation. In addition to reducing particle numbers at a given coagulant dose, the coagulant dose necessary to achieve a given settled water particle concentration was reduced. In contrast, preoxidation had a detrimental effect on the removal of *Cyclotella* with PACl. The coagulation results, combined with results from SEMs and particle size distributions, suggest that the oxidants attack the cell wall of *Scenedesmus,* causing changes to the cell surface and thus the cell stability, and also cause lysis of some cells. These factors lead to more effective coagulation and removal of the cells. *Cyclotella* cells showed more resistance to damage from the oxidants and thus did not benefit from changes in cell and coagulation characteristics.

The results of this research showed important impacts for water supply reservoirs treated with ozone. First, at low oxidant dose to cell number ratios (0.01–0.03 mg per 10^3 cells), algal cells may remain intact and must be removed by downstream treatment processes. At higher oxidant dose to cell number ratios (>0.05 mg per 10^3 cells), as might be expected in natural waters with low cell concentrations, some cells may be lysed. Increased DOC due to lysed cells and increased release of extracellular organic matter due to oxidant induced stress can result in poor coagulation of organic carbon when using a PACl coagulant, an increase in tastes and odours and increased disinfection by-products. Third, improved coagulation and settling for waters containing algae depend on the algal species.

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