Laboratory-scale simulations with hydrated lime and organic polymer to evaluate the effect of pre-chlorination on motile *Ceratium hirundinella* cells during conventional water treatment

H Ewerts[1,2], S Barnard[1*] and A Swanepoel[2]

[1]*Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa*
[2]*Rand Water Analytical Services, PO Box 3526, Vereeniging, 1930, South Africa*

ABSTRACT

Algal genera such as *Carteria*, *Chlamydomonas*, *Chlorogonium*, *Cryptomonas*, *Ceratium*, *Peridinium* and *Euglena* are motile and may disrupt unit processes and cause water treatment problems. Algal species belonging to these motile algal genera are known to interfere with coagulation and flocculation unit processes which are the main processes for algal removal. These cells are well adapted, by means of their motile structures, morphological shapes and storage products, to remain in the supernatant (by swimming or floating) until it is carried over to sand filters, where cells may cause filter-clogging problems. When organic material is released from algal cells as a result of physical-chemical impacts on the cells, it may result in taste- and odour-related problems or the formation of harmful organic products such as trihalomethanes (THM). The aims of this study were to: (i) determine chlorine concentrations required to immobilise *C. hirundinella* cells; (ii) determine the removal efficiencies of pre-chlorination; (iii) investigate the integrity of *C. hirundinella* cells; and (iv) identify trihalomethanes that are formed. Source water samples enriched with *C. hirundinella* cells were exposed to a pre-determined chlorine concentration range (0.05–0.45 mg/L). This study found that the half-maximal inhibitory concentration (IC_{50}-values) for chlorine < 0.20 mg/L is sufficient to render *C. hirundinella* cells immobile, while cells remain intact. Pre-chlorination did not have an impact on *C. hirundinella* removal when hydrated lime was used as a coagulant or coagulant aid. However, when organic polymer only was used as coagulant, removal efficiencies were improved by 20%. Chlorine by-products were measured, but posed no specific health risks to drinking water consumers due to the low concentration levels measured. Algal removal challenges that occur in water treatment plants when dosing organic polymers can be resolved by implementation of effective pre-chlorination strategies.

Keywords: algae, coagulation, dinoflagellate, pre-treatment, trihalomethanes (THM)

INTRODUCTION

Ceratium hirundinella (*C. hirundinella*) is a well-known fresh-water dinoflagellate. However, it has only been found to occur in extreme bloom-forming proportions in South Africa since 1999 (Van Ginkel et al., 2001a). *C. hirundinella* cells in source water treated for the production of drinking water have become a concerning issue to water treatment utilities, due to the fact that these cells may cause water purification related problems even at relatively low concentrations (Swanepoel et al., 2008a; Ewerts et al., 2013). The financial and operational impacts of *C. hirundinella* cells on the drinking water treatment industry are high, as this algal species is responsible for bad tastes and odours as well as clogging of sand filters (Van Ginkel et al., 2007). According to Pieterse et al. (2000) phytoplankton cells such as, *C. hirundinella* which contain flagella have the ability to: (i) interfere with conventional coagulation and flocculation by disrupting flocs and (ii) penetrate into the treated water. Studies undertaken by Ewerts et al. (2013) and Ewerts et al. (2014) support that this species is a problem-causing alga in conventional water treatment plants, and is responsible for floc disruption, filter clogging, increasing chlorine demand, taste and fishy-odour problems. Water treatment related problems caused by *C. hirundinella* cells can thus be ascribed to the fact that the dinoflagellate cells are large and highly motile, giving

cells the advantage to migrate through the water column better than other algae (Gligora et al., 2003).

A chlorophyll breakthrough event into the treated water was observed at South Africa's largest conventional water treatment plant (SALCWTP) during the summer of 2006 (Swanepoel et al., 2008a). This was the result of high numbers of *C. hirundinella* cells entering the final treated water (Swanepoel et al., 2008a). It was evident that the biggest problems regarding the break-through were encountered at the sand filtration step, from where an average of > 2 000 cells/mL penetrated into the potable water (Swanepoel et al., 2008a). Although coagulation, flocculation and sedimentation removed some of the biomass, an increase in phytoplankton concentrations was observed after the sand filtration (Swanepoel et al., 2008a). Organic polymer was dosed as coagulant at flocculators supplying a part of the station and hydrated lime in combination with activated silica was dosed at all of the other flocculators (Swanepoel et al., 2008a). With the increase in *C. hirundinella* cell concentrations in the source water, an increase in algal breakthrough into the drinking water may occur when unit processes are not optimised. This is due to its unique characteristics that can impede its removal by means of coagulation, flocculation and subsequently sedimentation (Swanepoel et al., 2008a). Therefore, from September to April, when these highly motile dinoflagellate cells occur in source water in South Africa, water treatment plants are required to optimise unit processes and coagulant dosages in order to remove these cells. However, not all coagulant treatment options used by water treatment plants are able to aid in the effective removal

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of such motile algae. Pre-treatment strategies such as pre-chlorination can be applied to immobilise cells in order to assist coagulation and flocculation unit processes, which will subsequently improve removal efficiencies.

The implementation of pre-oxidation with various oxidants (e.g. chlorination, potassium permanganate, ozone) is widely applied for various pre-treatment purposes, such as control of algae and oxidising of organic material during water treatment (Van der Walt et al., 2009). Oxidants have biocidal properties; therefore, they can be used to control extensive aquatic growths such as phytoplankton (algae and cyanobacteria) and are also used as primary disinfectants (Van der Walt et al. 2009). Pre-oxidation chemicals, such as chlorine and ozone, are well-known to enhance phytoplankton removal during the production of drinking water (Knappe et al., 2004; Van der Walt et al., 2009; Ma et al., 2012). Various studies have shown improved phytoplankton removal efficiencies by conventional processes (e.g. coagulation, flocculation and sedimentation) as a result of pre-chlorination (Steynberg et al., 1994; Henderson et al., 2008 and Zamyadi et al., 2012). It was reported that chlorine inactivates microorganisms by crossing cell membranes and damaging the intracellular organelles (Venkobacher et al., 1977). In their study on the effects of pre-chlorination on cell integrity of *Microcystis aeruginosa*, Ma et al. (2012) found that, although chlorination led to a leaky membrane, no change in cell morphology was observed.

Organic material that originates from algae can leak out when the cell membrane is damaged and act as an important precursor for trihalomethanes (THM) (Abdullah et al., 2009; Van der Walt et al., 2009; Lui et al., 2011; Zamyadi et al., 2012). The formation of THM poses a potential health risk to drinking water consumers, since THM are mutagens, carcinogens and toxic (Abdullah et al., 2009; Hebert et al., 2010). In light of these risks associated with pre-chlorination, this study in particular focused on the use of effective chlorine concentrations required to immobilise at least 50% of the *C. hirundinella* cells, while cells remain intact, as well as monitoring the concentration of THM that formed as a result of pre-chlorination.

The pre-chlorination practice mentioned can be applied to immobilise *C. hirundinella* cells in order to assist coagulation and subsequently reduce the financial and operational impact on the water treatment facility. Therefore, the aims of this study were to: (i) evaluate chlorine concentrations required to immobilise *C. hirundinella* cells prior to coagulation, (ii) determine the removal efficiencies of pre-chlorination in combination with different coagulants, (iii) investigate the integrity of *C. hirundinella* cells, and (iv) identify the formation of chlorine byproducts.

MATERIALS AND METHODS

Water samples: collection and preparation

Source water from Benoni Lake, South Africa, containing relatively high *C. hirundinella* concentrations (ranging between 1 000 and 4 000 cells/mL) was collected during 4 sampling occasions (November–December 2011). During periods of low *C. hirundinella* concentrations present in the source water, cells were concentrated using a 50 µm phytoplankton net to concentrate cells to ≥ 500 cells/mL. No adverse effects on the morphology of *C. hirundinella* cells were noticed due to this concentration step. All water samples were collected in pre-washed plastic containers and stored under laboratory conditions (± 22°C).

Determination of pre-chlorination concentrations using dose response fittings (half maximal inhibitory concentrations IC$_{50}$) and cell integrity (damage to cells)

Chlorine dosages

The pre-chlorination method used for this study was adapted from an in-house method (used at analytical laboratories of SALCWTP) that is used to determine the acute toxicity of chlorine to *Daphnia pulex* (Bungu, 2009). Chlorine measurement procedures were adapted from standard methods as described by APHA, (2013). Chlorine concentrations were dosed into jar beakers (containing experimental water or filtered source water) with glassware and equipment (e.g., pipettes and syringes) that were chlorine saturated (no chlorine demand). This was achieved by soaking the glassware in 4 mg/L chlorine (prepared with deionised water) for 24 h before use. After 24 h the glassware was rinsed thoroughly with distilled water and dried.

Chlorine solutions were prepared to range from 0.05 mg/L up to 0.45 mg/L (with equal increments). This dosage range were used to conduct chlorine exposure experiments, since most of the cells collected during the early stages of the *C. hirundinella* bloom lost their integrity (cell lysis occurred) at > 0.45 mg/L. Chlorine stock solutions were prepared from sodium hypochlorite, reagent grade, available chlorine 10–13% (Sigma-Aldrich). Chlorine measurements were performed using the HACH Pocket Colorimeter II Analysis System, using the diethyl-p-phenylene-diemine (DPD) tests, which can measure chlorine concentrations within the following ranges: 0.02 to 2.00 mg/L Cl_2 and 0.1 to 8.0 mg/L Cl_2. The typical pre-chlorination dosages (about 2–4 mg/L Cl_2 or 4–6 mg/L Cl_2) dosed for algal removal were not applicable to perform this method, but a new dosage range was determined as part of the study based on *C. hirundinella* cell immobilisation experiments. Therefore, no estimated detection limits for the pre-chlorination method used were known at the time of initiation of the experiment. Filtered source water enriched with a known concentration of *C. hirundinella* cells (target organisms) was exposed for approximately 5 min (T) to increasing concentrations (C) ranging from 0.05 mg/L to 0.45 mg/L (with equal increments of 0.05). After 5 min exposure, an inverted light microscope was used to enumerate the number of immobilised *C. hirundinella* cells (used to draw the dose response graphs). All chlorine exposure experiments with different chlorine concentrations were conducted in triplicate.

Water treatment simulations: pre-chlorination with and without coagulant treatments

Coagulant dosages

All coagulant chemicals were collected from the treatment plant that was simulated during this study by means of jar stirring tests. The use of hydrated lime in combination with activated silica (Ca(OH)-SiO$_2$) for destabilization and flocculation of suspended material is unique for the purification of Vaal Dam water in South Africa (Geldenhuys et al., 2000). South Africa's largest drinking water treatment plant selects coagulants and appropriate quantities based on a settling turbidity of < 5 NTU, during jar stirring tests. The following coagulant stock solutions and dosage ranges were prepared to perform jar stirring tests:

- Ca(OH)$_2$-SiO$_2$: Ca(OH)$_2$ dosages ranged from 60 to 160 mg/L (in increments of 20). A SiO$_2$ dosage of 4 mg/L was dosed as a coagulant aid.
- Ca(OH)$_2$-organic polymer: organic polymer (cationic high molecular weight) dosages ranged from 4 to 14 mg/L (in increments of 2). A Ca(OH)$_2$ dosage of 10 mg/L was added as a coagulant aid.
- Organic polymer: organic polymer dosages ranged from 4 to 14 mg/L with no coagulant aid (in increments of 2).

When dosing Ca(OH)$_2$-SiO$_2$ and Ca(OH)$_2$-organic polymer, the pH values of source water may increase from around pH 8.00–8.79 to ≥ pH 11.5 and ≥ pH 9.5, respectively, as recorded in the supernatant, while organic polymer alone may not be able to change the pH of the supernatant (Ewerts, 2015). During full-scale treatment, the pH of the supernatant is stabilised after sedimentation; however, this study only focuses on the improvement of flocculation and coagulation conditions as a result of pre-chlorination. The control of pH or removal of other impurities (from the supernatant) by subsequent unit processes such as chemical stabilisation and sand filtration (whereby supernatant is filtered) were not investigated during this study.

Pre-determined chlorine dosages (concentrations ranged between 0.05 mg/L and 0.45 mg/L) to establish the half maximal inhibitory concentrations (IC$_{50}$) were dosed 5 min prior to flash mixing into 2 000 mL experimental (filtered source) water samples containing 1 360 cells/mL, 2583 cells/mL, 3 746 cells/mL, and 1 481 cells/mL, during sampling occasion-a, occasion-b, occasion-c and occasion-d, respectively. The Phipps and Bird jar stirrer apparatus were used to conduct jar stirring tests. Different coagulant dosages were added with syringes and allowed to disperse uniformly at high-energy flash mixing conditions for another 30 s. Three decreasing energy stages of 125 s^{-1}, 54 s^{-1} and 14 s^{-1} were applied for 8 min, 1.5 min and 1 min respectively. Stirring paddles were switched off to allow flocs to settle for 20 min. Samples of the supernatants were collected to investigate the efficacy of coagulation, flocculation and sedimentation after dosing various coagulants.

Ceratium hirundinella cell enumeration

The sedimentation/centrifugation technique that was originally described by Lund et al. (1958) and adapted for Rand Water according to Swanepoel et al. (2008b), was used for identification and enumeration of *C. hirundinella* cells. The concentration (cells/mL) and physical integrity of *C. hirundinella* cells were determined and evaluated using an inverted light microscope.

Total photosynthetic pigments (TPP)

The presence of phytoplankton (algae and cyanobacteria) in water can be detected by a chlorophyll (chlorophyll-*a*, known as a photosynthetic pigment and other accessory pigments) analysis (Knappe et al., 2004; Swanepoel et al., 2008b). Analytical laboratories analysing samples for SALCWTP have developed a more sensitive method for the detection of chlorophyll in lower concentrations. In this study, this method is referred to as the total photosynthetic pigment (TPP) analysis, which can be used to detect chlorophyll concentration when relatively low phytoplankton concentrations (< 40 cells/mL) occur in the water, after different stages of water treatment as well as in the final treated water (Swanepoel et al., 2008b). After gravity sedimentation, the supernatant was analysed in terms of the content of TPP. Supernatant samples (100 mL) were filtered using membrane filters (< 0.45 µm). Membranes filters were boiled in 10 mL methanol to extract pigment into the methanol. Absorbance of the extract was measured at 650 and 750 nm using the Beckman Coulter (DU 65i) spectrophotometer (Swanepoel et al., 2008b).

Total organic carbon (TOC), dissolved organic carbon (DOC) and trihalomethanes (THM)

The content of organic matter was determined as total organic carbon (TOC) and dissolved organic carbon (DOC) using standard methods for examination of water and waste water samples (APHA 2013). TOC samples were collected headspace-free in 40 mL glass vials and preserved with 21% phosphoric acid and measurements performed (Phoenix and Fusion). Trihalomethanes (bromochloroform, bromoform, chloroform and dibromochloroform) were determined using a gas chromatograph linked to an electron capture detector (GCECD). The specific running conditions are listed in Table 1.

Statistical analysis

T-*Tests*

T-test analyses were performed to determine statistically significant differences between %TPP removed by various coagulant dosages and %TPP removed by coagulant dosages that were assisted by pre-chlorination (using Microsoft Office Excel 2007). The level of significance for all statistical analyses was set at *p*-values of 0.05 and the hypotheses were stated separately for each analysis with the hypothesised difference equal to zero.

TABLE 1
The Instrument configuration for GC-ECD parameters and running conditions

GC Oven	Front inlet	Capillary column (HP-1 methyl siloxane or equivalent)	Front detector (µECD)
Oven temperature: 40°C	Mode: Split	Maximum temperature: 300°C	Temperature: 315°C
Run time: 10 min	Initial temperature:	Mode: Constant flow	Mode: Constant makeup flow
	Pressure: 3.30 psi (22.75 KPa)	Initial flow: 5.1 mL/min	Makeup flow: 30.0 mL/min
	Split ratio: 10:1	Average velocity: 36 cm/sec.	
	Split flow: 51.1 mL/min		

Dose response graphs (hill slopes)

To determine the chlorine concentration where 50% of the initial *C. hirundinella* cells are immobilised, described as the half maximal inhibitory concentration (IC_{50}), the dose response graphs (Hill slopes of CurveExpert Pro Version 2.0.3) were used. The concentration-response curves (showing a typical sigmoidal shape) in the Hill Slopes models were fitted to evaluate the IC_{50} (Henlory et al., 2013; Gadagkar and Call, 2015). In order to plot sigmoidal curves, data were normalised to percentage of the maximum response, and subtracting a baseline. The y-axis indicated the percentage cell immobilisation by the chlorine dosages on the x-axis. The pre-chlorination dosages determined by dose response graphs were different (but remained below 1 mg/L) due to different sampling occasions (changes in water quality) and cell integrity considerations.

RESULTS

The average value, minimum value, maximum value and standard deviation of water quality parameters measured for 10 sampling occasions (*n*=10) in Table 2 represents a freshwater impoundment with a typical eutrophic status (based on nitrogen and phosphate values). The average turbidity of the water was relatively low at 7.32 NTU with TDS values ≥ 100 mg/L. Organic carbon (TOC) varied between 4.70 mg/L and 11.00 mg/L and should be considered as an important parameter to be evaluated during chlorine exposure experiments.

Effect of different chlorine concentrations on the motility and integrity of *C. hirundinella* cells

The effects of chlorine dosages on the motility and integrity of cells were observed microscopically. Figure 1a shows cells in the source water, while disruptions to cell integrity after chlorine exposure with dosages of > 0.20 mg/L are shown in Fig. 1c.

Pre-chlorination concentrations required to immobilise *C. hirundinella* cells, while maintaining the integrity of cells

Figure 2 shows the dose response models for chlorine exposure experiments when dosing chlorine concentrations within the range of 0.05 mg/L to 0.45 mg/L (the range indicated in Fig. 2, varied between 0.05 mg/L and 0.25 mg/L) to immobilise 50% of the initial *C. hirundinella* concentrations. During the different chlorine exposure experiments, the IC_{50} values and the impacts thereof on the integrity of cells were evaluated to determine the appropriate pre-chlorination dose (Table 3). Therefore, pre-chlorination values used during experiments may not necessarily correspond with the dose-response graphs due to cell integrity factors. The IC_{50} chlorine concentration values illustrated in the dose-response graphs a–b in Fig. 2 were below 0.20 mg/L with no specific correlations to the initial *C. hirundinella* concentrations. However, a poor sigmoidal graph (Hill slopes) illustration was observed for occasion-c which may be the result of cells becoming more sensitive to chlorine exposure; however, the pre-chlorination dose for immobilisation (0.121 mg/L) was similar to the dosage determined during occasion-a.

Table 3 lists the initial *C. hirundinella* concentrations in the source water used during these four chlorine exposure

TABLE 3
The initial *C. hirundinella* cell concentrations in source water used to determine the pre-chlorination concentrations during 4 chlorine exposure experiments.

	Occasion-a	Occasion-b	Occasion-c	Occasion-d
Initial cell concentrations (cells/mL)	1 360	2 583	3 746	1 481
Pre-chlorination concentration (mg/L)	0.121	0.186	0.121	0.106

TABLE 2
The key parameters summarizing the quality of source water collected from Benoni Lake during the sampling period. According to the Department of Water and Sanitation's trophic status criteria, the chemical determinants listed below can be used to determine the trophic status of freshwater impoundments such as Benoni Lake (Van Ginkel et al., 2001b).

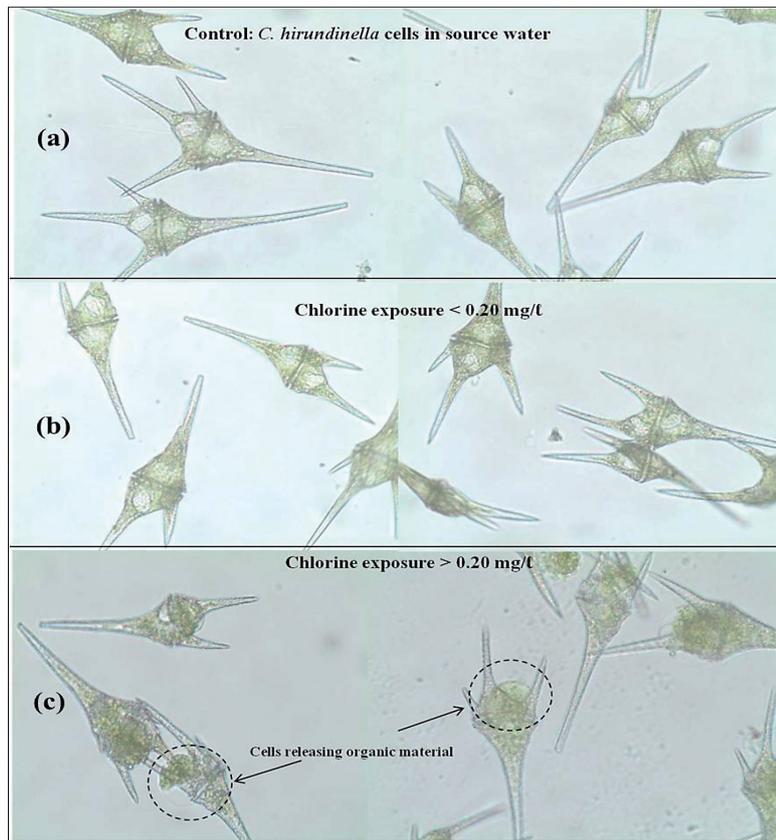|	Units	Average (*n* = 10)	Minimum value	Maximum value	Standard deviation
Nitrate (NO_3^-)	mg/L	0.95	0.00	8.60	2.69
Nitrite (NO_2^-)	mg/L	0.01	0.00	0.04	0.01
Total Kjeldahl nitrogen (TKN)	mg/L	2.41	1.20	5.90	1.32
Phosphorus (P)	mg/L	0.07	0.00	0.33	0.12
Total Phosphate (TP)	mg/L	0.01	0.00	0.09	0.03
Chemical oxygen demand (COD)	mg/L	22.00	16.00	33.00	4.90
Hardness as $CaCO_3$	mg/L	75.80	57.00	92.00	9.80
Total dissolved solids (TDS)	mg/L	148.50	100.00	200.00	31.63
Turbidity	NTU	7.32	3.90	14.00	2.97
M-alkalinity as $CaCO_3$	mg/L	83.40	53.00	260.00	62.38
pH		6.59	6.24	6.99	0.24
Conductivity	mS/m	26.30	21.00	39.00	5.27
Total organic carbon (TOC)	mg/L	7.15	4.70	11.00	2.01

Figure 1

Light microscopy images of C. hirundinella *cells in source water (a) and the effects of chlorine concentrations after exposure to chlorine concentrations below 0.20 mg/L (b) and chlorine concentrations ≥ 0.20 mg/L (c).*
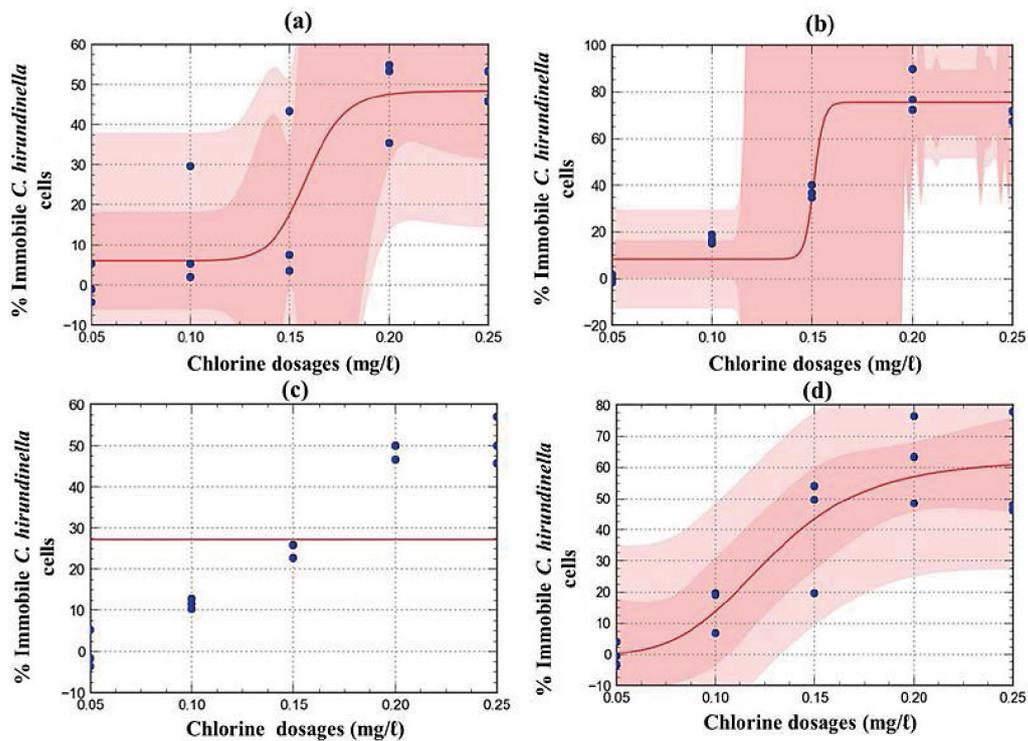

Figure 2

The dose-response models (Hill slopes) for 4 chlorine exposure experiments and the relationships between the percentages (%) measured for immobilised C. hirundinella cells and increasing chlorine concentrations.

experiments together with pre-chlorination concentrations. Although no specific correlations between the number of cells and pre-chlorination concentrations can be observed, the pre-chlorination concentrations used in this study were below 0.20 mg/L.

The effect of pre-chlorination on the percentage (%) TPP removal during coagulation and flocculation

Relatively low pre-chlorination concentrations (below 0.20 mg/L) are indicated in Table 3 (0.121 mg/L, 0.186 mg/L, 0.121 mg/L and 0.106 mg/L). These chlorine concentrations were dosed prior to coagulation, flocculation and sedimentation unit processes. Results obtained after sedimentation illustrate the removal efficiencies of *C. hirundinella* cells (indicated as %TPP removal) when the following coagulants were dosed in combination with pre-determined chlorine concentrations: $Ca(OH)_2SiO_2$; $Ca(OH)_2$ organic polymer and organic polymer. These coagulant dosages were also dosed without pre-chlorination as a control to evaluate the removal efficiencies.

The effects of pre-chlorination (chlorine dose of 0.121 mg/L) on %TPP removal when dosing $Ca(OH)_2$-SiO$_2$ and

$Ca(OH)_2$-organic polymer showed poor *C. hirundinella* cell removal as indicated by the %TPP removal. The effect of pre-chlorination on *C. hirundinella* removal was better illustrated by the %TPP removal when organic polymer alone was used as a coagulant option (Table 4). These results have indicated that organic polymer may not be the best coagulant option to remove *C. hirundinella*, but the effect of *C. hirundinella* immobilisation as a result of pre-chlorination is better shown when dosing organic polymer. On the other hand, when pre-chlorination was used prior to $Ca(OH)_2$ treatment options, no improvement in the %TPP was observed due to the effect of pH adjustments caused by $Ca(OH)_2$ on *C. hirundinella* cell removal. The effect of pH changes immobilised the cells; therefore, the effects of pre-chlorination on cell removal as a result of immobilisation could not be observed in the %TPP removal. Due to these previous findings, organic polymer without a coagulant aid was dosed as the primary coagulant during experimental occasion-b; occasion-c and occasion-d to illustrate the effect of pre-chlorination (Table 5). Results in Table 5 were obtained using the chlorine concentrations of 0.186 mg/L, 0.121 mg/L and 0.106 mg/L that were able to assist the lowest organic coagulant dosage of 4 mg/L to improve the percentage TPP removal

TABLE 4
The %TPP removal by various coagulants compared to %TPP removal achieved when the same coagulants were assisted by pre-chlorination. Pre-chlorination concentration of 0.121 mg/L as previously determined during occasion-a (Table 3).

Ca(OH)$_2$-SiO$_2$ Ca(OH)$_2$ dosages (60–160 mg/L increments of 20)			Ca(OH)$_2$-organic polymer Organic polymer dosages (4–14 mg/L increments of 2)			Organic polymer Dosages (4–14 mg/L increments of 2)		
Control %TPP removal	Pre-Cl$_2$ %TPP removal	Removal success	Control %TPP removal	Pre-Cl$_2$ %TPP removal	Removal success	Control %TPP removal	Pre-Cl$_2$ %TPP removal	Removal success
58.90	60.27	✓	43.15	33.56	✗	28.08	38.36	✓
74.66	63.01	✗	50.68	45.89	✗	30.14	34.25	✓
93.15	86.99	✗	52.74	60.96	✓	34.93	43.15	✓
96.44	95.00	✗	64.38	65.75	✓	50.00	55.48	✓
97.74	96.37	✗	76.03	73.97	✗	60.27	65.07	✓
98.29	97.67	✗	78.77	76.71	✗	61.64	68.49	✓
p-value		0.37	*p*-value		0.44	*p*-value		0.23

Removal success: *The effect of pre-chlorination on %TPP removal is indicated with a tick (✓), while no improved %TPP removal is indicated with a cross (✗). The 6 percentages in the table for %TPP removal were obtained from 6 increasing coagulant dosages (with equal increments) for each treatment option.*

TABLE 5
The %TPP removal by organic polymer compared to %TPP removal achieved when organic polymer were assisted by pre-chlorination (control). Organic polymer dosages (4–14 mg/L increments of 2). See occasion-b, occasion-c and occasion-d (Table 3).

Occasion-b (Pre-chlorination concentration 0.186 mg/L)			Occasion-c (Pre-chlorination concentration 0.121 mg/L)			Occasion-d (Pre-chlorination concentration 0.106 mg/L)		
Control %TPP removal	Pre-Cl$_2$ %TPP removal	Removal success	Control %TPP removal	Pre-Cl$_2$ %TPP removal	Removal success	Control %TPP removal	Pre-Cl$_2$ %TPP removal	Removal success
32.31	41.54	✓	31.90	52.16	✓	29.82	31.58	✓
36.92	44.62	✓	41.81	53.02	✓	31.58	40.35	✓
35.38	49.23	✓	48.71	64.22	✓	35.09	43.86	✓
49.23	63.08	✓	63.36	76.72	✓	49.12	57.89	✓
53.85	67.69	✓	72.41	83.62	✓	59.65	66.67	✓
49.23	69.23	✓	76.72	87.07	✓	29.82	31.58	✓
p-value		0.03	*p*-value		0.09	*p*-value		0.22

Removal success: *The effect of pre-chlorination on %TPP removal is indicated with a tick (✓), while no improved %TPP removal is indicated with cross (✗). The 6 percentages in the table for %TPP removal were obtained from 6 increasing coagulant dosages (with equal increments) for each treatment option.*

TABLE 6
The minimum (min.) and maximum (max.) values for THM (bromodichloroform and chloroform) after chlorine exposure experiments and organic carbon (TOC and DOC) concentrations measured in samples collected during 4 sampling occasions (a–d).

| | THM | | | | Organic carbon | |
| | Bromodichloroform (µg/L) SANS 241 guideline for Bromo-dichloroform ≤ 100 µg/L | | Chloroform (µg/L) SANS 241 guideline for chloroform ≤ 300 µg/L | | TOC (mg/L) SANS 241 guideline for TOC ≤ 10 mg/L | DOC (mg/L) SANS 241 guideline for DOC (included in TOC) |
Occasions	min.	max.	min.	max.		
a	5.70	6.20	15.00	22.00	10.00	8.20
b	3.90	5.80	13.00	22.00	6.30	5.40
c	1.70	6.50	3.60	23.00	11.00	9.90
d	0.00	0.00	1.00	2.50	7.20	5.80

South African National Standards for drinking water (SANS 241)

by 9%, 20% and 2% respectively. The highest dosage (14 mg/L), on the other hand, improved the TPP removal percentages by 20%, 10% and 2%, respectively.

The formation of trihalomethanes (THM) during pre-chlorination

Large algae, such as *C. hirundinella* cells, contribute to significant quantities of the total organic carbon (TOC) in source water (Table 2). When chlorine reacts with TOC in the source water, it results in the formation of harmful chlorine by-products known as trihalomethanes. TOC and DOC concentrations measured in source water during all sampling occasions varied from 6.3–11 mg/L and 5.4–9.9 mg/L, respectively. The concentrations of organic carbon compounds measured during different sampling occasions of this study were relatively similar, while increased THM concentrations were measured as a result of increasing chlorine concentrations.

Four THM were present after chlorine exposure, namely; bromodichloroform, bromoform, chloroform and dibromochloroform. The THM consisted mostly of bromodichloroform and chloroform, since bromoform and dibromochloroform were below the detection limits of the method used in this study. The minimum (min) and maximum (max) values measured for bromodichloroform and chloroform is given in Table 6. These values were obtained after dosing a chlorine range of 0.05 to 0.45 mg/L.

DISCUSSION

Extreme blooms of *C. hirundinella* in South African impoundments have become more frequent since it was first observed in 1999 in the Hartbeespoort Dam (Van Ginkel et al., 2001a, Barnard et al., 2014). A breakthrough event observed at SALCWTP highlighted the problems that can be experienced during the production of potable water when blooms of *C. hirundinella* occur in source waters (Swanepoel et al., 2008). When such environmental changes (algal blooms) occur that have an impact on source water quality, a conventional water treatment plant should be able to optimise unit processes or have effective pre-treatment options to ensure good drinking water quality.

Water samples enriched with *C. hirundinella* cells were collected from a freshwater lake (Benoni Lake, South Africa) from early spring (September) until late autumn (March) months. During early spring months cells appeared highly motile with no damaged cells (as observed with light microscope after sampling), but become less active by means of motility towards the late autumn months, with a few damaged cells that will release organic material into the source water. Therefore, effective monitoring and optimisation strategies are required to assist water treatment plant managers and operators during events of *C. hirundinella* blooms, especially by treatment facilities where pre-chlorination is used to improve the removal of algae.

This study has shown that pre-chlorination can be used effectively, prior to water treatment, to assist coagulation and flocculation unit processes, specifically when organic polymers are used as principle coagulant chemical. However, when implementing pre-chlorination, the integrity of cells should be monitored to avoid cell lysis which may lead to taste and odour problems as well as an increase in TOC and DOC and the related formation of THM in the drinking water. Studies have shown that different algal genera differ in their resistance to cell damage caused by pre-oxidation treatments (Steynberg et al., 1994; Liao et al., 2015; Coral et al., 2013; Lin et al., 2009; Ma et al., 2012).

Most of these studies investigated the effects of pre-chlorination or pre-ozonation on the cell integrity of cyanobacteria, diatoms and green algae. The presence of *C. hirundinella* recorded during warmer months of spring (September to November), summer (December – February) and autumn (March) in various South African impoundments such as the Benoni Lake, highlighted the fact that very little is known about the effect of pre-chlorination on the cells as well as on the flocculation of dinoflagellates. Dinoflagellates maintain their position in the water column by swimming, which gives cells the ability to interfere with conventional coagulation and flocculation by disrupting flocs (Pieterse et al., 2000).

Unlike previous studies (Lin et al., 2009; Ma et al., 2012; Zamyadi et al., 2012), where algal or cyanobacterial cells were collected from culture media, the algal cells used during this study were collected from natural source water and re-suspended in natural filtered source water to conduct chlorine exposure experiments. During this study the pre-chlorination dosages required to achieve 50% immobility of cells were much lower than previously recorded (0.121 mg/L, 0.186 mg/L, 0.121 mg/L and 0.106 mg/L respectively) (Steynberg et al., 1994; Zamyadi et al., 2012). According to Zamyadi et al. (2012), the industry dosages determined as Cl_2 to DOC ratios are low (< 1.5 Cl_2: DOC). During this study ratios of between 0.012 and 0.03 (Cl_2: DOC) were applied for the different sampling occasions. These results indicate that the relatively low chlorine dosage range (< 1 mg/L) required for immobilisation purposes

may reduce the treatment costs significantly. The effects of different pre-chlorination dosages on the integrity of cells were observed microscopically. Disruptions to cell integrity after chlorine exposure were observed with dosages 5 to 10 times lower (0.20 mg/L) than observed in other studies (1–5 mg/L) (Lin et al., 2009; Zamyadi et al., 2010; Zamyadi et al., 2012).

When considering pre-chlorination to immobilise algal cells such as *C. hirundinella*, the primary coagulant dosed during coagulation also plays a major role, since coagulant options containing Ca(OH)$_2$ can also render motile cells immobile. Ca(OH)$_2$ increases the pH levels of water, which have similar impacts as chlorine on flagellated organisms, such as *C. hirundinella* (Ferreira and Du Preez, 2012). Pre-chlorination implemented to assist Ca(OH)$_2$-SiO$_2$ and Ca(OH)$_2$-organic polymer was not effective in enhancing the decrease of %TPP removal. When dosing organic polymer only, no pH adjustments occurred and the treatment subsequently removed less *C. hirundinella* cells when compared to other coagulant options. This means that *C. hirundinella* cells can remain motile and disrupt the coagulation and flocculation processes. Table 5 confirmed the good removal efficiencies of *C. hirundinella* cells as a result of pre-chlorination when dosing organic polymer as the only coagulant. These results confirmed that continuous *C. hirundinella* cell removals can be achieved by conventional unit processes (coagulation, flocculation) when coupled to pre-chlorination. Steynberg et al. (1994) and Henderson et al. (2008) also stated that improvements in removal efficiencies of up to 95% can be expected when immobilisation assists coagulation and flocculation, depending on the chemical used.

Organic material excreted or released from algae, such as algal hydrophilic protein, is difficult to remove during coagulation and flocculation and may pose risks when penetrating the final disinfection stage of water treatment when chlorine is used as a disinfectant (Lui et al., 2011; Shen et al., 2011). It is well-known that organic material arising from algae serves as precursor material for the formation of harmful chlorine by-products, such as THM, and may cause unpleasant tastes and odours in drinking water (Goslan et al., 2009; Zamyadi et al., 2012). The most commonly found THM (bromodichloroform, bromoform, chloroform and dibromochloroform) were identified during this study; however, only bromodichloroform and chloroform were measured above the method limit of detection. Lui et al. (2011) stated that algal hydrophilic protein has a high chloroform formation potential when dosing chlorine which could explain the fact that chloroform was the most abundant THM found.

The guidelines set for South African drinking water are similar to guidelines set by the WHO, as indicated by SANS 241:2015 (SANS 241: 2015, 2015). THM concentrations that are formed during pre-chlorination of this study are not an issue for concern, since concentrations were measured at levels of < 100 µg/L. However, higher THM may form as a result of increased chlorine concentrations (e.g. chlorine overdose, or during higher dosages for disinfection after sand filtration). Therefore, water treatment plants that use pre-chlorination can reduce the THM entering the treatment plant by using an additional aeration step or allowing pre-chlorinated water to be exposed to ambient temperature in order to release THM into the atmosphere.

CONCLUSIONS

The integrity of *C. hirundinella* cells remains undisrupted when dosing the appropriate pre-chlorination dosage (e.g. IC$_{50}$) as required for rendering cells immobile, which will subsequently

result in effective flocculation when dosing organic polymer. These immobile cells are also much more sensitive to cell damage by oxidation when using pre-chlorination; therefore, light microscopy investigations should be used to monitor the integrity of cells. Pre-chlorination practices aimed at algal cell immobilisation can be implemented at a relatively low treatment cost when the DOC and TOC concentrations are relatively low. The low pre-chlorination dosages in combination with low carbon content (DOC and TOC) observed during this study are not a matter of concern for water quality, since the THM concentrations measured after pre-chlorination were lower than the SANS guideline values. However, careful consideration should be given by plant managers and process controllers when using elevated pre-chlorination dosages to treat source water characterised by high DOC and TOC concentrations.

When source water used for the production of drinking water is enriched with phytoplankton cells (algae and cyanobacteria), treatment options which include hydrated lime (Ca(OH)$_2$) may result in aggravation of water treatment problems such as tastes and odours. The elevated pH levels (≥ pH 10) are sufficient to inactivate organisms such as algae and cyanobacteria. These water treatment plants should therefore avoid using additional treatment steps such as pre-chlorination. On the other hand, water treatment plants that dose organic polymer as the only coagulant may experience treatment problems associated with motile algae (e.g. flagellated algal genera such as *Carteria*, *Chlamydomonas*, *Chlorogonium*, *Cryptomonas*, *Ceratium*, *Peridinium* and *Euglena*). To resolve floc disruption, especially by flagellated algal genera, it is recommended to implement pre-chlorination (to render cells immobile) prior to coagulation and flocculation unit processes to ensure effective removal of algae by gravity sedimentation or dissolved air floatation.

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