

Laboratory-scale evaluation of different aspects related to *Ceratium hirundinella* removal during simulation of a conventional water treatment plant which includes sedimentation

H Everts

20101066

Thesis submitted for the degree *Philosophiae Doctor* in Environmental Sciences at the Potchefstroom Campus of the North-West University

Promoter: Prof S Barnard

Co-promoter: Dr S Janse van Vuuren

October 2015



The laboratory-scale water treatment processes and techniques used during this research simulated Rand Water's conventional water treatment processes.

Many other processes exist that can remove algae effectively if the process designs for new plants are considered such as dissolved air flotation (DAF), Cocodaff and ultrafiltration (UF).

Laboratory investigations such as scanning electron microscopy (SEM) and Zeta Potential should only be considered by water treatment utilities where the capacity exists.

Table of Content

Table of Content

I	Summary.....	i
II	Opsomming	iii
III	Lists of Figures	v
IV	List of Tables	vii
V	List of Acronyms and Abbreviations	ix
VI	Acknowledgements	xi

Chapter 1

General Introduction and Aims of Study	1
1.1 General Introduction	1
1.2 Aims of Study	3

Chapter 2

Literature Review	5
2.1 Introduction	5
2.2 The Occurrence of the Dinoflagellate Group with Emphasis on the Freshwater Species <i>C. hirundinella</i>	6
2.3 South African Case Studies: Conventional Water Treatment Processes and Algal Removal.....	7
2.4 The Effects Phytoplankton on Conventional Water Treatment Processes and the Removal Challenges of Phytoplankton by Different Unit Processes.	8
2.5 The Morphological Structures of <i>Ceratium hirundinella</i> Cells and the Relationship between Structural Features and Removal Difficulties	13

Table of Content

2.6	Phytoplankton Removal Strategies and Mechanisms to Reduce the Impacts of <i>C. hirundinella</i> and Other Phytoplankton on Conventional Water Treatment as Employed by Rand Water to Produce Good Quality Drinking Water.....	14
2.6.1	Coagulant Selection and Zeta Potential	14
2.6.2	Pre-chlorination to Assist Coagulation	16
2.7	The Impacts Taste and Odour Organic Compounds (associated with <i>C. hirundinella</i> and Other Algae) on Water Quality.....	17
2.8	The Formation of Trihalomethanese (THMs) and the Impacts on Water Quality	20
2.9	Conclusions	21

Chapter 3

	Strategies of Coagulant Optimization to Improve the Removal of Turbidity and <i>Ceratium hirundinella</i> Cells during Conventional Drinking Water Treatment.....	22
3.1	Introduction	22
3.2	Material and Methods.....	24
3.2.1	Source water	24
3.2.2	Coagulant chemicals used to conduct jar stirring test experiments.....	25
3.2.3	Experimental design	25
3.2.4	Turbidity and TPP analyses.....	27
3.2.5	Phytoplankton (<i>Ceratium hirundinella</i> , other algae and cyanobacteria) analyses (Identification and enumeration)	27
3.2.6	Determination of appropriate coagulant dosages	27
3.2.7	Statistical analysis	28
3.3	Results	28

Table of Content

3.3.1 PCA analyses of turbidity, TPP and <i>C. hirundinella</i> and coagulants to determine appropriate indicators of coagulant dosages.....	28
3.3.2 The efficacy of turbidity, TPP and <i>Ceratium hirundinella</i> removal when the parameters turbidity and TPP were used respectively as indicators of appropriate coagulant dosages	29
3.3.3 Impacts of different coagulants on the physical integrity of <i>C. hirundinella</i> cells after sedimentation	33
3.4 Discussion.....	35
3.5 Conclusions	38

Chapter 4

An Investigation Into The Zeta Potential Changes of <i>C.hirundinella</i> Cells When Simulating Conventional Coagulation Using Inorganic nd Organic Coagulants.....	39
4.1 Introduction	39
4.2 Materials and Methods	40
4.2.1 Source water and sample collections.....	40
4.2.2 Coagulants	40
4.2.3 Jar stirring tests and sampling for analytical determinants	40
4.2.4. Zeta potential and pH.....	41
4.2.5 <i>Ceratium hirundinella</i> cell identification and enumeration	41
4.2.6 Statistical analyses.....	41
4.3 Results	42
4.3.1 Changes in zeta potential when dosing various coagulant treatments	42
4.3.2 Using a constant appropriate coagulant dose to treat source water enriched with different <i>Ceratium hirundinella</i> cell concentrations with different settling times.....	45
4.4 Discussion.....	51

Table of Content

4.5	Conclusions	55
-----	-------------------	----

Chapter 5

Physical and Chemical Impacts of Conventional Unit Processes and Coagulants on <i>Ceratium hirundinella</i>	56
--	-----------

5.1	Introduction	56
-----	--------------------	----

5.2	Materials and Methods.	58
-----	-----------------------------	----

5.2.1	Source water sampling and water treatment simulations.	58
-------	---	----

5.2.2	Sampling and preparation of biological material for scanning electron microscopy (SEM) investigations.	59
-------	---	----

5.3	Results	59
-----	---------------	----

5.3.1	The physical appearance of <i>Ceratium hirundinella</i> cells in source water and after flash mixing.....	59
-------	---	----

5.3.2	Hydrated lime (Ca(OH) ₂) in combination with activated silica (SiO ₂) treatment.....	63
-------	--	----

5.3.3	Ca(OH) ₂ in combination with organic polymer treatment	63
-------	---	----

5.3.4	Organic polymer treatment.....	64
-------	--------------------------------	----

5.4	Discussions	68
-----	-------------------	----

5.5	Conclusions	70
-----	-------------------	----

Chapter 6

Organic Compounds Associated with the Freshwater Dinoflagellate, <i>Ceratium hirundinella</i> and the Impact Thereof on Water Quality and Consumers	71
--	-----------

6.1	Introduction	71
-----	--------------------	----

6.2	Materials and Methods	72
-----	-----------------------------	----

6.2.1	Source water sample collection	72
-------	--------------------------------------	----

Table of Content

6.2.2 Chemicals and reagents	73
6.2.3 Sample preparation and derivatization procedure	73
6.2.4 Gas chromatography mass spectrometry (GC-MS) conditions.....	73
6.2.5 Analyte quantification equations.....	74
6.3 Results	74
6.3.1 Gas chromatography mass spectrometry (GC-MS) fragments	74
6.3.2 Organic acid abundance and identification	75
6.3.3 The major organic compounds associated with <i>Ceratium hirundinella</i>	75
6.4 Discussion.....	79
6.5 Conclusions	81

Table of Content

Chapter 7

Investigating <i>Ceratium hirundinella</i> Cell Immobilization Using Pre-Chlorination Prior to Conventional Coagulation and The Formation Of Total Trihalomethanes.....	82
7.1 Introduction	82
7.2 Material and Methods.....	83
7.2.1 Water samples: collection and preparation.....	83
7.2.2 Determination of pre-chlorination dosages using cell integrity and dose response fitting (half maximum inhibitory concentrations).....	84
7.2.3 Pre-chlorination and jar stirring test experiments	84
7.2.4 Coagulant dosages	85
7.2.5 Analytical determinants	85
7.2.6 Total organic carbon (TOC), dissolved organic carbon (DOC) and Trihamethanes (THMs)	85
7.2.7 Dose response graphs (Hill slopes)	85
7.2.8 Statistical analysis	86
7.3 Results	86
7.3.1 Effects of pre-chlorination on the integrity of <i>C. hirundinella</i> cells	86
7.3.2 Pre-chlorination dosages required to immobilize <i>C. hirundinella</i> cells, while keeping the integrity of cells	88
7.3.3 The effect of pre-chlorination on the percentage (%) TPP removal during coagulation and flocculation.....	89
7.3.4 The formation of total trihalomethanes (TTHMs) during pre-chlorination	93

Table of Content

7.4	Discussion.....	95
7.5	Conclusions	98

Chapter 8

General Conclusions	100
----------------------------------	------------

Chapter 9

Recommendations and Operational Guidelines.....	103
--	------------

9.1	Introduction	103
9.2	Recommendations and Operational Guidelines: Source water quality containing algae as <i>C. hirundinella</i>	104
9.3	Recommendations and Operational Guidelines: Pre-chlorination to immobilize <i>C. hirundinella</i> cells	105
9.4	Recommendations and Operational Guidelines: Coagulation, Flocculation and Sedimentation	106
9.5	Recommendations and Operational Guidelines: Rapid sand filtration (Chapter 3)	106
9.6	Recommendations and Operational Guidelines: <i>C. hirundinella</i> organic compounds associated with taste and odour (Chapter 6).....	107
9.7	Recommendations and Operational Guidelines: The use of laboratory analyses to assist plant operators and managers with optimisation strategies (Chapter 4, 5)	107

Chapter 10

References	109
-------------------------	------------

Appendices.....	119
Appendices.....	Refer to attached CD

Summary

The freshwater dinoflagellate species, *Ceratium hirundinella* (*C. hirundinella*) possesses unique characteristics, such as a thecal-plate cell covering of cellulose, spines and flagella. Unlike most other algae and cyanobacteria, *C. hirundinella* cells are relatively large in size (up to 450 µm in length and 50 µm in width). These unique characteristics (e.g. cell covering and flagella) and adaptations (e.g. spines) give the dinoflagellate cells the ability to reduce their sinking rate from the euphotic zone and to migrate easily through the water column. When source water contains high concentrations of *C. hirundinella* cells, water treatment problems and poor aesthetic water quality can be expected. These water treatment problems may include 1) the disruption of coagulation and flocculation, 2) clogging of sand filters and 3) taste and odour problems when cells penetrate into the final water. In Chapter 9 of this study, a list of operational guidelines (including alert levels) and recommendations to assist managers and operators of plants when *C. hirundinella* cells are causing water treatment problems.

During events of high *C. hirundinella* concentrations in source water, managers and operators of conventional water treatment plants need strategies to optimize coagulants and unit processes. Thus when source water contains motile nuisance algae, such as *C. hirundinella*, in moderate or abundant quantities, it is advisable to conduct jar stirring test experiments using both turbidity and total photosynthetic pigment (or chlorophyll-*a*) analyses as indicators of appropriate coagulant choice and dosages. The aims of this study are summarized as follows:

- To optimize coagulants and conventional water treatment processes by implementing relevant algal removal strategies and indicators during jar stirring test experiments,
- To investigate the changes in surface charge (known as zeta potential) on *C. hirundinella* cells before and after adding coagulants as part of the treatment processes,
- To investigate the physical and chemical impacts on the morphology of *C. hirundinella* cells after coagulation, flocculation and sedimentation,
- To identify organic compounds that may be responsible for taste and odour problems associated with *C. hirundinella*,
- To investigate the efficiency of pre-chlorination on the removal *C. hirundinella* cells when dosing various coagulants, and
- Give recommendations and operational guidelines relevant for a conventional water treatment plant to improve *C. hirundinella* removal

A combined water treatment system (Phipps and Bird Model), consisting of a six paddle jar test apparatus and six sand filter columns, was used to simulate conventional processes (coagulation, flocculation, sedimentation and rapid sand filtration). Source water samples containing relatively high *C. hirundinella* concentrations (> 500 cell/ml) were collected from Benoni Lake

(26°10'50.40''S; 28°17'50.11'' E) in plastic containers and stored as a homogenous sample in a 200 litre container under laboratory conditions (± 22 °C). Samples were collected from the source water as well as after sedimentation (from the supernatant or sludge) to determine turbidity, total photosynthetic pigment analyses (chlorophyll) and for phytoplankton analyses. Flocs (containing *C. hirundinella* cells) were collected from the sludge or sediment for scanning electron microscopy investigations and to perform zeta potential analyses. Concentrated *C. hirundinella* samples were frozen at -80 °C according to the proposed sampling protocol for organic compound analyses.

Results obtained from this study proved that using the relevant indicators to determine the appropriate coagulant dosages during jar stirring tests may generally improve the removal of problem-causing algae, such as *C. hirundinella* cells. Improved algal removal efficiencies will subsequently ensure final water with good aesthetic quality. The surface charge (zeta potential) on *C. hirundinella* cells can be used to evaluate the best coagulation conditions within an operating window of -10 mV to +3 mV when dosing various coagulants. Scanning electron microscopy investigations revealed major damaging effects to *C. hirundinella* cells when dosing high Ca(OH)_2 concentrations. However, when dosing lower Ca(OH)_2 concentrations, in combination with organic polymer, better *C. hirundinella* cell removal efficiencies with less damaging effects to cells was observed.

This study also indicated that the pre-chlorination, without causing cell lyses, can be applied to render the highly motile cells immobile which will subsequently assist the coagulation unit process. The aesthetic quality (e.g. tastes and odours) of drinking water may be influenced when *C. hirundinella* cells release organic material into the water as a result of cell lyses. Organic compounds, such as fatty acids and dicarboxylic acids can lead to taste and odour problems which associate with the presence of *C. hirundinella*. Organic compounds also serve as precursors for the formation of harmful chlorine by-products formed during chlorination.

Key words: *Ceratium hirundinella*, Conventional Water Treatment, Coagulants, Zeta Potential, Organic Acids and Pre-chlorination

Opsomming

Die varswater dinoflagellaatspesie, *Ceratium hirundinella* (*C. hirundinella*) beskik oor unieke karaktereienskappe soos 'n teka-plaat selbedekking van sellulose, horings en flagellums. In teenstelling met ander alge en sianobakterieë is *C. hirundinella* selle relatief groot (omtrek 450 µm in lengte en 50 µm breedte). Hierdie unieke karaktereienskappe (bv. selbedekking en flagellums) en aanpassings (bv. horings) gee aan die dinoflagellaatselle die vermoë om stadiger uit te sak vanuit die eufotiese sone en om maklik deur die waterkolom te migreer. Wanneer bronwater hoë konsentrasies van *C. hirundinella* selle bevat, kan watersuiweringsprobleme verwag word wat 'n invloed op die estetiese kwaliteit van drinkwater kwaliteit kan hê. Hierdie watersuiweringsprobleme sluit die volgende in: 1) onderbreking van koagulering en flokkulering, 2) verstopping van sandfilters en 3) smaak- en reukprobleme wanneer selle na die gesuiwerde water penetreer. Hoofstuk 9 van hierdie studie lys operasionele riglyne (insluitende waarskuwing vlakke) en aanbeveling om bestuurders en operateurs van suiwingaanlegte te help wanneer *C. hirundinella* selle watersuiweringsprobleme veroorsaak.

Gedurende hoë *C. hirundinella* selkonsentrasies benodig bestuurders en operateurs van konvensionele watersuiweringsaanlegte strategieë om koagulante en eenheidsprosesse te optimaliseer. Dus, wanneer bronwater hoogs beweeglike alge, soos *C. hirundinella*, in matige of groot hoeveelhede bevat, word aanbevelings gemaak om bekerroertoetseksperimente uit te voer waar beide troebelheid en totale fotosinterende pigment (of chlorofil-*a*) analyses gebruik word as indikatore om beide die geskikte koagulant- en doseringskonsentrasie te kies. Gebaseer op die probleme en uitdagings waarmee konvensionele prosesse te kampe het wanneer *C. hirundinella* selle in matige of groot hoeveelhede voorkom, is die doelwitte van die studie as volg:

- Om koagulante en konvensionele watersuiweringsprosesse te optimaliseer deur die implementering van relevante algverwyderingstrategieë en aanwysers van toepaslike koagulant dosisse gedurende bekerroertoetseksperimente.
- Om die verandering in oppervlaklading (bekend as zeta potensiaal) op *C. hirundinella* selle voor en na die byvoeging van koagulante te ondersoek as deel van die watersuiweringsproses.
- Om die fisiese en chemiese impakte op die morfologie van *C. hirundinella* selle na koagulering, flokkulering en sedimentering te ondersoek.
- Om die organiese verbindings wat met *C. hirundinella* geassosieer is en moontlik vir reuk- en smaakprobleme verantwoordelik mag wees, te identifiseer.
- Om die effektiwiteit van voor-chlorinering op die verwydering van *C. hirundinella* selle te ondersoek indien verskillende koagulante gedoseer word, en
- Om aanbevelings te maak en om relevante operasionele riglyne te gee te maak vir 'n konvensionele watersuiweringsaanleg om die verwydering van *C. hirundinella* te verbeter.

'n Gekombineerde watersuiweringsstelsel (Phipps and Bird Model), wat uit 'n ses spaan bekerroertoetsapparaat en ses sandfilteringskolomme bestaan, is gebruik om konvensionele prosesse (koagulering, flokkulering, sedimentering en vinnige sandfiltrering) te simuleer. Bronwater wat relatief hoë *C. hirundinella* konsentrasies bevat (> 500 selle/ml) is vanuit die Benoni meer ($26^{\circ}10'50.40''$ S; $28^{\circ}17'50.11''$ E) versamel in plastiese houers en gestoor as 'n homogene monster in 'n 200 liter houer onder laboratorium toestande ($\pm 22^{\circ}\text{C}$).

Alle watermonsters wat versamel is vanuit die bronwater asook na sedimentering (van die bo-vloeistof of slyk), is gebruik om die volgende analises uit te voer: troebelheid, totale fotosinterende pigment (chlorofil) en fitoplankton konsentrasies. Vlokkie (wat *C. hirundinella* selle bevat) is versamel vanuit die slyk of sediment vir skandeerelektronmikroskopiese ondersoeke en zeta potensiaal analises. Gekonsentreerde *C. hirundinella* monsters is gevries en by -80°C gestoor volgens die voorgestelde monster versamelingsprotokol vir organiese verbindingsanalises.

Resultate wat tydens hierdie studie verkry is, het bewys dat die gebruik van relevante aanwysers om die toepaslike koagulantdosisse te bepaal tydens bekerroertoetse oor die algemeen verbeterde verwydering van problematiese alge soos *C. hirundinella* selle tot gevolg sal hê. Verbeterde algverwyderingsdoeltreffendheid sal bydra tot die goeie estetiese kwaliteit van finaal gesuiwerde water. Die oppervlaklading (zeta potensiaal) op *C. hirundinella* selle kan gebruik word om die beste koagulerings toestande te bepaal wat plaasvind tussen -10 mV tot $+3$ mV indien verskillende koagulant gedoseer word. Skandeerelektronmikroskopiese ondersoeke het 'n groot mate van beskadiging aan *C. hirundinella* selle gewys, veral wanneer hoë dosisse $\text{Ca}(\text{OH})_2$ gedoseer is. Wanneer laer $\text{Ca}(\text{OH})_2$ konsentrasies saam met die organiese polimeer toegedien word, het beter *C. hirundinella* plaasgevind en was daar minder skade aan die selle.

Hierdie studie het ook aangedui dat voor-chlorinering sonder selbreking uitgevoer kan word waartydens die beweeglike algselle geïmmobiliseer word om koagulering te ondersteun. Die estetiese kwaliteit (bv. smaak en reuke) van drinkwater mag beïnvloed word wanneer *C. hirundinella* selle organiese materiaal in die water vrystel as gevolg van selbreking. Organiese verbindings, soos vetsure en dikarboksielsure, kan lei tot smaak- en reukprobleme, en die voorkoms word dikwels geassosieer met die teenwoordigheid van *C. hirundinella*. Organiese verbindings dien ook as voorlopers vir die vorming van skadelike chloor-byprodukte tydens chlorinering.

Sleutelwoorde: *Ceratium hirundinella*, Konvensionele watersuiweringsaanleg, Koagulant, Zeta Potensiaal, Organiese Sure en Voor-chlorinering

List of Figures

Figure 3.1	The experimental setup for removing <i>C. hirundinella</i> cells (a) by using a combined water purification apparatus, consisting of jar beakers (b) connected to sand filter columns (c) with a filtered water outlet (d)26
Figure 3.2	The PCA biplot ordination of appropriate coagulant dosages and response parameters, turbidity, TPP and <i>C. hirundinella</i> cells after sedimentation.28
Figure 3.3	Light microscopy images of <i>C. hirundinella</i> cells in (a) source water, (b) after treatment with $\text{Ca(OH)}_2\text{-SiO}_2$, (c) Ca(OH)_2 -organic polymer and (d) organic polymer.34
Figure 4.1	The comparison between pH levels and zeta potential values after treating the filtered same source water (control) containing <i>C. hirundinella</i> cells with the following coagulants: $\text{Ca(OH)}_2\text{-SiO}_2$, Ca(OH)_2 -organic polymer and organic polymer. Dotted lines indicate the zeta potential range required for good coagulation.44
Figure 4.2	The number of <i>C. hirundinella</i> cells removed after settling times of 20 minutes (a), 2 hours (b) and 4 hours (c) with increasing organic polymer dosages (1-6) per cell concentrations (from 5.5 ng/cell to 1.5 ng/cell).47
Figure 4.3	The changes in zeta potential after different settling times (20 minutes, 120 minutes and 240 minutes) when dosing the same coagulant dosages ($\text{Ca(OH)}_2 = 10 \text{ mg/l}$; Organic polymer = 11 mg/l) to treat water with increasing <i>C. hirundinella</i> cell concentrations48
Figure 4.4	<i>C. hirundinella</i> cells in the supernatant after different settling times (20 minutes, 120 minutes and 240 minutes) when dosing the same coagulant dosages to treat49
Figure 4.5	pH levels measured in the supernatant after different settling times (20 minutes, 120 minutes and 240 minutes) when dosing the same coagulant dosages ($\text{Ca(OH)}_2 = 10 \text{ mg/l}$; Organic polymer = 11 mg/l) to treat water with increasing <i>C. hirundinella</i> cell concentrations50
Figure 5.1	Line diagram representing the structure of the freshwater dinoflagellate, <i>C. hirundinella</i> (adapted from Prescott <i>et al.</i> , 1978)57

Figure 5.2	The six paddle Phipps and Bird jar stirring test apparatus (Model 7790-704) with six 2 ℓ beakers.	58
Figure 5.3	Dorsal view of <i>C. hirundinella</i> (a), the apical horn with flattened end (b) and antapical horn with pointed end (c).	61
Figure 5.4	Ventral view of <i>C. hirundinella</i> (a) illustrating perforations in thecal plates and organic material released in the sulcus area (b) and broken cells observed after flash mixing (c).	62
Figure 5.5	SEM images showing the morphology of <i>C. hirundinella</i> cells embedded in Ca(OH) ₂ -SiO ₂ flocs (a) and damage to spherical part of cells (b) and bending of horns (c).	65
Figure 5.6	SEM images showing the morphology of <i>C. hirundinella</i> cells embedded in Ca(OH) ₂ -organic polymer flocs where organic material is released through the sulcus grooves (a-c).	66
Figure 5.7	SEM images showing the morphology of <i>C. hirundinella</i> cells in organic polymer flocs, which are smaller (a) appear more smooth (b) with only a few intact cells (c) embedded.	67
Figure 6.1	GC-MS results indicating the analyte separation and abundance over a retention period of approximately 40 minutes for four replicates (a-d).	77
Figure 7.1	Light microscopy images of <i>C. hirundinella</i> cells in source water (a) and the effects of chlorine dosages after exposure to chlorine dosages below 0.20 mg/ℓ (b) and chlorine dosages above 0.20 mg/ℓ (c).	87
Figure 7.2	The dose response models (Hill slopes) for three chlorine exposure experiments (occasion c is not shown) and the relationships between the percentages (%) recorded for immobilized <i>C. hirundinella</i> cells and increasing chlorine dosages.	88

List of Tables

Table 1.1	A list of important parameters to be monitored as production specification for Rand Water compared to SANS guidelines for drinking water.....	3
Table 2.1	Phytoplankton genera belonging to various groups possess different cell sizes (in μm) as well as different additional characteristics such as appendages, organelles and special adaptations that to influence the performance of water treatment processes in different ways (Janse van Vuuren <i>et al.</i> , 2006).....	11
Table 2.2	Conventional water treatment processes and its objectives during water treatment together with the characteristics of phytoplankton that can affect each process (Van der Walt <i>et al.</i> , 2009).	12
Table 2.3	The nature of taste and odours produced by different phytoplankton genera (adapted for Degrémont, 2007).....	19
Table 3.1	The average values for parameters measured ($n=4$) in the source water, after sedimentation and after filtration when turbidity was used as indicator of appropriate coagulant dosages. The TPP values of $0\ \mu\text{g}/\ell$ indicates concentrations below $0.5\ \mu\text{g}/\ell$ or concentration values below the detection limit of method used. <i>C. hirundinella</i> cells are covered with theca-plates that may remain intact after organic material is released. Furthermore, 1-2 cells/ $\text{m}\ell$ may occur with $0\ \mu\text{g}/\ell$ or low pigment concentrations.	30
Table 3.2	The average values for parameters measured ($n=4$) in the source water, after sedimentation and after filtration when TPP was used as indicator of appropriate coagulant dosages. The TPP values of $0\ \mu\text{g}/\ell$ indicates concentrations below $0.5\ \mu\text{g}/\ell$ or concentration values below the detection limit of method used. <i>C. hirundinella</i> cells are covered with theca-plates that may remain intact after organic material is released. Furthermore, 1-2 cells/ $\text{m}\ell$ may occur with $0\ \mu\text{g}/\ell$ or low pigment concentrations.	32
Table 4.1	The zeta potential of <i>C. hirundinella</i> cells, pH and cell concentrations recorded during five sampling occasions in source water collected from the Benoni Lake (Sampling 11 January – 8 February 2012).	42

Table 4.2	The zeta potential and pH measurements ($n=5$) of the six pre-determined initial <i>C. hirundinella</i> concentrations that were used in simulated jar stirring test experiments with $\text{Ca}(\text{OH})_2$ -organic polymer. The amount of organic polymer (0.011 mg/ml) per cell ranging from 5.5 ng/cell to 1.5 ng/cell is also reported.	46
Table 6.1	Analytical conditions for the GC-MS analysis	74
Table 6.2	The m/z 60 and m/z 76 fragments with the TMA-N-oxide ratio for GC-MS results in Figure 6.1.	75
Table 6.3	The organic compound values (mean, min and max) recorded for four replicates of an unknown concentrated <i>C. hirundinella</i> sample. The percentages (%) for organic compound of the total organic compound composition were determined and are classified into dicarboxylic acids (DAs) and fatty acids (FAs).	78
Table 7.1	The initial <i>C. hirundinella</i> cell concentrations in source water used to determine the pre-chlorination dosages during four chlorine exposure experiments.	89
Table 7.2	The % TPP removal by various coagulants compared to % TPP removal achieved when the same coagulants were assisted by pre-chlorination. (Pre-chlorination dosage of 0.121 mg/l as previously determined during occasion a Table 7.1).	91
Table 7.3	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, c and d (Table 7.1).	92
Table 7.4	The minimum (min) and maximum (max) values for THMs (bromodichloroform and chloroform) after chlorine exposure experiments and organic carbon (TOC and DOC) concentrations measured in samples collected during four sampling occasions (a-d).	94

List of Acronyms and Abbreviations

°C	Degrees Celsius
µg/ℓ	Micro-gram per Litre
ANOVA	A Single Factor Analysis of Variance
APHA	American Public Health Association
AWWA	American Water Works Association
BIC	Brookhaven Instruments Corporation
BSTFA	BiS(trimethysulyl)-TriFluoroAcetamide
<i>C. hirundinella</i>	<i>Ceratium hirundinella</i>
Ca(OH) ₂	Hydrated Lime
cells/ml	Cells per Millilitre
Chl- <i>a</i>	Chlorophyll- <i>a</i>
CO ₂	Carbon Dioxide
COD	Chemical Oxygen Demand
CT	Concentration Contact Time
DAs	Dicarboxylic Acids
DBPs	Disinfection By-products
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DWA	Department Of Water Affairs (of South Africa), Now referred to as DWS*
DWAF	Department Of Water Affairs and Forestry (of South Africa)
DWS*	Department of Water and Sanitation (South Africa).
ECD	Electron capture detector
EOM	Extracellular Organic Matter
FAs	Fatty Acids
Fe ³⁺	Iron (III) ion
FTN	Flavour Threshold Number
GC-MS	Gas Chromatography Mass Spectrometry
HAA	haloacetic acids
IC ₅₀	Half Maximal Inhibitory Concentration
IEP	Isoelectric Point
IS	Internal Standard
IWA	International Water Association
kPa	Kilopascals
M/ℓ	Mega Litre
m/z	Mass/Charge
mg/ℓ	Milligram per Litre
MIB/2-MIB	2-Methylisoborneol

List of acronyms and abbreviations

mm	Millimetre
mV	Millivolts
NDMA	N-nitrosodimethylamine
ng/cell	Nanogram per Cell
ng/ℓ	Nanno-gram per Litre
nm	Nanometre
NOM	Natural Organic Matter
NTU	Nephelometric Turbidity Units
P	Phosphorus
PAC	Powered Activated Carbon
PCA	Principal Component Analysis
Pt	Platinum
R ²	Square Correlation Coefficient
Rf	Retention Factor
RPM/rpm	Revolutions per Minute
SANAS	South African National Accreditation System
SANS	South African National Standards
SEM	Scanning Electron Microscopy
SiO ₂	Silicon Dioxide (Activated Silica)
THMs	Trihalomethanes
THMs	Trihalomethanes
TMA/N	trimethylamine/nitrogen
TMCS	TriMethylChloroSilane
TOC	Total Organic Carbon
TON	Threshold Odour Number
TPP	Total Photosynthetic Pigments
T-tests	Statistical Significance (Student T-tests) to compare difference between averages
Turb	Turbidity
UE	European Union
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation

Acknowledgements

I would like to take the opportunity to sincerely thank the following people and institutions for their input, support and advice during this study:

- Rand Water for supplying the data for the project as well as allowing access to research tools and supplying a two year bursary for this study.
- The North West University for the platform to enrol for this study as well as excellent off-campus support regarding access to lecturers, scientific literature and financial support.
- Prof. Sandra Barnard from the North West University for guidance, support, excellent advice, scientific input and being a great promoter.
- Dr. Sanet Janse van Vuuren from the North West University for guidance, support, advice and scientific input and being a great co-promoter.
- Ms. Annelie Swanepoel from Rand Water for moral support, guidance, encouragement, scientific input and influencing the focus of the project and being a great mentor.
- Prof. Hein du Preez from Rand Water for moral support, guidance, encouragement, scientific input and influencing the focus of the project.
- Dr. Rahzia Hendricks from Rand Water for guidance, support, advice and scientific input.
- Prof. J. Mienie and Mr. Jano Jacobs from the North West University for scientific input and organic compound analyses.
- Dr. L. Tiedt from the North West University for scientific input and electron microscopy investigations.
- Ms. Azelda Schwartz Du Plessis from the North West University for moral support, guidance, encouragement, scientific input and influencing the focus of the project.
- Ms. Ashvena Ramcharan, Ms. Ashvita Ramcharan, Ms. Carna Joubert, Mrs. Elmarí Krüger, Ms. Ishana Dusrath, Ms. Lelethu Bungu, Mr. Michael Sekonyela, Mr. Petrus Mofokeng and Ms. Rita Guglielmi from the Hydrobiology laboratory at Rand Water, for accommodating me during the time I spent on this project.
- The Technical Team of Rand Water Academy, Mrs Poppie Sera, Ms Nokukhanya Sishi, Mrs Dawn Maluleka, Mrs Fezeka Zwane and Mr. Gerald Molley, for your moral support.
- My friends Geronomow Frans, Grant Jefthas, Preveen Daya and Bernadine Beukes, Roxanne Cornellissen, Rufane and Rochelle Carelse, Friedl and Natasha Taylor, Deodat and Herchell Willemse, Eljon and Elzhane Simeon, Jermaine and Monique Lee, Dwaine and Kaylin Smith, Luchaan and Monique Schreiner, Keenan Smith, Monique Bock, Avril de Wee, Morné Fillies, Michelle Walters, Ernestine February, Kirby Klaasen and Shanice Botha for moral support and being a positive influence during this study.
- The Du Plessis, Jacobs and Schwartz family – Aunty Azelda, Aunty Retta, Uncle Jakes, Leighton, Andiveve, Arejuan, Chante, Ouma Torrie, Oupa Abe and Aunty Connie Visser.
- My family from Great Brak River, Uncle Paul (R.I.P. Paula, till we meet again) and Aunty Jenny Jansen.
- The Pienaar family and Uncle Ronnie Coetzee, for moral support.

Chapter 1

General Introduction and Aims of the Study

Chapter 1

1.1 General Introduction

South Africa can be classified as a water scarce country with an uneven distribution of rainfall from east to west and alternating periods of drought. Furthermore is this valuable resource under constant pollution pressure from industry, mining, agriculture, energy use and accidental water pollution. The overall water quality of freshwater bodies is therefore declining. Given the current growth of the population, South Africa is facing a variable water supply challenges.

The listed anthropogenic impacts lead to eutrophication, acidification, sedimentation, salinization and microbial pollution and causing an ever increasing deterioration of the available water quality. Phytoplankton (algae and cyanobacteria) blooms in freshwater bodies caused by eutrophication remain an issue of critical concern for the water treatment fraternity. Another concerning issue, is the increasing salinity rates of fresh water bodies. Roos and Pieterse (1995) stated that salinity is increasing at an alarming rate in South African freshwater bodies. Increased salinity and eutrophication create environmental conditions that are favourable for the growth of specific salinity-tolerant freshwater algal species, such as the dinoflagellate species *Peridinium* sp. and *Ceratium hirundinella* (*C. hirundinella*).

C. hirundinella is currently the most commonly found freshwater dinoflagellate species in numerous South African water bodies. Algal blooms recorded in oligotrophic or hypertrophic impoundments are often dominated by cyanobacteria, but occasionally the alga *C. hirundinella* dominates the phytoplankton composition (Van Ginkel *et al.*, 2001). The ability of *C. hirundinella* cells to swim gives them an advantage over other immobile algae, such *Scenedesmus* and *Oocystis* to utilize nutrients and optimal sunlight for photosynthesis. This behavioural feature of *C. hirundinella* makes it a good competitor, especially during summer months in shallow water bodies as observed by Grigorzyk *et al.* (2003) in a shallow oxbow lake in Hungary (46°57'13.96'' N and 20° 49'28.73''E).

The presence of *C. hirundinella* in the Vaal River catchment was recorded for the first time in November 2000 (Swanepoel *et al.*, 2008a). South Africa's largest conventional drinking water treatment plant receives source water for drinking water treatment purposes from the Vaal Dam, situated within the Vaal River catchment. Rand Water's Zuikerbosch conventional water treatment plant employs the following processes after screening: coagulation, flocculation, sedimentation, stabilization (using carbon dioxide) and rapid sand filtration before chlorination. Unlike dissolved air flotation (DAF) that removes algae by flotation, in a conventional plant the removal is by sedimentation and therefore dependent on the formation of flocs. Flagellated algae, such as

Chapter 1

General Introduction and Aims of the Study

C. hirundinella interfere with floc formation when pre-treatment chemicals (e.g. pre-chlorination) and coagulants are dosed to immobilize cells prior to floc formation which will result in poor removal efficiencies during sedimentation (Van der Walt, 2012).

During the summer of 2006, the Zuikerbosch plant experienced a chlorophyll breakthrough into the final water, which proved to be the result of *C. hirundinella* cells penetrating into the final water. The penetration of *C. hirundinella* cells into the final water may have occurred due to the following characteristics of cells: 1) motility of cells by means of two flagella and 2) complex morphological structures, such as horns or spines. Henderson *et al.* (2008) also confirmed that the aforementioned characteristics contribute to the removal difficulties of algal species during conventional water treatment processes. Highly motile algal genera, such as *Ceratium*, *Chlamydomonas* and *Euglena* are therefore well-known for their ability to disrupt floc formation during coagulation and flocculation (Pieterse *et al.*, 2000).

Dissolved air flotation (DAF) is generally considered more efficient than sedimentation when source water used for drinking water production contains relatively high concentrations of algae (Teixeira and Rosa, 2006). The physical design of a conventional water treatment plant such as what is simulated in this study does not allow different configurations (e.g. from sedimentation to dissolved air flotation) to improve the removal efficiencies of problem-causing algae. Although the Zuikerbosch water treatment plant (Rand Water) is not employing DAF for algal removal, the following South African water treatment plants employ DAF to remove problem-causing algae: Roodeplaat and Vaalkop water treatment plants (Van der Walt *et al.*, 2009). The Rietvlei water treatment plant was the first water treatment facility in South Africa to change its process configuration from sedimentation to full scale DAF plant as a result eutrophication of source water used for the production of drinking water (Van der Walt *et al.*, 2009).

When problem-causing algae entering a water treatment plant such as Zuikerbosch, where the process configuration only allows sedimentation through gravity, additional treatment strategies or process optimization are crucial. Therefore, to improve algal removal, pre-treatment strategies, such as pre-chlorination can however be implemented to enhance the removal of motile algae during coagulation, flocculation and sedimentation (Steynberg *et al.*, 1994). Removal efficiencies may improve chemically by dosing the proper or appropriate coagulant, such as hydrated lime ($\text{Ca}(\text{OH})_2$) and organic polymer to immobilize algal cells and destabilize their surface charge to promote floc formation.

When source water supplied to a conventional drinking water treatment plant contains *Ceratium hirundinella* cells in either moderate or abundant quantities, it may interfere with the conventional water treatment processes. The relatively large dinoflagellate cells may cause major

Chapter 1

General Introduction and Aims of the Study

water treatment problems such as 1) disruption of coagulant and flocculation unit processes; 2) tastes and odours when cells accumulate in the sludge of sedimentation tanks; 3) filter clogging or reduced filter runtimes; 4) the formation of harmful disinfection by-products and 5) discoloration of water when present in abundant quantities. South Africa's largest drinking water supplier uses stringent specifications for drinking water to meet a minimum of 95 % compliance according to South African National Standards (SANS) for drinking water. The important parameters to be monitored (with Rand Water specifications and SANS guidelines for drinking water) that is associated with the occurrence of algae such as *C. hirundinella* cells listed in Table 1.1 below.

Table 1.1

A list of important parameters to be monitored as production specification for Rand Water compared to SANS guidelines for drinking water.

Parameter	Rand Water Internal Production Specifications	SANS Guideline for Drinking Water (2011) (Class 1 recommended operational limit)
Turbidity	< 0.5 NTU	<1 NTU
Colour	< 15 mg/ℓ Pt	<20 mg/ℓ Pt
Taste	<1 FTN	<5 FTN
Odour	<1 TON	<5 TON
DOC	<7 mg/ℓ	<10 mg/ℓ
TOC	<1 µg/ℓ	None
TPP/Chlorophyll-665	<1 µg/ℓ	None
Total Trihalomethanes	None	<200 µg/ℓ

None = Drinking water internal production specification or guideline is not included

1.2 Aims of Study

Based on the aforementioned challenges and water treatment related problems that may occur when *C. hirundinella* cells are present in source water used for drinking water production, the aims of this study were to:

1. Investigate the use of turbidity and total photosynthetic pigments (TPP) as indicators for the selection of appropriate coagulant dosages during screening jar stirring tests; compare the efficacy of appropriate coagulant dosages determined by these indicators in removing turbidity, TPP and *C. hirundinella* cells simultaneously and evaluate the removal abilities put on each unit process when dosing various coagulants (Chapter 3).
2. Investigate the change in zeta potential of *C. hirundinella* cells during coagulation using various coagulants to simulate conventional coagulation, flocculation and sedimentation (Chapter 4).

Chapter 1

General Introduction and Aims of the Study

3. Investigate the physical and chemical impacts on the morphology of *C. hirundinella* cells after coagulation, flocculation and sedimentation and to describe the physical appearance of flocs containing *C. hirundinella* cells; 2) evaluate the potential of cells to recover their natural surface charge after sedimentation (Chapter 5).
4. Identify the organic compounds excreted by *C. hirundinella* that may be responsible for taste and odour problems as well as to highlight the possible impacts on drinking quality and consumers (Chapter 6).
5. Investigate the effect of pre-chlorination on the integrity of *C. hirundinella* cells; evaluate the efficacy *C. hirundinella* cell removal when various coagulants are by assisted by pre-chlorination; and investigate the formation of THMs when using pre-chlorination to immobilize *C. hirundinella* cells (Chapter 7).
6. Give recommendations and highlight operational guidelines relevant for a conventional water treatment plant to improve *C. hirundinella* removal

For the purpose of this study, a combined water treatment apparatus, composed of a six paddle jar stirring apparatus and column filtration system was used (Phipps and Bird model). Coagulants, such as Ca(OH)_2 and organic polymer, dosed at South Africa's largest conventional drinking water treatment plant, were used for laboratory simulation experiments. The use of Ca(OH)_2 in combination with activated silica (SiO_2) for destabilization and flocculation of suspended material is unique for the treatment of Vaal Dam water in South Africa (Geldenhuys *et al.*, 2000). In this Chapter, Ca(OH)_2 is used as primary coagulant as well as a coagulant aid to treat source water with opposite algal content (measured as chlorophyll) and turbidity levels when compared to Vaal Dam source water that is characterised by low chlorophyll concentrations ($< 10 \mu\text{g/l}$) and relatively high turbidity levels ($> 70 \text{ NTU}$). Water treatment indicators such as TPP and turbidity will not only optimise various coagulants, but reduce the impacts on the algae which can be evaluated by morphological investigations. The stability of suspended particles such as algae (recorded as zeta potential and pH) can be monitored and corrected, especially when dosing Ca(OH)_2 . This study will also highlight the organic material associated with *C. hirundinella* that may cause poor tastes and odours in drinking water as well as to identify harmful chlorine by-products when pre-treatment such pre-chlorination is employed.

Chapter 2

Literature Review

Primary clarification may involve either gravity sedimentation or dissolved air flotation (DAF). Conventional drinking water treatment usually includes coagulation (C), flocculation (F) and sedimentation (S) which can be simulated by a jar stirrer apparatus (known as a jar tester). When source water used for drinking water production contains high concentrations of algae, DAF is generally considered more effective than S. When the dinoflagellate *Ceratium hirundinella* (*C. hirundinella*) occurs in freshwater (source water) used for drinking water production, many water-related problems can be expected due to the “robust” and motile nature of these phytoplankton cells. Little is known about the impacts of conventional water treatment processes (including coagulants) on *C. hirundinella* or the impacts thereof on the unit processes. However, research published on related impacts between other algae and cyanobacteria can be used as background literature to undertake investigations. Literature published on aspects related dinoflagellates, other algae and cyanobacteria are used in this Chapter to understand the removal difficulties of phytoplankton such *C. hirundinella* during conventional water treatment.

2.1 Introduction

The unique surface characteristics of *Ceratium hirundinella* (*C. hirundinella*) cells (theca-plate cell covering, spines) may have a negative impact on floc formation during coagulation and flocculation. Van Ginkel *et al.* (2007) and Swanepoel *et al.* (2008a) also referred to the robustness of *C. hirundinella* cells and their ability to avoid removal by conventional coagulation and flocculation. Swanepoel *et al.* (2008a) also found that more algae and cyanobacteria cells are penetrating into the final treatment water when relatively high number of *C. hirundinella* cells is present in source water used for drinking water production.

Eutrophication and cyanobacterial blooms are widespread in South African water bodies, which remain is of concern for water quality (Matthews, 2014). Eutrophication and phytoplankton blooms present an increasing threat to the health of freshwater ecosystems and humans who use these resources for drinking and recreation (Matthews *et al.*, 2010). In the light of these water-related problems that may occur when abundant quantities of phytoplankton occur in the source water, conventional drinking water treatment plants should have strategies in place to optimize coagulant treatments and unit processes (Knappe *et al.*, 2004). Therefore, optimized coagulant treatments and unit processes will ensure good phytoplankton removal and subsequently improve water quality.

Three main aims were selected for this Literature Review Chapter to review articles published regarding; **1)** the occurrence of the dinoflagellate group with emphasis on the freshwater species *C. hirundinella*, **2)** the effects of phytoplankton on conventional water treatment processes and the removal difficulties of phytoplankton by different unit processes, **3)** the morphological structures of *C. hirundinella* cells and the relationship between structural features and removal difficulties, **4)** phytoplankton removal strategies and mechanisms to reduce the impacts of *C. hirundinella* and other phytoplankton on conventional water treatment to subsequently ensure drinking water with good quality, **5)** the impacts taste and odour organic compounds (associated with *C. hirundinella* and other algae) on water quality and **6)** the formation of trihalomethanes (THMs) and the impacts on water quality.

2.2 The Occurrence of the Dinoflagellate Group with Emphasis on the Freshwater Species *C. hirundinella*

Dinoflagellates are unicellular eukaryotic organisms, living in both marine and freshwater ecosystems (Canter-Lund and Lund, 1995). In the marine ecosystems, dinoflagellates (e.g. *Gymnodinium*, *Gonyaulax*) are responsible for the so called “toxic red tides” that may result in the death of marine life as well as humans that consume contaminated marine animals (Canter-Lund and Lund, 1995). The frequency, magnitude and geographical extent of red-tide outbreaks produced by dinoflagellates (both toxic and non-toxic) have been reported globally, especially in marine environments (Anderson, 1997). In recent decades various dinoflagellate blooms were documented globally in both freshwater and marine ecosystems (Smalley and Coats 2002; Silke *et al.*, 2005; Shear and Ross, 2009; Matsuyama, 2012; Feki *et al.*, 2013). The genera *Ceratium* and *Peridinium* are the most commonly found freshwater dinoflagellates, while the greatest diversity occurs in marine ecosystems (Canter-Lund and Lund, 1995). Though no “toxic red tide blooms” in freshwater ecosystems have been reported, when these freshwater genera (*Ceratium* and *Peridinium*) form blooms, it often produces a brownish discoloration of surface water as a result of cellulose theca-plate cell. covering.

In South Africa, the occurrences of dinoflagellates were also recorded in both freshwater (e.g. Hartbeespoort Dam, Klipvoor Dam, Boskop Dam) and marine ecosystems (e.g. Saldanha Bay) *hirundinella* were reported in various South African freshwater impoundments such as the Hartbeespoort Dam (Van Ginkel *et al.*, 2001; Van Ginkel and Silberbauer, 2007), Vaal River (Swanepoel *et al.*, 2008a) and Albert Falls Dam (Hart and Wragg, 2009) over the last decades.

The Vaal Dam situated in the Vaal River catchment is an important impoundment, since it acts as a source for drinking water treatment by South Africa’s largest conventional drinking water treatment works. The phytoplankton assemblages of the Vaal River have been documented by various

Chapter 2

Literature Review

scientists before the year 2000 with no records confirming the occurrence of *C. hirundinella* (Visser and Pieterse, 1999; Janse van Vuuren and Pieterse 2000). Swanepoel *et al.* (2008a) recorded the occurrence of *C. hirundinella* in the Vaal River catchment for the first time during November 2000. After recording the freshwater dinoflagellate, *C. hirundinella*, it was observed by Swanepoel *et al.* (2008a), Van der Walt (2012) and Ewerts *et al.* (2014) that the frequency of occurrence as well as the concentrations thereof in the Vaal River catchment have increased, especially during warmer spring and summer periods.

Extensive studies into the occurrence of *C. hirundinella* in blooms as well as the ecological drivers behind the occurrence of the dinoflagellate species have been studied worldwide in freshwater sources (Pfiester, 1971; Nakano *et al.*, 1999; Gligora *et al.*, 2003; Grigorszky *et al.*, 2003; Van Ginkel *et al.*, 2001; Hedger *et al.*, 2004; Van Ginkel and Silberbauer, 2007; Hart and Wragg, 2009). The occurrence of the freshwater dinoflagellate *C. hirundinella* has been found to be periodically dominant in South African impoundments (Van Ginkel *et al.*, 2001). Van Ginkel *et al.* (2001) recorded high *C. hirundinella* cell concentrations under bloom forming conditions in hyper-eutrophic South African impoundment, which correlated well with total nitrogen, dissolved inorganic nitrogen and ortho-phosphate. It can therefore be speculated that the availability of nutrients together with other environmental factors such as temperature and light penetrations are the drivers behind the occurrence of *C. hirundinella* in freshwater impoundments during specific seasonal periods. Van Ginkel *et al.* (2001) also confirmed that the occurrence of phytoplankton blooms is an indication that enough nutrients (e.g. nitrates and phosphates) are available as well as favourable conditions such as light penetration and temperature.

2.3 South African Case Studies: Conventional Water Treatment Processes and Algal Removal

Visser and Pieterse (1999) investigated the occurrence of phytoplankton in the Vaal River at Balkfontein drinking water treatment plant (South Africa) as well as the penetration of genera into the different unit processes. Findings made by Visser and Pieterse (1999) indicated that penetration of phytoplankton were related to specific treatability of genera. Genera such as *Aulacoseira* and *Cyclotella* (centric diatom) are covered with silica-frustules and can be effectively removed by Ca(OH)_2 treatment options (Visser and Pieterse, 1999). Green algae such as *Chlamydomonas* on the other hand can be effectively removed by FeCl_3 treatment options (Visser and Pieterse, 1999). Flagellated algae such as *Chlamydomonas*, *Cryptomonas*, *Ceratium hirundinella* and *Peridinium* can be rendered immobile by pH shock created when dosing coagulants such as Ca(OH)_2 to assist coagulation (Van der Walt, 2012). In the light of various surface characteristics and motile adaptations of the different genera, specific coagulant requirements and coagulation conditions are needed to ensure their removal.

During the summer of 2006-2007 South Africa's largest conventional water treatment utility experienced a chlorophyll breakthrough into the final water. Analytical laboratories of the drinking water utility analysed samples (invertebrate identification and enumeration) collected from the final water and discovered an unusual brown residue at the bottom of samples (Swanepoel *et al.*, 2008a). A phytoplankton analyses confirmed that the brown residue was pure *C. hirundinella* cells. Swanepoel *et al.* (2008a) found that the break-through of *C. hirundinella* suggested that more phytoplankton penetrate into potable water than what was previously suspected in the conventional water treatment plant investigated. It has become evident from these findings that unit processes or coagulant used during the event of this *C. hirundinella* breakthrough were not optimized remove these highly motile phytoplankton cells. In conventional water treatment, coagulants are critical for effective removal of microorganisms (especially microbial pathogens) and can be achieved by coagulation, flocculation and sedimentation (LeChevallier and Au, 2004).

Ewerts *et al.* (2013) investigated the efficacy of South Africa's largest conventional water treatment plant that abstract source water from the Vaal Dam to produce approximately 3400 M/ℓ drinking water per day. Findings made from this research stated that, although the conventional water treatment plant is able to remove phytoplankton effectively from the source water, genera such as *Anabaena* and *C. hirundinella* pose risks to unit processes and water quality. Small sized *Anabaena* cells can float and can easily penetrate into the final water, while large *C. hirundinella* cells can disrupt floc formation. In the light of these threats, both *Anabaena* and *C. hirundinella* were identified as problem-causing phytoplankton.

2.4 The Effects Phytoplankton on Conventional Water Treatment Processes and the Removal Challenges of Phytoplankton by Different Unit Processes.

Table 2.2 gives the sequence and objectives of conventional water treatment processes of a South African water treatment plant as suggested by Van der Walt *et al.* (2009). Henderson *et al.* (2007) describes the characteristics of phytoplankton and how it can affect specific conventional water treatment processes. Poor removal of phytoplankton poses high risks and challenges for drinking water utilities such as the production of tastes and odours in drinking water as well as toxic substances (Du Preez and Van Baalen, 2006). Each unit process is designed to remove phytoplankton cells and other suspended particles, but may be influenced by the species present, since different species have different morphologies and motility

Phytoplankton cells are suspended in surface water (source water) as part of suspended particles (Linde *et al.*, 2001; Bolto and Gregory, 2007). In a water treatment plant all algal genera have the potential to cause problems such as the disruption of flocs, filter clogging or may produce tastes and odours. The Cyanophyceae genera can produce cyanotoxins or release organic material when the

Chapter 2

Literature Review

integrity of cells is disrupted (Knappe *et al.*, 2004). Furthermore, the Cyanophyceae genera (surrounded with a mucous layer) possess gas vacuoles which add to removal difficulties during conventional water treatment (Janse van Vuuren *et al.*, 2006).

The Bacillariophyceae group known as diatoms (e.g. centric or pennate diatoms) has characteristic frustules as cell covering (Janse van Vuuren *et al.*, 2006). This group also possesses spines, projections on their cell walls which may add a challenges and removal difficulties during water treatment (Janse van Vuuren *et al.*, 2006 and Henderson *et al.*, 2007). Diatoms are able to cause taste and odour problems, clogging filters but produce no toxic substances (Knappe *et al.*, 2004).

The groups Chrysophyceae and Cryptophyceae are known as golden-brown algae and cryptomonads respectively (Janse van Vuuren *et al.*, 2006 and Knappe *et al.*, 2004). The golden-brown algae are covered in fine silica scales, while the cryptomonads possess firm pellicle plates that may add removal difficulties during water treatment (coagulation/flocculation) processes. Genera within these two groups may cause filter clogging and produce tastes and odours during the production of drinking water (Knappe *et al.*, 2004). No production of toxic substances has been recorded for these groups (Knappe *et al.*, 2004).

The algal group, Chlorophyceae consist of the largest variety of species that possess different adaptations (e.g. mucilaginous sheets and extensions) which may add different removal difficulties and challenges during agglomeration. Green algae can produce tastes and odours and clog filters, but no toxic substances are produced (Knappe *et al.*, 2004). Another challenge for agglomeration is the ability of the Euglenophyceae cells that are motile by means of metabolic movements (Janse van Vuuren *et al.*, 2006).

Members belonging to the dinoflagellate group are cover with theca plates and use flagellate to migrate through the water column, which give them the ability to disrupt unit processes and avoid removal during coagulation and flocculation unit processes (Pieterse *et al.*, 2000; Janse van Vuuren *et al.*, 2006). When source water used for drinking water production contains relatively high numbers dinoflagellates, it is possible that these algal cells will occur in the abstracted water due their ability to migrate to different depths or zones within lakes or dams.

Source water used for drinking water production can be abstracted at certain depths to avoid high concentrations of phytoplankton cells (especially cyanobacteria) entering the treatment plants (Du Preez and Van Baalen, 2006). When high concentrations of phytoplankton are present in the source water used for drinking water production, the removal efficiency is poor resulting in phytoplankton concentrations (Pieterse *et al.*, 2000). The agglomeration phase where the appropriate coagulant dosages are added to the water is considered as the main process for phytoplankton

Chapter 2

Literature Review

removal (Henderson *et al.*, 2007); therefore these processes (coagulation/flocculation) involved should function optimally. However, phytoplankton removal during the production of drinking water remains a challenge to be removed by conventional water treatment due to various removal difficulties (Henderson *et al.*, 2007). It is difficult to remove high concentrations of phytoplankton using the conventional water treatment process. Some of the characteristics that could cause removal challenges are presented in Table 2.1. The specific cell, colony or filament size and characteristics are also listed. Characteristics such as morphology, motility, extracellular organic matter (EOM) and surface charge are important aspects, since they contribute to removal difficulties (Henderson *et al.*, 2007). Therefore, knowledge of phytoplankton removal difficulties can add value to water treatment strategies (e.g. coagulants) when specific genera become problematic to a water treatment plant.

Chapter 2

Literature Review

Table 2.1

Phytoplankton genera belonging to various groups possess different cell sizes (in μm) as well as different additional characteristics such as appendages, organelles and special adaptations that to influence the performance of water treatment processes in different ways (Janse van Vuuren *et al.*, 2006).

Phytoplankton		Specific Size	Additional appendages, organelles and special adaptations
Cyanophyceae	⁽¹⁾ <i>Anabaena</i> ⁽²⁾ <i>Microcystis</i>	7-12 μm . 0.5-9 μm .	Gas vacuoles, surrounding mucous layer provide buoyancy.
Chrysophyceae	<i>Synura</i>	30-45 μm long and 7-17 μm broad. Colonies may be 30-300 μm across.	Covered with fine silica scales. Most of the anterior scales have spines.
Bacillariophyceae	⁽¹⁾ <i>Aulacoseira</i> ⁽²⁾ <i>Cyclotella</i> ⁽³⁾ <i>Navicula</i> ⁽⁴⁾ <i>Nitzschia</i>	⁽¹⁾ 4-20 μm . ⁽²⁾ 5-30 μm . ⁽³⁾ 6-42 μm long/4-12 μm wide. ⁽⁴⁾ 5-100 (exceptionally 600) μm long and 2.5-12 μm .	⁽¹⁾ Spines are usually present on the end walls. ⁽²⁾ May have wart-like projections. ⁽³⁾ Raphe in both valves. ⁽⁴⁾ Raphe is supported by bars (fibulae) diagonally opposite.
Cryptophyceae	<i>Cryptomonas</i>	15-8 μm long.	1-3 Contractile vacuoles. Underneath cell membrane are firm pellicle plates.
Dinophyceae	<i>C. hirundinella</i>	Large cells up 450 μm long and 30-100 μm wide.	One anterior horn and 2-3 posterior horns.
Euglenophyceae	⁽¹⁾ <i>Euglena</i> ⁽²⁾ <i>Trachelomonas</i>	⁽¹⁾ 20-540 μm long and 5-50 μm wide. ⁽²⁾ Loricas are usually 14-60 μm and 9-35 μm wide.	⁽¹⁾ Capable of contraction (metabolic movement). ⁽²⁾ Loricas with a distinct collar arising near the apical pore. Red anterior eye spots (some species).
Chlorophyceae	⁽¹⁾ <i>Chlamydomonas</i> ⁽²⁾ <i>Scenedesmus</i> ⁽³⁾ <i>Coelastrum</i>	⁽¹⁾ 2-50 μm long (mostly 5-20 μm wide) and 8-22 μm wide. ⁽²⁾ 5-30 μm long and 2-10 μm wide. Spines may be up to 200 μm in length. ⁽³⁾ 2-30 μm in diameter and colonies 100 μm across.	⁽¹⁾ Stigma (red eye spot). ⁽²⁾ Cells may be arranged zigzag in a single or double row. Ornamentation pattern on cell walls (spines, ribs, granulation, etc.). ⁽³⁾ Protuberances arise from mucilaginous sheath surrounding each cell from extensions of the cell wall.

Chapter 2

Literature Review

Table 2.2

Conventional water treatment processes and its objectives during water treatment together with the characteristics of phytoplankton that can affect each process (Van der Walt *et al.*, 2009).

Process	Objectives of processes	Characteristics of phytoplankton that can effect processes
Coagulation	Flocculation of particles.	Morphology, motility, EOM and surface charge.
Flocculation	Flocculation of suspended particles.	Morphology, motility, EOM and surface charge.
Sedimentation	Removal of heavier flocculated particles.	Morphology, motility and density.
Rapid sand filtration	Final barrier for micro-organisms and removal of remaining fine particles.	Morphology and motility.
Carbonation/Stabilization	Stabilize water with CO ₂ gas to adjust pH.	No conclusive relationship with phytoplankton characteristics.
Chlorination/Disinfection	Removal of harmful micro-organisms, disinfection.	No conclusive relationship with phytoplankton characteristics.
Chloramination	Providing residual disinfectant in long distance systems.	No conclusive relationship with phytoplankton characteristics.

2.5 The Morphological Structures of *C. hirundinella* Cells and the Relationship between Structural Features and Removal Difficulties

The surface morphology of phytoplankton genera are described by many authors as complex structures, with emphasis on the surface characteristics of cells which may also impact on water treatment processes (Canter-Lund and Lund, 1995; John *et al.*, 2002; Janse van Vuuren *et al.*, 2006 and Henderson *et al.*, 2008). Amongst other phytoplankton groups, the dinoflagellates are covered with unique cell covering known as theca plates and are mostly divided into upper and lower halves; where the upper half of the cells is known as the epitheca and the lower part of the cells is known as the hypotheca (Harland, 1988). The cell wall consists partly of cellulosic plates of thecae (Harland, 1988). A few dinoflagellate species are naked or without a cell wall (e.g. *Gymnodinium*); however, a conspicuous separation of the cell wall (epi- and hypotheca) is described in *Ceratium* species (Wetzel, 1983).

The main characteristics of cells such as the cell size with horns found on the epi- and hypotheca as well as the ability of the robust cells to migrate through the water column with two flagella are well described in literature (Canter-Lund and Lund, 1995; Van Ginkel *et al.*, 2001; John *et al.*, 2002; Gligora *et al.*, 2003 and Grigorszky *et al.*, 2003). Morphological variability that occurs in dinoflagellates is described by Taylor (1987) and has been correlated to buoyancy and cell resistance to sinking (Wetzel, 1983 and Hamlaoui *et al.*, 1998). The morphology forms of dinoflagellates are diverse; they differ from cell size, shape, ornamentation and capacity to form chains (Smayda, 2010).

The adaptive significance (e.g. cyclomorphism and polymorphism) of the morphology diversity in the dinoflagellate genera are known to promote growth and to keep the position of cells in the water column for optimum photosynthesis (Smayda, 2010). It has been reported that *C. hirundinella* also undergo seasonal polymorphism or cyclomorphism, which are usually a result of the cells to reduce sinking rate (Hamlaoui *et al.*, 1997; Gligoral *et al.*, 2003 and Wetzel, 1983). Appendages such as horns can also reduce the necessary contact between phytoplankton cells for successful agglomeration during floc formation, since aggregation is only effective when sufficient floc is present (Knappe *et al.*, 2004). Apart from the horns that reduce the sinking rate of cells, dinoflagellates possess two flagella (directed perpendicular to one another); namely, a transversal flagellum and longitudinal flagellum (Fenchel, 2001) by means of which they can swim to avoid sedimentation. The transversal flagellum is situated within an equatorial (slightly descending) groove, while the longitudinal flagellum is situated freely in the sulcus groove (Fenchel, 2001). Poor removal of *C. hirundinella* may occur as a result of

the complex morphology and motility when coagulants and unit processes are not optimized to remove these robust flagellated cells (see Chapters 3 and 4 for a more detailed discussion).

2.6 Phytoplankton Removal Strategies and Mechanisms to Reduce the Impacts of *C. hirundinella* and Other Phytoplankton on Conventional Water Treatment as Employed by Rand Water to Produce Good Quality Drinking Water

2.6.1 Coagulant Selection and Zeta Potential

A wide variety of particulate impurities which include inorganic substances such as clays and metal oxides, various organic colloids and microbes (viruses, bacteria, protozoa and phytoplankton) occur in natural waters (Duan and Gregory, 2003). These suspended particles in solutions such as surface water often have a charge on the surface (mostly negatively charged) and the magnitude thereof can be determined electrophoretically (Linde *et al.*, 2001; BIC, 2002). The conversion of particles in a solution from a stable state to an unstable state (destabilization) can be accomplished in the conventional water treatment processes coagulation and flocculation (Linde *et al.*, 2001 and BIC, 2002).

When coagulants are added to source water during treatment, positively charged coagulants react with suspended particles (colloidal and dissolved NOM) to form flocs that are removed during separation processes such as sedimentation (Edzwald *et al.*, 2000). The removal of these substances from the source water requires a specific coagulant demand, which affect the required coagulant dose (Edzwald *et al.*, 2000). Interactions of particles are due to the charge on their surface and will only be effective below a certain size range and any size range above the range will be subjected to gravity forces (Linde *et al.*, 2001). For smaller particles, separation efficiency can be greatly enhanced by aggregation; because of their surface charge these aquatic particles are often colloiddally stable and resistant to aggregation (Duan and Gregory, 2003).

After coagulation, the flocculation process occurs when neutralized particles agglomerate into microflocs. Microflocs then comes together to form flocs that can settle (Degrémont, 2007). The different mechanisms involved with coagulation and flocculation can be defined in four zones for coagulant dosage, and give the following consequences for negatively charged particles (Duan and Gregory, 2003); 1) very low coagulant dosage - particles still negatively charged and hence stable; 2) dosage sufficient - give charge neutralization and hence coagulation; 3) higher dosage - giving charge neutralization and re-stabilization; and 4) still higher dosage - giving hydroxide precipitation and sweep flocculation.

Chapter 2

Literature Review

Coagulants are needed to destabilize the particles (Duan and Gregory, 2003). The basic mechanism of coagulation (colloid stability) can be described as the concept of an electrical double layer which surrounds a colloid and factors of ionization, ion adsorption and zeta potential are emphasized by a physical theory that deals with particulate material which require removal (Linde *et al.*, 2001).

The key parameter of double layer repulsion for individual particles is the zeta potential and can usually be used to interpret the trend of coagulation efficiency (Yang *et al.*, 2005; Taki *et al.*, 2008). Colloidal particles should have zero net surface charge (isoelectric point, IEP) for agglomeration (Yang *et al.*, 2005). Although the iso-electric point is at 0 mV, there is no single value of zeta potential that will guarantee good coagulation for every water treatment plant (Jefferson *et al.*, 2004). However, when the zeta potential of particles is approaching zero, coagulation efficiency is generally improved (Jun *et al.*, 2001). Coagulants destabilize colloidal particles and provide a medium with multivalent cations that are either free or bonded to an organic molecule (cationic poly-electrolyte). Cations are absorbed to and attached to the first Stern layer; the zeta potential then rises until it reaches zero or as neutralization of all the particle's electronegative charge is achieved (Degrémont, 2007).

The zeta potential when using different optimum coagulant dosages may be within certain range for optimum coagulation conditions (Jefferson *et al.*, 2004 and Eikenbrokk *et al.*, 2006). The zeta potential at optimum coagulation conditions can also deviate from zero (0 mV), indicating that other mechanisms than charge neutralization is involved or relevant (Eikebrokk *et al.*, 2006). Eikebrokk *et al.* (2006) determined that it is difficult to use zeta potential as the only tool to indicate optimum dosages of coagulants. Increased coagulant dosages will subsequently increase the sludge (sediment) volumes; however, beyond the optimum coagulant dosage, there is little improvement in particle removal (Duan and Gregory, 2003).

Aggregation can be achieved since phytoplankton cells are negatively charged and must be destabilized by charge neutralization to allow effective treatment and removal of cells (Yan and Jameson, 2004). Therefore, optimization of processes such as coagulation and flocculation play a major role during water treatment with respect to phytoplankton removal (Pieterse *et al.*, 2000; Knappe *et al.*, 2004; Teixeira and Rosa, 2006). The water treatment plant that on which laboratory simulations are based uses the following processes: 1) coagulation, flocculation, sedimentation and sand filtration for the removal of suspended and colloidal matter; 2) carbon dioxide gas for chemical stabilisation of water to prevent corrosion of pipelines and other structures as well as to protect distribution systems from scale formation; 3) disinfection to produce water that is safe to drink using chlorination and chloramination (Schutte, 2006) .

2.6.2 Pre-chlorination to Assist Coagulation

It has been reported that the coagulation and flocculation processes can be improved when using pre-treatment options such as pre-chlorination to enhance the removal of phytoplankton cells (Geldenhuis *et al.*, 2000; Shehata *et al.*, 2002; Hoko and Makado, 2011 and Van der Walt, 2012).

Phytoplankton cells that penetrate into the final water will increase the organic content in the water that may increase the chlorine demand when chlorine is used as a disinfectant.

Pre-oxidants such as ozone, chlorine, potassium permanganate and potassium ferrate has shown in many instances to improve phytoplankton removal as a result of movement or metabolism inactivation (Steynberg *et al.*, 1994; Henderson *et al.*, 2008). Ma *et al.* (2012) found that the pre-chlorination substantially affected the optimum coagulant dosages for effective removal of *Microcystis* cells and that inactivated cells have a positive influence on removal efficiencies. Henderson *et al.* (2008) also stated that pre-oxidation prior to coagulation and flocculation is able to achieve relatively good phytoplankton removal. Amongst the aforementioned pre-oxidants, chlorine is widely used by water treatment plants due to relatively lower costs (Van der Walt *et al.*, 2009). Therefore, pre-chlorination is often considered as a primary disinfectant in water treatment plants where chemical oxidation is desired for control of colour, taste and odour, and phytoplankton growth (Van der Walt *et al.*, 2009).

During phytoplankton outbreak periods or blooms, conventional technology to enhance removal of phytoplankton by increasing coagulant dosage and chlorine can be applied (Shen *et al.*, 2011). Pre-or advanced water treatment options may increase the cost of water treatment significantly (Pieterse *et al.*, 2000 and Van der Walt *et al.*, 2009). To minimize treatment costs, pre-chlorination of recycled water (sludge and filtered backwash) to remove organic compounds (taste and odour) and phytoplankton can be employed before water is channelled back to the abstracted source water destined for water treatment. Water utilities often add oxidants early in the treatment process to (WHO and IWA, 2004) in order to; 1) maximize the contact time with the oxidant; 2) oxidize compounds for subsequent removal by the treatment process (e.g. iron or manganese); 3) provide initial treatment in sufficient time for water to be further treated if necessary (e.g. oxidation of taste and odour compounds); and 4) improve particle removal in subsequent clarification and filtration processes.

The key challenge with pre-chlorination is that high taste and odour compounds from sludge dams often coincide with high chlorine demand (Van der Walt *et al.*, 2009). In these cases where taste and odour problems occur at the same time as high iron and manganese concentrations, either pre-chlorination or powered activated carbon (PAC) can be used (Van der Walt *et al.*, 2009). In many instances

phytoplankton species are successfully removed as a result of “inactivation”; however, the use pre-chlorination may also lead to the formation of disinfection by-products, such as trihalomethanes (THMs) (Prévost *et al.*, 2005; Henderson *et al.*, 2008 and Van der Walt *et al.*, 2009). THMs are carcinogenic which pose health risks to drinking water consumers (Van der Walt *et al.*, 2009); therefore, sufficient knowledge on the release of organic compounds associated with *C. hirundinella* is required to reduce the formation of harmful products when dosing chlorine. Refer to Chapter 7 for a more detailed discussion.

2.7 The Impacts Taste and Odour Organic Compounds (associated with *C. hirundinella* and Other Algae) on Water Quality

Organic compounds in water may act as substrates for blood worms, nematodes, copepods, freshwater sponges, bryozoan and other undesirable organisms (Palmer, 1980). Phytoplankton can also impart their characteristic odours to the stored water and these can be carried into the remainder of the distribution system. It is therefore of critical importance to reduce the organic load sufficiently during drinking water treatment, in order to eliminate these possibilities. Some phytoplankton genera are known to produce specific distinctive taste and odours in drinking water. Taste and odours originate from specific organic compounds when phytoplankton cell lyses occur during water treatment processes. Common compounds causing taste and odour problems in drinking water are as follows (Knappe *et al.*, 2004):

- Geosmin (produced by *Anabaena*, *Aphanizomenon*, *Fischerella*, *Lyngbya*, *Oscillatoria*, *Phormidium*, *Schizothrix* and *Symplocaspp.*) causing earthy-corn-musty taste and odour.
- 2-methylisoborneol (MIB) causing earthy-musty taste and odour (produced by *Oscillatoria*, *Phormidium*, *Pseudanabaena* and *Synechococcus spp.*).
- 2t,4c,7c-decatrienal causing fishy taste and odour (produced by *Synura* and *Dinobryon spp.*).
- 2t,6c-nonadienal causing cucumber taste and odour (produced by *Synura*)
- Linoleic acid causing sweet-melon-water melon taste and odour (*Microcystis*, *Oscillatoria* and *Chlamydomonas spp.*).
- B-cyclocitral causing sweet-fruity-chocolate-pipe tobacco taste and odour (*Microcystis* and *Oscillatoria spp.*).
- Isovaleric acid causing rancid-cheesy-dirty socks-sour odour (*Chlamydomonas*)
- *trans*, *trans*-2-4-heptadienal and *trans*, 4-heptenal causing cucumber-fishy odours when in moderate quantities and a rotten-medicinal (dinoflagellates, e.g. *Ceratium* and *Peridinium*)

Table 2.3 also gives a list of the most commonly found phytoplankton groups and important genera together with the potential nature of odours that may occur in the water when specific phytoplankton are

Chapter 2

Literature Review

present in either moderate or abundant quantities. All these groups and genera Vaal Dam is an important water source, since it supplies South Africa's largest conventional water treatment plant with source water for drinking water treatment. It is often a challenge for water treatment plants such as South Africa's largest conventional water treatment plant to effectively remove all different algal species (Ewerts *et al.*, 2013). Ewerts *et al.* (2013) identified at least two of the genera listed below as problem-causing phytoplankton; namely *Anabaena* and *Ceratium* due various problems that occur in water treatment plants when cells are present in the source water.

A fishy odour is often produced by the same phytoplankton cells that are responsible for the aromatic odours, but where these organisms are present in large numbers (Palmer, 1980). One such alga is *C. hirundinella* that can produce fishy taste and odour compounds in drinking water (Van der Walt, 2012) as well as brown discoloration of water during bloom formation (Janse van Vuuren *et al.*, 2006). During drinking water treatment, the dinoflagellates *Ceratium* and *Peridinium* are commonly associated with taste and odour problems (Knappe *et al.*, 2004). The main compounds identified were two unsaturated aldehydes (*trans, trans*-2-4-heptadienal and *trans*, 4heptenal). One ketone (1-penten-3-one) was tentatively identified. Young and Suffet (1999) also recorded *trans, trans*-2-4-heptadienal as a newly found fishy taste and odour causing compound.

Robinson *et al.* (1987) isolated steroidal derivatives, fatty acid alkyl esters and triacylglycerols in two cultured dinoflagellates (*Woloszynskia coronata* and *Ceratium furcoides*) and natural populations of two *Peridinium* species (*P. lomnickii* and *P. cinctum*). The same sterol moieties (in free and esterified forms) occurred within each species. However, 4*a*-methyl steroidal ketones were present in *Woloszynskiacoronata* and *Ceratium furcoides*, a unique organic compound among dinoflagellates. Refer to Chapter 6 for a more detailed discussion on organic compounds associated with *C. hirundinella*.

Chapter 2

Literature Review

Table 2.3

The nature of taste and odours produced by different phytoplankton genera (adapted for Degrémont, 2007).

Phytoplankton Groups	Main genera involved	Phytoplankton Quantities	
		Moderate	Abundant
Cyanophyceae	<i>Anabaena, Aphanizomenon, Gloeotrichia, Gomphosphaeria, Microcystis, Oscillatoria</i>	grassy, earthy-musty	rotten, grassy, musty, spicy
Chrysophyceae	<i>Dinobryon, Mallomonas, Synura</i>	fishy, cucumber, rotten	Fishy
Bacillariophyceae	<i>Asterionella., Cyclotella, Diatoma, Fragellaria, Melosira, Stephanodiscus, Synedra</i>	grassy, spicy geranium	fishy, musty
Cryptophyceae	<i>Cryptomonas</i>		Fishy
Dinophyceae	<i>Ceratium, Peridinium</i>	cucumber, fishy	rotten, fishy
Euglenophyceae	<i>Euglena</i>	-	Fishy
Chlorophyceae	<i>Chlamydomonas, Chlorella, Cladophora, Closterium, Cosmarium, Eudorina, Pandorina, Pediasstrum, Scenedesmus, Spirogyra, Straurastrum</i>	Grassy	grassy, musty, fishy

2.8 The Formation of Trihalomethanes (THMs) and the Impacts on Water Quality

Chlorine is the commonly used disinfectant in countries such as North America and South Africa and has been reported over the last two decades to be responsible for the formations of disinfection by-products (Van der Walt, 2009 and McCormick *et al.*, 2010). Most water treatment plants in South Africa use chlorine as a disinfectant during the production of potable water (Van der Walt *et al.*, 2009). The two prevalent classes of disinfection by-products, trihalomethanes (THM's) and haloacetic acids (HAAs) have been reported and identified to have potential adverse health risks to consumers, such as increased incidence of cancer (Dodds *et al.*, 2004; Van der Walt *et al.*, 2009 and Shen *et al.*, 2011). THMs are formed when chlorine or bromine reacts with organic compounds in the water (Van der Walt *et al.*, 2009). However, THMs can be removed from water by removing the precursor material prior to the disinfection stage of water treatment (Linde *et al.*, 2001).

The use of oxidants for disinfection, taste and odour removal, or for decreasing coagulant demand also produces undesirable organic by-products (EPA, 1999). Organic oxidation by-products such as aldehydes and ketones are primarily associated with strong oxidants such as ozone, chlorine and advanced oxidation (Van der Walt *et al.*, 2009). Aldehydes and ketones form part of organic oxidation- and halogenated organic by products, respectively (Van der Walt *et al.*, 2009). The formation of disinfection by-products (DBPs) is generally associated with the use of chlorine in the presence of natural organic matter (NOM); however, alternative disinfectants can also produce DBPs as a result of reactions between disinfectants and organic compounds (Van der Walt *et al.*, 2009). Monochloramine is the species most suited for drinking water disinfection, but chloramines cause taste and odour problems and should be avoided due to their ability to cause phytoplankton cells lyses (Van der Walt *et al.*, 2009). Organic material released during lysis of phytoplankton cells may also raise issues related to organic oxidation by-products such as haloacetic acids (HAA) and N-nitrosodimethylamine (NDMA) during pre-chlorination or disinfection (Zamyadi *et al.*, 2012). Therefore, the formation of chlorine by-products should be regularly monitored at different stages for water treatment when algae and cyanobacteria occur in source water to comply with World Health Organisation guidelines and South African National Standards (SANS) for drinking water.

2.9 Conclusions

The occurrence of dinoflagellates well described in this literature review, with the majority of members occurring in marine environments. Members living in marine environments are often responsible for toxic algal blooms known red tides, while members found in freshwater are causing a brownish discoloration of the water which is not reported to be toxic. When a brownish discoloration of the water occurs, it is often the result of a dinoflagellate bloom caused by either *C. hirundinella* or the genus *Peridinium*. Although freshwater blooms were not reported in literature to be toxic, the most commonly found species; *C. hirundinella* can cause major water-related problems when source water used for drinking water production is enriched with this alga. The alga is relatively large in cell size, highly mobile and covered with robust theca plates which give the algal cells the following abilities: 1) to contribute to large quantities of organic material in water, 2) to migrate easily through the water column to utilize nutrients and optimal sunlight for photosynthesis and 3) to resist changing environment conditions. The presence *C. hirundinella* cells in drinking water treatment plants will interfere with coagulation and flocculation unit processes, since cells can swim out of flocs and will subsequently clog sand filters when cells are carried over to sand filter beds. When these cells are damaged due to various treatment processes it will result in taste and odour problems or form chlorine by-products during disinfection that are harmful to consumers. To resolve the problems that occur when *C. hirundinella* cells are present in source water used for drinking water production, a number of algal removal strategies and mechanisms are described in literature.

The application of phytoplankton removal strategies should also include jar testing experiments to optimize coagulation and flocculation in order to effectively remove suspended particles, such as turbidity and phytoplankton during the sedimentation stage (Prévost, 2005). Pre-oxidation is also widely described as a pre-treatment step to be considered when problem-causing algae such as *C. hirundinella* are present in source water. The control of surface charge known as zeta potential can also effectively be used to improve the removal efficiencies of problem-causing algae. It has also become evident from this literature review that damage to algal cells, notably cell lysis, may result in taste and odour problems that originates from organic compounds produced by algae. This literature also highlights that algae such as *C. hirundinella* produce organic compounds that are responsible for fishy to rotten tastes odours (Refer to Chapter 6 for a more detailed discussion).

Chapter 3

Strategies of coagulant optimization to improve the removal of turbidity and Ceratium hirundinella cells during conventional drinking water treatment

Chapter 3

Strategies of Coagulant Optimization to Improve the Removal of Turbidity and *Ceratium hirundinella* Cells during Conventional Drinking Water Treatment

3.1 Introduction

Different water treatment processes are used in sequence in order to produce drinking water of desired quality (Schutte, 2006). Water treatment plants can either be conventional or advanced in design and operation. In conventional water treatment works, suspended matter is removed in clarifiers or sedimentation tanks and sand filters after coagulation and flocculation (Schutte, 2006). The primary aim of coagulation and flocculation is to remove suspended and dissolved particles that may be undesirable in the final effluent (Leopold and Freese, 2009). Research on conventional water treatment processes such as coagulation, flocculation, sedimentation and filtration contribute to a better understanding of these processes and improves performance (Schutte, 2006).

The choice of treatment processes to produce drinking water will be influenced or governed by the quality of source water and the quality standards that must be met (Prévost *et al.*, 2005). The quality of source water may be influenced by a number of factors, such as suspended matter; clay, fine particulate matter, colloids, organic and inorganic precipitates (Schutte, 2006). Algae and cyanobacteria (phytoplankton) form part of organic suspended matter in water. Phytoplankton cells such as the dinoflagellate *Ceratium hirundinella* (*C. hirundinella*) are known to cause major water treatment problems, especially during bloom forming periods (Pieterse *et al.*, 2000; Swanepoel *et al.*, 2008a).

Increasing frequencies of occurrence and concentrations of the freshwater dinoflagellate *Ceratium* were recorded in various South African impoundments (e.g. Hartbeespoort Dam, Vaal Dam and Albert Falls Dam) from the year 2000 until recently (Van Ginkel *et al.*, 2001; Swanepoel *et al.*, 2008a; Hart and Wragg, 2009). Monitoring the occurrence of this problem-causing alga is priority to Rand Water, due to a previously recorded event of chlorophyll breakthrough into the drinking water. *C. hirundinella* cells are described to be relatively large in size, up to 450 µm long and 30-100 µm wide (Janse van Vuuren *et al.*, 2006). Due to large cell size, it contributes significantly to total photosynthetic and accessory pigment concentrations in the source water (Van Ginkel *et al.*, 2001; Swanepoel *et al.*, 2008a; Hart and Wragg, 2009). Photosynthetic and accessory pigments associated with the dinoflagellate, *C. hirundinella* are green pigments (e.g. chlorophyll-*a* and *b*) and golden-brown pigments known as peridinin (Janse van Vuuren *et al.*, 2006).

Chapter 3

Strategies of coagulant optimization to improve the removal of turbidity and Ceratium hirundinella cells during conventional drinking water treatment

Characteristics of phytoplankton cells such as morphology, motility, surface charge and cell density have been reported to influence the performance of water treatment works (Henderson *et al.*, 2007). Coagulation is mostly affected by the changes in phytoplankton characteristics, but is considered as the main process in removal of phytoplankton during water treatment (Henderson *et al.*, 2007). Pieterse *et al.* (2000) found that flagellated cells, such as *C. hirundinella* are more difficult to remove, because they prevent floc formation and consequently avoid removal by sedimentation. When *C. hirundinella* is present in high concentrations in the source water, many water treatment problems arise, like the disruption of floc formation, clogging of sand filters, and the production of fishy taste and odours that may penetrate into the final drinking water.

Turbidity removal experiments by means of jar stirring tests are often applied to determine the appropriate coagulant dosages (Bolto, 1995; Zhu *et al.*, 1995; Eikebrokk *et al.*, 2006; Liu *et al.*, 2006). However, the settling turbidity after sedimentation achieved by jar stirring test experiments or in water treatment works does not necessarily indicate the removal of phytoplankton cells (e.g. *C. hirundinella*). In contrast, it can be expected that total photosynthetic pigment (TPP) removal should have a better correlation with *C. hirundinella* cell removal (when compared to turbidity after sedimentation). TPP measurements the well-known photosynthetic pigment, chlorophyll-*a* as well as other accessory pigments such as pheophytin and chlorophyll-*b*.

Although, chlorophyll-*a* analysis is described as an easy and accurate method to determine phytoplankton removal from source water (Knappe *et al.*, 2004), chlorophyll-*a* analysis is less suitable to use when phytoplankton concentrations decrease as a result of removal efficiencies after different stages of water treatment (Swanepoel *et al.*, 2008b). A methods manual compiled by Swanepoel and co-workers for the water research commission (WRC) compares the TPP method (described in method manual as chlorophyll-665 method) to the chlorophyll-*a* method. The methods manual describes the TPP method as more appropriate for treated water, since it is more sensitive to detect low photosynthetic pigment in water (Swanepoel *et al.*, 2008b). This method is currently in use at the Hydrobiology Laboratory, Analytical Services of Rand Water where this research was conducted. TPP analyses rather than settling turbidity measurements may thus be a better indicator of appropriate coagulant dosages when water contains high *C. hirundinella* concentrations. It is recommended that TPP analyses after jar stirring tests should be included into water treatment strategies when phytoplankton blooms occur in source water.

South Africa's largest conventional water treatment plant (Zuikerbosch water treatment plant) dose hydrated lime (e.g. $\text{Ca}(\text{OH})_2$), activated silica (SiO_2) and organic coagulants (e.g. organic polymer) during coagulation and flocculation to remove turbidity, phytoplankton and other impurities. Turbidity and *C. hirundinella* removal when dosing a combination of coagulants may be a challenge

Chapter 3

Strategies of coagulant optimization to improve the removal of turbidity and Ceratium hirundinella cells during conventional drinking water treatment

to water treatment utilities if coagulant dosages and processes are not optimized. Therefore, the aims of this research were to: 1) investigate the use of turbidity and TPP as indicators for the selection of appropriate coagulant dosages during screening jar stirring tests; 2) compare the efficacy of appropriate coagulant dosages determined by these indicators in removing turbidity, TPP and *C. hirundinella* cells simultaneously and 3) evaluate the removal abilities put on each unit process when dosing various coagulants.

3.2 Materials and Methods

3.2.1 Source water

Source water samples containing relatively high *C. hirundinella* concentrations (> 500 cell/ml) were collected from Benoni Lake, South Africa (26°10'50.40''S; 28°17'50.11'' E) in plastic containers. Source water quality parameters are summarized in Appendix Table B and are typical of eutrophic water sources in South Africa according to DWAF (now DWS) criteria for trophic statuses of freshwater impoundments (Van Ginkel *et al.*, 2001b).

Appendix Table C gives a comparison between the source waters of Benoni Lake and Vaal Dam. The concentrations measured for nitrates and total phosphates in Benoni Lake and Vaal Dam were relatively similar, with mean values that compare as follow: 0.95 mg/l to 0.34 mg/l and 0.01 mg/l to 0.12 mg/l respectively. Lower chemical oxygen demand (COD) concentrations were recorded in Vaal Dam water, while the pH values in Benoni Lake water were slightly lower. Swanepoel (2014) classified the Vaal Dam as mesotrophic according to the system used by the South African Department of Water and Affairs (DWA). Ewerts *et al.* (2014) made a further comparison between the significant phytoplankton genera found in the water sources, which indicated that the TPP concentrations in Benoni Lake were significantly higher in comparison to the concentrations measured in Vaal Dam water (Appendix Table C). Algal species that may have a potential impact on the results of this research are indicated in Appendix Table C. High TPP concentrations that occurred in the low turbid Benoni Lake can be mainly ascribed to the presence of dinoflagellates that are relatively large in cell size when compared to the cyanobacterial cells that are mostly found in the Vaal Dam. Although the water quality of the two water bodies is relatively similar, the trophic statuses will vary due to the difference in TPP (or chlorophyll-*a*) concentrations.

Results from ten sampling occasions were used to perform a principal component analysis (PCA) and four sampling occasions were used to simulate the conventional water treatment processes employed at Rand Water's Zuikerbosch water treatment plant.

3.2.2 Coagulant chemicals used to conduct jar stirring test experiments

The following coagulant chemicals and dosages were used to perform screening jar stirring tests to select appropriate dosages:

- (a) **Hydrated Lime in combination with activated silica ($\text{Ca(OH)}_2\text{-SiO}_2$):** Ca(OH)_2 dosages ranged from 60 to 160 mg/ℓ (with increments of 20). SiO_2 dosages of 4 mg/ℓ were dosed as a coagulant aid.
- (b) **Hydrated Lime in combination with organic polymer:** Organic polymer dosages ranged from 4 to 14 mg/ℓ (with increments of 2). Ca(OH)_2 dosages of 10 mg/ℓ were added as a coagulant aid.
- (c) **Organic Polymer:** Organic polymer dosages ranged from 4 to 14 mg/ℓ (with increments of 2).

3.2.3 Experimental design

Figure 3.1 illustrates the experimental setup used to simulate conventional water treatment processes (coagulation, flocculation, sedimentation and sand filtration). The experimental setup consisted of a six paddle jar stirrer apparatus and a column sand filtration system (Phipps and Bird models). Jar stirring methods were performed according to procedures performed at Rand Water's Process Technology Department. The sand filtration processes of Rand Water is simulated that uses filter media with an effective size of 0.7 mm and a uniformity coefficient of 1.4 or less (Figure 3.1 illustration ii).

Chapter 3

Strategies of coagulant optimization to improve the removal of turbidity and *Ceratium hirundinella* cells during conventional drinking water treatment

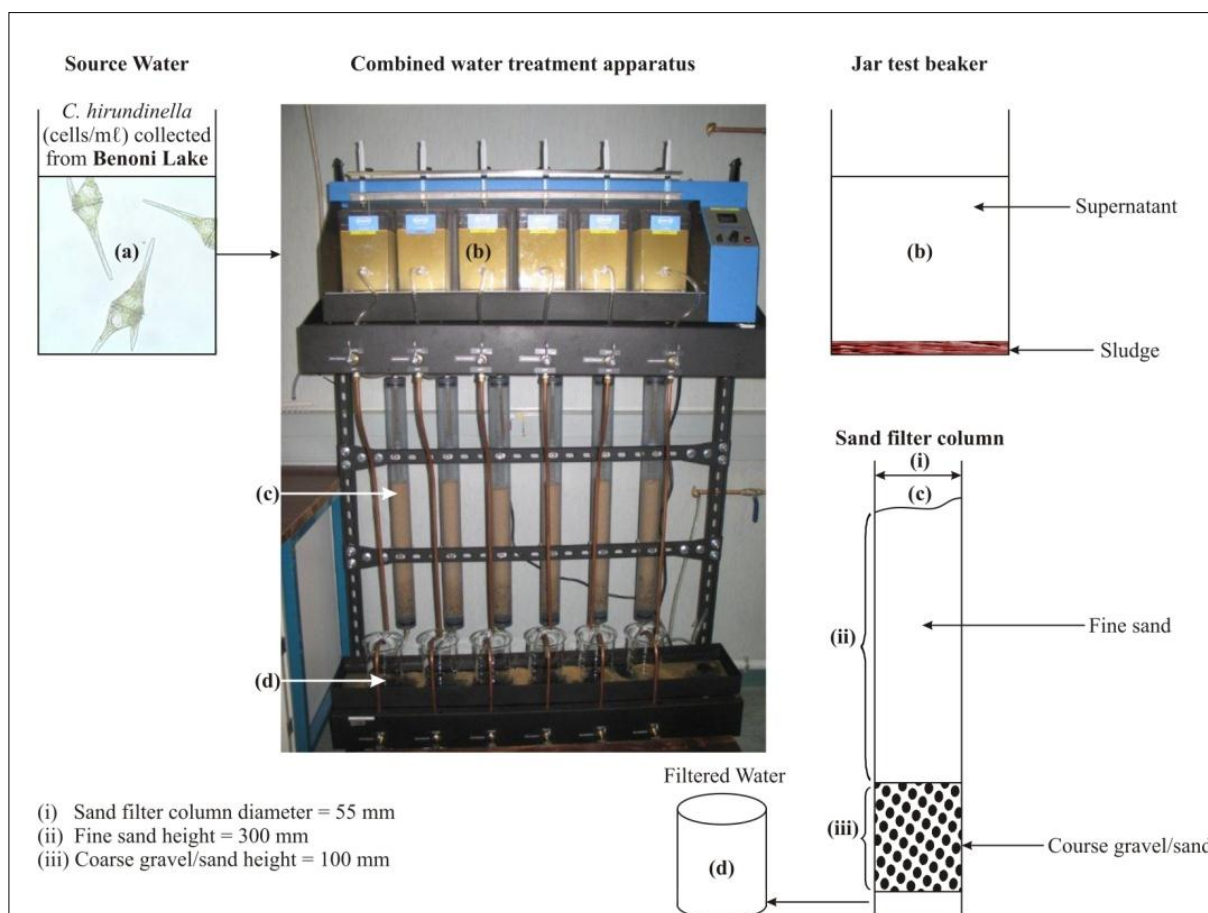


Figure 3.1

The experimental setup for removing *C. hirundinella* cells (a) by using a combined water treatment apparatus, consisting of jar beakers (b) connected to sand filter columns (c) with a filtered water outlet (d).

Benoni Lake source water samples containing a known number of *C. hirundinella* cells were used to simulate the treatment process of the water treatment works at South Africa largest conventional water treatment plant on a laboratory scale under laboratory conditions ($\pm 22^\circ\text{C}$). Conventional coagulation, flocculation and sedimentation unit processes was carried out in 2ℓ jar stirring test beakers (Figure 3.1 b). The 2ℓ source water samples were subjected to high energy flash mixing conditions of 300 rpm (G -value of 400s^{-1}) for 30 seconds. Different coagulant dosages were added with syringes and allowed to disperse uniformly at high energy flash mixing conditions for another 30 seconds. Three decreasing energy stages of 125s^{-1} , 54s^{-1} and 14s^{-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 minutes. Samples of the supernatants were collected to investigate the efficacy of coagulation, flocculation and sedimentation after dosing various coagulants. After sedimentation, the supernatant in the jar beakers (Figure 3.1 b) were drained through the sand filter beds (Figure 3.1 c) at a filtration

rate of 4 metres/hour. Subsequently, the filtrate was collected in glass beakers (Figure 3.1 d) to investigate the efficacy of sand filtration.

3.2.4 Turbidity and TPP analyses

Turbidity and TPP analyses were used to determine the efficacy of coagulant dosages. Turbidity measurements were determined using a HACH 2100AN model. TPP analyses were determined by extracting pigments from phytoplankton cells using methanol and analysed with a Beckman spectrophotometer (650i) as described by Swanepoel *et al.* (2008b). For the purpose of this research, no chlorophyll-*a* analyses were performed. The TPP method is more sensitive to determine chlorophyll pigments in treated water (containing lower chlorophyll contents) than the chlorophyll-*a* method only. The chlorophyll-*a* concentration can be determined as follows: (TPP/chlorophyll-665 = 1.24*chlorophyll-*a*) by Swanepoel (2014).

3.2.5 Phytoplankton (*C. hirundinella*, other algae and cyanobacteria) analyses (Identification and enumeration)

Source water (200 mL) samples were collected in dark plastic bottles. Samples were pressure-deflated in a steel chamber with a rubber stopper using a specially-designed hammer that exerted a pressure of approximately 49.5 kPa on a sample, which is needed to collapse the gas vacuoles of cyanobacteria (Janse van Vuuren, 1996). Treated water (1000 mL) samples were collected and filtered through 0.45 µm filter paper to trap phytoplankton. Phytoplankton trapped on filter paper were recovered and re-suspended in 10 mL using a vortex mixer.

All algal samples were preserved with Lugol's solution or formaldehyde (2% final concentration). The sedimentation/centrifugation technique, originally described by Lund *et al.* (1958) and adapted for Rand Water according to Swanepoel *et al.* (2008a) was used for identification and enumeration. The concentration (cells/mL) and physical integrity of *C. hirundinella* cells were determined and evaluated using an inverted light microscope.

3.2.6 Determination of appropriate coagulant dosages

Within the screening coagulant dosage range, the dosage that is the most cost effective in removing turbidity to values below 5 NTU were selected as an indicator of appropriate coagulant dosage. Good TPP removal usually occurs at the highest coagulant dosage which is not always the most cost effective; however, to achieve the best removal of *C. hirundinella*, TPP should be included as an indicator during the selection of an appropriate coagulant dosage.

3.2.7 Statistical analysis

Principal component analysis (PCA) was carried out to determine the correlation between appropriate coagulant dosages and parameters (turbidity, TPP and *C. hirundinella*) after sedimentation. The computer package CANOCO, version 4.5 was used (TerBraak, 1988). Ordinations were interpreted using the following rationale: parameters are positively correlated with each other if their arrows subtend a small angle, no correlations if their arrows are 90° and negatively correlated if their arrows are directed in opposite directions. Parameters with the longest arrow relative to an axis have the greatest influence on that axis.

3.3 Results

3.3.1 PCA analyses of turbidity, TPP and *C. hirundinella* and coagulants to determine appropriate indicators of coagulant dosages

Samples ($n=10$) were analysed for appropriate coagulant dosages (based on turbidity as indicator), and the parameters; turbidity, TPP and *C. hirundinella* cell counts.

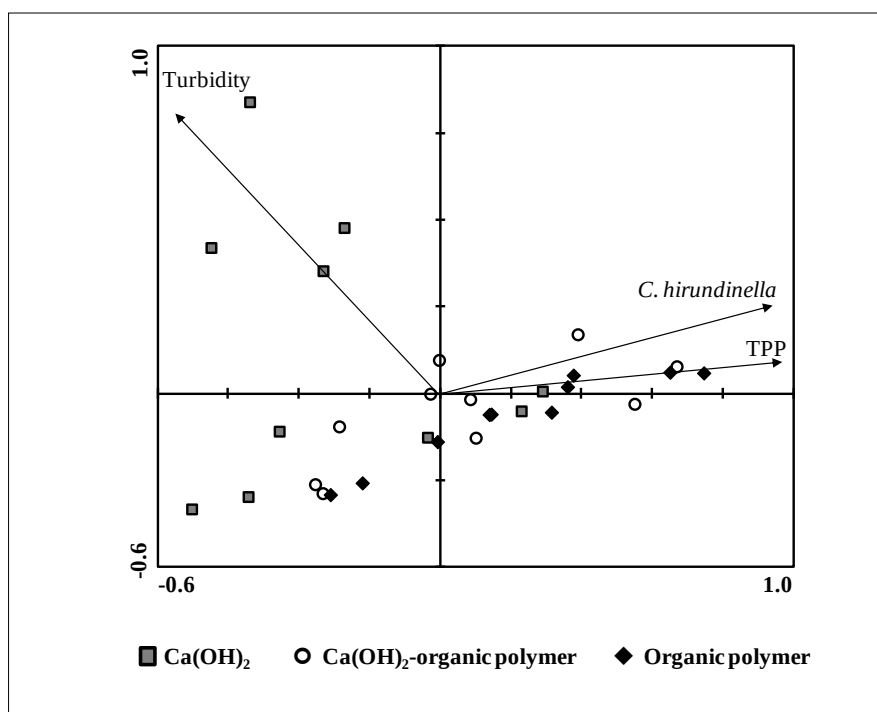


Figure 3.2

The PCA biplot ordination of appropriate coagulant dosages and response parameters, turbidity, TPP and *C. hirundinella* cells after sedimentation.

The first axis (x-axis) of the PCA ordination (Figure 3.2) described 61.7 % of the variance in the data. The second axis (y-axis) of the PCA ordination described another 28.9 % of variance in the data (Figure 3.2). A close correlation was observed between the parameters TPP and *C. hirundinella* cell counts. $\text{Ca(OH)}_2\text{-SiO}_2$ as coagulant correlated strongly with turbidity, while appropriate dosage samples for Ca(OH)_2 -organic polymer and organic polymer as coagulants associated mostly with TPP and *C. hirundinella* cell counts. This indicates that the coagulant option of $\text{Ca(OH)}_2\text{-SiO}_2$ resulted in high levels of turbidity after sedimentation. Ca(OH)_2 -organic polymer correlated with the presence of *C. hirundinella* cells, while organic polymer correlated with the TPP content in the supernatant.

3.3.2 The efficacy of turbidity, TPP and *Ceratium hirundinella* removal when the parameters turbidity and TPP were used respectively as indicators of appropriate coagulant dosages

Turbidity is mostly used as an indicator when choosing appropriate coagulant dosages. The South African standard/guideline for aesthetic turbidity values of treated water are set at ≤ 5 NTU (SANS 241-1, 2011). In this Chapter, the unit processes used to treat Vaal Dam source water were simulated; therefore, turbidity levels and algal concentrations (TPP) measured in Benoni Lake water were compared to contents in Vaal Dam water. Thus, Benoni Lake water can be characterised as low turbidity levels and high algal concentrations (Appendix Table C). The TPP concentration in Benoni Lake water originated mostly from *C. hirundinella* cells. Water bodies with low turbidity levels (high transparency) as recorded in Benoni Lake water allows efficient light penetration that is beneficial for algal growth when environmental conditions are favourable (Wetzel, 2001). The use of Ca(OH)_2 as coagulant in appropriate dosage to treat source water with low turbidity can improve both turbidity and *C. hirundinella* removal; however, an over dosage of Ca(OH)_2 may impact negatively on water treatment.

The $\text{Ca(OH)}_2\text{-SiO}_2$ associated with higher Ca(OH)_2 dosages was responsible for a major increase in turbidity levels after sedimentation putting strain on the subsequent sand filtration step. Better turbidity removal was achieved by Ca(OH)_2 -organic polymer (-17 %), although it has also increased the initial turbidity levels. Both these coagulant options managed to remove TPP and *C. hirundinella* cells fairly well (83 % and 51 % respectively). The organic polymer achieved the best turbidity removal. However, this coagulant option was not effective in removing TPP or *C. hirundinella* cells, removing only 38 % and 39 % respectively.

Chapter 3

Strategies of coagulant optimization to improve the removal of turbidity and Ceratium hirundinella cells during conventional drinking water treatment

Table 3.1

The average values for parameters measured ($n=4$) in the source water, after sedimentation and after filtration when turbidity was used as indicator of appropriate coagulant dosages. The TPP values of 0 $\mu\text{g}/\ell$ indicates concentrations below 0.5 $\mu\text{g}/\ell$ or concentration values below the detection limit of method used. *C. hirundinella* cells are covered with theca-plates that may remain intact after organic material is released. Furthermore, 1-2 cells/ $\text{m}\ell$ may occur with 0 $\mu\text{g}/\ell$ or low pigment concentrations.

Coagulants and Average dosages	Parameters	Units	Source Water	After Sedimentation	After Filtration	Percentage Removal	
						After Sedimentation	After Filtration
Ca(OH) ₂ -SiO ₂ [135 mg/ ℓ Ca(OH) ₂ - 4 mg/ ℓ SiO ₂]	Turbidity	NTU	6	29	1	-383	83
	Total Photosynthetic Pigments	$\mu\text{g}/\ell$	41	7	0	83	100
	<i>Ceratium hirundinella</i>	cells/ $\text{m}\ell$	510	130	2	75	100
Ca(OH) ₂ -Organic Polymer [10 mg/ ℓ Ca(OH) ₂ – 6.5 mg/ ℓ Organic Polymer]	Turbidity	NTU	6	7	0	-17	100
	Total Photosynthetic Pigments	$\mu\text{g}/\ell$	41	20	1	51	98
	<i>Ceratium hirundinella</i>	cells/ $\text{m}\ell$	510	125	0	75	100
Organic Polymer [4 mg/ ℓ Organic Polymer]	Turbidity	NTU	6	4	1	33	83
	Total Photosynthetic Pigments	$\mu\text{g}/\ell$	41	25	1	39	98
	<i>Ceratium hirundinella</i>	cells/ $\text{m}\ell$	510	314	0	38	100

Chapter 3

Strategies of coagulant optimization to improve the removal of turbidity and Ceratium hirundinella cells during conventional drinking water treatment

Although no specific guideline for TPP after sedimentation is currently set, the coagulant dosage that achieved the best TPP removal should be taken into account during the selection of an appropriate dosage. The largest portion of the average TPP concentration originates from *C. hirundinella* cells, and was (41 µg/ℓ) in the source water. Therefore, TPP can be used as indicator of appropriate coagulant dosages to ensure good *C. hirundinella* removal (Table 3.2). Results obtained when using turbidity as indicator of appropriate coagulant dosage (Table 3.1) are compared to results obtained when using TPP as indicator of coagulant dosage (Table 3.2) to investigate removal efficacies.

When TPP was used as indicator of appropriate coagulant dosages, an increase in supernatant turbidity was again recorded when dosing $\text{Ca(OH)}_2\text{-SiO}_2$ (Table 3.2). In the light of this observation, increasing turbidity is rather a result of residual high Ca(OH)_2 dosages used during $\text{Ca(OH)}_2\text{-SiO}_2$ treatment. However, when using TPP as indicator of appropriate dosages, both Ca(OH)_2 -organic polymer and organic polymer achieved higher removals of turbidity (50 % in both cases), TPP (76 % and 51 % respectively) as well as *C. hirundinella* cells (82 % and 84 % respectively).

Chapter 3

Strategies of coagulant optimization to improve the removal of turbidity and Ceratium hirundinella cells during conventional drinking water treatment

Table 3.2

The average values for parameters measured ($n=4$) in the source water, after sedimentation and after filtration when TPP was used as indicator of appropriate coagulant dosages. The TPP values of 0 $\mu\text{g}/\ell$ indicates concentrations below 0.5 $\mu\text{g}/\ell$ or concentration values below the detection limit of method used. *C. hirundinella* cells are covered with theca-plates that may remain intact after organic material is released. Furthermore, 1-2 cells/ $\text{m}\ell$ may occur with 0 $\mu\text{g}/\ell$ or low pigment concentrations

Coagulants and Average dosages	Parameters	Units	Source Water (Actual Values Recorded)	After Sedimentation	After Filtration	Percentage Removal	
						After Sedimentation	After Filtration
Ca(OH) ₂ -SiO ₂ [150 mg/ ℓ Ca(OH) ₂ - 4 mg/ ℓ SiO ₂]	Turbidity	NTU	6	22	1	-267	83
	Total Photosynthetic Pigments	$\mu\text{g}/\ell$	41	6	0	85	100
	<i>Ceratium hirundinella</i>	cells/ $\text{m}\ell$	510	217	1	57	100
Ca(OH) ₂ -Organic Polymer [10 mg/ ℓ Ca(OH) ₂ – 14 mg/ ℓ Organic Polymer]	Turbidity	NTU	6	3	0	50	100
	Total Photosynthetic Pigments	$\mu\text{g}/\ell$	41	10	1	76	98
	<i>Ceratium hirundinella</i>	cells/ $\text{m}\ell$	510	90	0	82	100
Organic Polymer [13.5 mg/ ℓ Organic Polymer]	Turbidity	NTU	6	3	0	50	100
	Total Photosynthetic Pigments	$\mu\text{g}/\ell$	41	20	0	51	100
	<i>Ceratium hirundinella</i>	cells/ $\text{m}\ell$	510	83	0	84	100

High variability in percentage removal was recorded for turbidity, TPP and *C. hirundinella* after sedimentation when dosing various appropriate coagulants dosages as determined by different indicators (Tables 3.1 and 3.2). However, improved TPP and *C. hirundinella* removal (indicated as percentage removal) were recorded when using TPP as indicator. Irrespective of the percentage removal achieved by various appropriate coagulant dosages or indicators after sedimentation, the sand filtration step removed almost all of the remaining turbidity, TPP and *C. hirundinella* contents. In both cases when using turbidity and TPP as indicator (Tables 3.1 and 3.2), maximum percentage removals were achieved (83-100 %) for the full-scale conventional water treatment process.

3.3.3 Impacts of different coagulants on the physical integrity of *C. hirundinella* cells after sedimentation

The appearance of *C. hirundinella* cells in the source water (Figure 3.3 b) is used as a control for the impacts of coagulants on the physical integrity of cells. The coagulant $\text{Ca(OH)}_2\text{-SiO}_2$ is characterized by relatively high dosages of Ca(OH)_2 which increase the pH levels to 11 and above. This coagulant treatment caused major damage to the cells (Figure 3.3 b). Broken cells of *C. hirundinella* may also have contributed to increasing turbidity levels after sedimentation (Tables 3.1 and 3.2). This therefore could explain the negative values obtained for percentage turbidity removals recorded after sedimentation. Less damaging effects to *C. hirundinella* cells were observed when Ca(OH)_2 -organic polymer and organic polymer coagulant treatment options were used (Figures 3 c and 3 d) respectively. Less damaging effects to cells may be a result of lower Ca(OH)_2 dosages used during Ca(OH)_2 -organic polymer treatment. Ca(OH)_2 -organic polymer and organic polymer treatment options were also characterized with an overall good turbidity removal.

Chapter 3

Strategies of coagulant optimization to improve the removal of turbidity and Ceratium hirundinella cells during conventional drinking water treatment

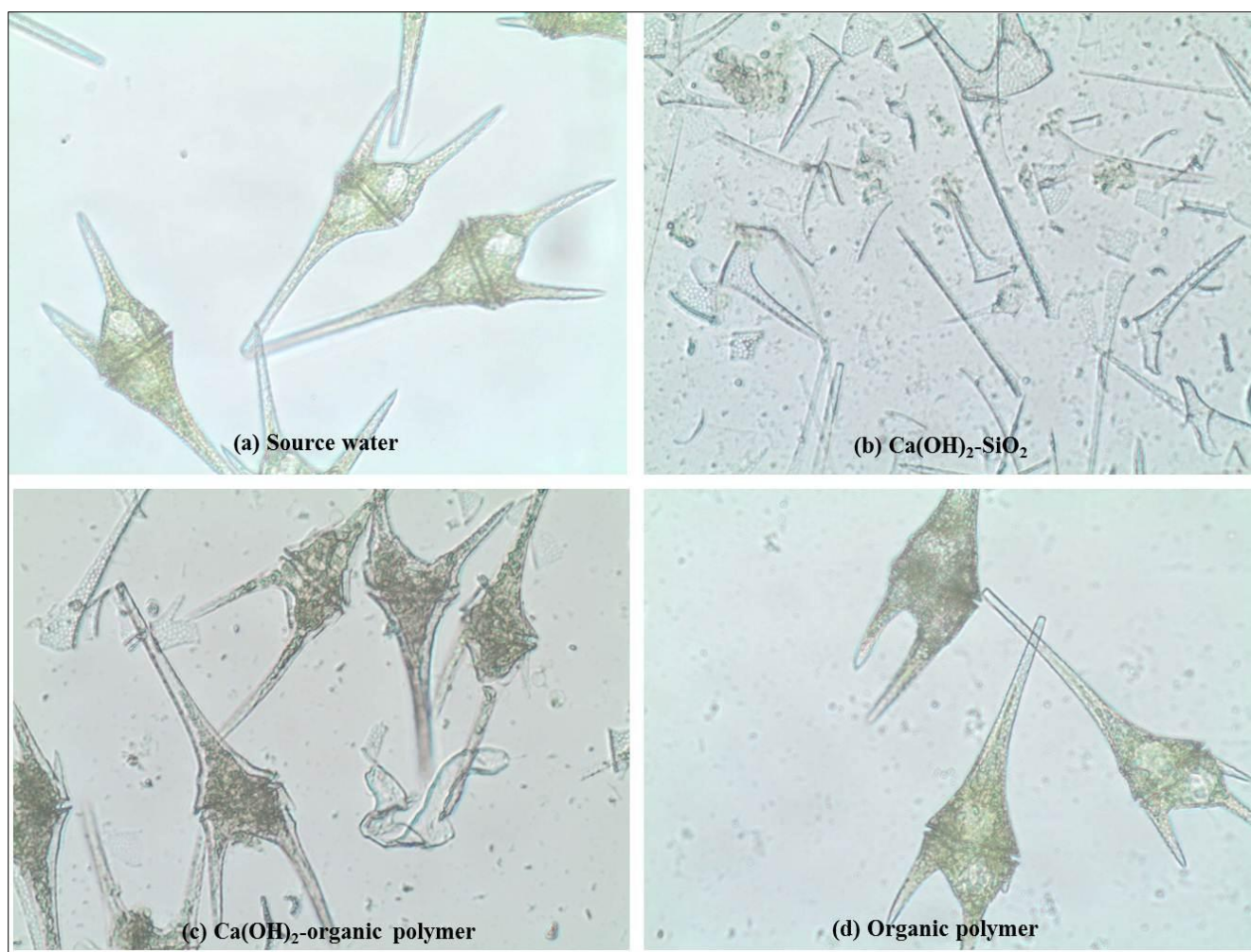


Figure 3.3

Light microscopy images of *C. hirundinella* cells in source water (a) and after sedimentation when dosing the following coagulants: $\text{Ca(OH)}_2\text{-SiO}_2$ (b), $\text{Ca(OH)}_2\text{-organic polymer}$ (c), and organic polymer (d).

3.4 Discussion

It is a well-known fact that the occurrence of phytoplankton (cyanobacteria and algae) in eutrophic source water used for drinking water production may lead to many water-related problems (Knappe *et al.*, 2004). *Ceratium* genera have been identified as a problem-causing organism for water treatment plants and may pose risks to water quality (Knappe *et al.*, 2004; Ewerts *et al.*, 2013). Swanepoel *et al.* (2008a) reported the penetration of *C. hirundinella* cells into the final treated water at South Africa's largest conventional water treatment works. Findings made by Swanepoel *et al.* (2008a) indicate that highly motile *C. hirundinella* cells are able to disrupt coagulation and flocculation, and therefore, these cells remain in the supernatant after sedimentation (Ewerts *et al.*, 2013).

In order to address these challenges to drinking water treatment, managers and operators should implement strategies to assist unit processes to remove problem-causing algae, like *C. hirundinella*. The application of phytoplankton removal strategies should include jar stirring test experiments to optimize coagulation and flocculation in order to effectively remove phytoplankton cells from source water during the sedimentation stage. In this Chapter, a laboratory-scale conventional water treatment apparatus was used to simulate the conventional water treatment processes using different coagulant options.

Due to their large cell size, *C. hirundinella* cells contribute to significant amounts of chlorophyll and accessory pigments in water (Janse van Vuuren *et al.*, 2006 and Swanepoel *et al.*, 2008a). Since *C. hirundinella* cells contribute to large quantities of chlorophyll and other accessory pigments, TPP measurements can be used as an indication of *C. hirundinella* concentrations present in source water, and after different stages of the conventional water treatment process. In Tables 3.1 and 3.2 it is evident that the TPP no specific correlations occur between the number of cells and the TPP concentrations in the supernatant. The impacts of treatment on the cells and the potential correlations between *C. hirundinella* cells and TPP will be discussed in Chapter 5. Therefore, when source water used for drinking water production contains *C. hirundinella* cells in either moderate or abundant quantities, TPP measurements after sedimentation should be included during jar screening tests to select appropriate coagulant dosages for the removal of *C. hirundinella* (as indicated by the strong correlation between TPP and *C. hirundinella* in Figure 3.2).

Turbidity measurements, traditionally used as indicator of appropriate coagulant dosages, correlated positively with $\text{Ca(OH)}_2\text{-SiO}_2$ (Figure 3.2). This suggests that the, high turbidity levels in the supernatant showed after sedimentation, may have originated from Ca(OH)_2 and other impurities

Chapter 3

Strategies of coagulant optimization to improve the removal of turbidity and Ceratium hirundinella cells during conventional drinking water treatment

(Figure 3.2). High Ca(OH)_2 (80-160 mg/l) dosages remove *C. hirundinella* and TPP effectively, but cause major damaging effects to cells and increase turbidity.

Organic polymer dosages were dosed as primary coagulant during Ca(OH)_2 -organic polymer and organic polymer treatments respectively. The purpose of Ca(OH)_2 in combination with organic polymer is mostly for pH adjustment (WHO, 2004). The use of organic polymer is not linked to pH adjustments and only low dosages are required for effective turbidity removal (Ebeling *et al.*, 2005). However in both these coagulant choices, a positive correlation was recorded with *C. hirundinella* and TPP (Figure 3.2) when turbidity was used as indicator. Thus although the use of organic polymer achieved maximum turbidity removal after sedimentation, it was not effective in removing *C. hirundinella*.

The chlorophyll concentrations after sedimentation and filtration should be lower than concentrations in source water (Tables 3.1 and 3.2). Therefore, the determination of TPP is an appropriate indicator to determine coagulant choice for the removal of *C. hirundinella* cells. In both cases where either turbidity or TPP was used as indicator, turbidity increased, or the removal thereof was poor in the events where the coagulant $\text{Ca(OH)}_2\text{-SiO}_2$ was dosed. The coagulant treatment option, Ca(OH)_2 -organic polymer performed better in removing turbidity, TPP and *C. hirundinella*, where the parameter TPP was used as indicator. Organic polymer also functions as an appropriate coagulant for turbidity removal when using TPP as indicator of coagulant dosages. The use of coagulants together with dosages of Ca(OH)_2 , results in a “pH-shock”. Similar observations with regards to “pH-shock” and the removal enhancement of micro-organisms during coagulation are also described by Hoko and Makado (2011) and Ferreira and Du Preez (2012). This Chapter showed that the coagulant option $\text{Ca(OH)}_2\text{-SiO}_2$ increased pH levels to above 11, with major damage to the physical integrity of *C. hirundinella* (which is will be described in more detail in Chapter 5). Although pH shock caused to organisms are described in previous studies when dosing Ca(OH)_2 related coagulants, no evidence of cell damage were observed or no disruptions to cells morphology were investigated. In this Chapter is unique evidence of cell disruption or damage to the morphology of cells when dosing coagulants that increase the pH to levels above 10. Broken cells (e.g. theca plates) may be carried over to sand filters and can cause filter clogging and reduced filter run-times.

Furthermore, when Ca(OH)_2 -organic polymer was used as coagulant the Ca(OH)_2 dosage only increased the pH to between 9-10, leaving the cells intact. It therefore can render cells immobile and assist the coagulation process without contributing to turbidity levels. Consequently, operators and managers of water treatment plants should investigate or implement water quality monitoring schedules to evaluate the impacts of high Ca(OH)_2 dosages on the integrity of algae entering the treatment facilities to avoid water treatment related problems. In addition, cell damage which results

Chapter 3

Strategies of coagulant optimization to improve the removal of turbidity and Ceratium hirundinella cells during conventional drinking water treatment

in the release of organic cell content into the supernatant, may pose major health risks to consumers. Dissolved organic carbon (DOC) that arises from algae and other organic sources can form harmful organic by-products such as trihalomethanes when reacting with chlorine at the disinfection stage of water treatment (Van der Walt *et al.*, 2009). Therefore, maximum removal of DOC should be achieved before the disinfection stage of water treatment to keep levels disinfection by-products below the guidelines.

Using turbidity as indicator of appropriate coagulant dosages is more cost effective than using TPP as an indicator. However, turbidity as an indicator potentially results in a higher organic loading onto the sand filters, which may increase treatment costs significantly. Increased treatment costs can be attributed to increased back-wash frequencies, intensified filter backwash water treatment and the probability of discarding high volumes of filter backwash water.

Recycled water from filter backwash and sludge treatment may contain high levels of inorganic (including Ca(OH)_2 particles) and organic (algae and micro-organisms) impurities (Van der Walt *et al.*, 2009); therefore, plant operators should carefully consider the use of recycled water when algal blooms occur in source water. Van der Walt *et al.* (2009) suggest that water the quality of all waste streams should be monitored when recycled to the inlet of the treatment plant. When dosing coagulant such as Ca(OH)_2 that increases the pH of water, stabilisation of water should be taken into consideration. The stability of water can be determined by using the parameter Calcium Carbonate Precipitation Potential that gives the actual mg/l CaCO_3 that would precipitate from the water (Schutte, 2006). The CCPP value of about 4 mg/l is suggested to give adequate protection against corrosion (Schutte, 2006).

3.5 Conclusions

Strategies used for optimizing conventional water treatment processes should not only be based upon treatment costs but also upon consumer health aspects and aesthetic water quality. Thus when source water used for drinking water production contains highly motile nuisance algae such as *C. hirundinella* in either moderate or abundant quantities, it is advisable to conduct jar stirring test experiments using both turbidity and TPP analyses as indicators of appropriate coagulant choice and dosages. Unlike chlorophyll-*a* analysis, a TPP analysis is more sensitive to detect algal pigments as the content thereof decrease after different of water treatments. The application of TPP analyses to evaluate the removal efficacy of water treatment processes can thus be considered as better practice. This research showed that TPP was the most effective indicator of coagulant choice and dosage when source water contain high concentrations of *C. hirundinella* cells and that Ca(OH)_2 dosages can assist the coagulation and flocculation process of the algal cells by rendering them immobile. When dosing coagulants such as Ca(OH)_2 , water stabilisation is essential and can be monitored using CCPP as parameter to ensure positive results in order to avoid corrosion of pipes and distribution systems. Water treatment plant that recycles water from filter backwash and sludge treatment should monitor water quality frequently from source to drinking water.

Chapter 4

An investigation into the zeta potential changes of Ceratium hirundinella cells when simulating conventional coagulation using inorganic and organic coagulants

Chapter 4

An Investigation into the Zeta Potential Changes of *Ceratium hirundinella* Cells When Simulating Conventional Coagulation Using Inorganic and Organic Coagulants

4.1 Introduction

Within conventional treatment plants the processes of coagulation and flocculation are considered to be principally responsible for removing negatively charged algae (Henderson *et al.*, 2008). Coagulation and flocculation is the result of the particle collisions and charge interactions between coagulants and algal cell surfaces (Gerde *et al.*, 2014). Coagulation and flocculation is followed by sedimentation as particles cluster together and their settling rates increase (Williams *et al.*, 2010).

The surface charge of algal particles, is represented by their zeta potential. This is an important parameter determining their behavior during coagulation and flocculation (Yang *et al.*, 2005). When the zeta potential of particles approaches zero, coagulation efficiency is generally improved (Yang *et al.*, 2005). Therefore the removal of algae during coagulation and flocculation requires that treatment processes are capable of neutralizing algal surface charges to ensure that they are effectively incorporated into flocs (Knappe *et al.*, 2004). Optimization strategies that were discussed in the previous Chapter play an important role to ensure the removal of algae during coagulation, flocculation and sedimentation unit processes to reduce algal penetration into the final water. Zeta potential was employed to verify these findings.

A dosage increase of coagulant is one way of improving coagulation efficiency. Unfortunately, this can create challenges including increased cost per volume of water and increased sludge volumes (Takaara *et al.*, 2007). To ensure the best coagulation conditions it is important to consider zeta potential in order to not only select the best coagulants but also to confirm their optimum dosages (Ministry of Health, 2001). Poor neutralization practices can result in poor floc formation where cells can restore their surface charge, swim out of flocs and penetrate into the final water. Therefore, the aims of this Chapter were to 1) investigate the change in zeta potential of *C. hirundinella* cells during coagulation when using various coagulants to simulate conventional coagulation, flocculation and sedimentation as well as to 2) evaluate whether cells were sufficiently neutralized to remain in aggregation (flocs), or are cells able restore the natural surface charge after sedimentation and swim out of the flocs.

4.2 Materials and Methods

4.2.1 Source water and sample collection

Source water samples (approximately 100 ℓ) containing high concentrations *C. hirundinella* cells were collected from the Benoni Lake in South Africa. Two litre samples were prepared for jar tests using the following approach: 1) most algae and bacteria were first removed by filtration using 0.45 µm filters, 2) *C. hirundinella* cells were then recovered from a separate sample using a 50 µm sieve and 3) the *C. hirundinella* cells recovered with the 50 µm sieve were re-suspended in the 0.45 µm filtered water prepared in the first step. This resulted in a source water matrix enriched with *C. hirundinella* cells. Water samples (containing living *C. hirundinella* cells) were stored under laboratory conditions at ± 22°C. Six different *C. hirundinella* cell concentrations were prepared from a stock sample (containing 7000 cells/mL) to obtain a dilution range from 2000 cells/mL to 7000 cells/mL (with increments of 1000).

4.2.2. Coagulants

Hydrated lime, activated sodium silicate and organic polymer stock solutions were prepared as described in Chapter 3. The following coagulants options were dosed within different dosage ranges: 1) Hydrated lime in combination with activated silica (Ca(OH)₂-SiO₂) in the range of 60 to 160 mg/ℓ Ca(OH)₂ and constant SiO₂ dosages (4 mg/ℓ); 2) Ca(OH)₂ in combination with organic polymer: with the organic polymer in the range of 4 to 14 mg/ℓ and a constant Ca(OH)₂ dosages (10 mg/ℓ) and 3) organic polymer in a range of 4 to 14 mg/ℓ.

The calculations that were used in this Chapter are follows to determine:

1. The amount of organic polymer per cell =
$$\frac{\text{Number of cells } (\frac{\text{cells}}{\text{mL}})}{\text{Organic polymer dosage } (\frac{\text{mg}}{\text{mL}})}$$
 and
2. The percentage cell removal =
$$\frac{\text{Initial cell concentration} - \text{Cell concentration in supernatant}}{\text{Initial cell concentration}} \times 100$$

Initial cell concentrations varied from 2000 cells/mL to 7000 cells/mL (increment of 1000).

4.2.3 Jar stirring tests and sampling for analytical determinations

The conventional water treatment processes coagulation, flocculation and sedimentation were simulated with a jar stirring apparatus (Phipps and Bird, Model 7790-704). Jar stirring experiments were conducted at room temperature (± 22°C); using six 2 litre beakers filled with filtered source water. Known dosages of different coagulants were added with syringes. The 2 litre jar beakers with filtered source water containing pre-determined *C. hirundinella* cell concentrations were

Chapter 4

An investigation into the zeta potential changes of Ceratium hirundinella cells when simulating conventional coagulation using inorganic and organic coagulants

subjected to high energy jar stirring test conditions as described in Chapter 3. After conducting jar stirring tests the following samples were collected to perform specific analyses:

- Samples were collected from the supernatant after treatment with different coagulants to perform pH and *C. hirundinella* cell count analyses.
- Supernatant samples containing flocs were collected to perform zeta potential analyses after water treatment with various coagulants after 20 minutes and again after 2 hours and 4 hours.

4.2.4 Zeta potential and pH

The Brookhaven Instruments Corporation (BIC) ZetaPlus zeta potential analyser (range of ± 150 mV) was used to perform zeta potential measurements. Approximately 1.6 ml of sample was transferred into square cuvetts and the electrodes of the ZetaPlus analyser were submerged into the sample. The surface charge of *C. hirundinella* cells was measured using the Zeta Potential Analyser software. Each zeta potential value is the average of 10 readings. The pH meter built into the ZetaPlus Analyser (calibrated pH probe) was used to determine the pH of samples simultaneously with zeta potential analyses.

4.2.5 *C. hirundinella* cell identification and enumeration

C. hirundinella cell identification and enumeration was performed with the sedimentation technique (phytoplankton method), originally described by Lund *et al.* (1958) and adapted for Rand Water as the phytoplankton identification and enumeration method according to Swanepoel *et al.* (2008 b). The complete phytoplankton method is described in Chapter 3. Each sample was analyzed in triplicate.

4.2.6 Statistical analyses

Normality tests were performed in XLSTAT version 2014.1.07 for pH and zeta potential data. The following normality tests indicated a normal distribution for all data: Shapiro-Wilks, Anderson-Darling, Lilliefors and Jarque-Bera.

A single factor analysis of variance (ANOVA) was performed to determine if there was a statistically significant difference between the average values of zeta potential and pH measurement determined in the source water (control) and after different coagulant treatments or after different settling times. ANOVA was performed using GraphPad Prism for Windows version 4.00. The level of significance for all statistical analyses was set at 0.05 (*p*-value) and the hypotheses were stated separately for each analysis with the hypothesised difference equal to zero.

4.3 Results

4.3.1 Changes in zeta potential when dosing various coagulant treatments

The same coagulant dosages that were optimized in Chapter 3 were used to investigate the changes in zeta potential of *C. hirundinella* cells. Table 4.1 reports the zeta potential of *C. hirundinella* cells and pH values of filtered source water collected from Benoni Lake. The concentrations of *C. hirundinella* cells in filtered source water varied between 392-616 cells/ml with an average zeta potential and pH values of -13.38 and 8.79 respectively.

Figure 4.1 reports the changes in pH levels and zeta potential values when dosing different coagulant dosage ranges for $\text{Ca}(\text{OH})_2\text{-SiO}_2$, $\text{Ca}(\text{OH})_2$ -organic polymer and organic polymer. The average zeta potential and pH values for the five different sampling occasions were reported for the complete jar test (six different dosage concentrations as shown in Chapter 3) were reported. The proposed zeta potential range required for good coagulation (-10 mV to +3 mV) is indicated by the dotted line in Figure 4.1 b and will be used to evaluate the efficacy of coagulants to meet optimum coagulation conditions. After simulating the conventional water treatment processes the initial zeta potential values of *C. hirundinella* cells and pH values of filtered source water either changed significantly or remained unchanged. The zeta potential measured may remain unchanged until the treated water is stabilised.

Table 4.1

The zeta potential of *C. hirundinella* cells, pH and cell concentrations recorded during five sampling occasions in source water collected from the Benoni Lake (Sampling 11 January – 8 February 2012).

Sampling occasions	zeta potential (mV)	pH	<i>C. hirundinella</i> (cells/ml)
1	-12.43	8.63	392
2	-9.67	8.95	594
3	-14.90	8.82	538
4	-14.83	8.75	515
5	-15.05	8.81	616
Average values	-13.38	8.79	531

Chapter 4

An investigation into the zeta potential changes of Ceratium hirundinella cells when simulating conventional coagulation using inorganic and organic coagulants

The pH measurements showed a significant increase at the lowest Ca(OH)_2 dosage in combination with SiO_2 from pH 8.79 (control) to pH levels above 10.50, where-after an increasing pH trend was recorded as a result of increased coagulant dosages (Figure 4.1 a). When dosing Ca(OH)_2 in combination with SiO_2 , the lowest Ca(OH)_2 dosage of 60 mg/l caused the initial zeta potential to decrease (Figure 4.1 b; from -13.38 mV to -16.71 mV). However, the subsequent increasing Ca(OH)_2 dosages showed an increasing trend in zeta potential, but it remained below the proposed zeta potential range required for good coagulation conditions.

Increased pH levels ($> \text{pH } 9.70$) were recorded for the lowest organic polymer dosage which can be ascribed to the low constant Ca(OH)_2 dosage. The organic polymer dosage of 4 mg/l in combination with Ca(OH)_2 (10 mg/l) kept the initial zeta potential of -13.38 mV relatively constant at a value of -13.57 mV, but an increase in zeta potential values were recorded when organic polymer dosages were increased. Zeta potential values for organic polymer (4-14 mg/l) in combination with Ca(OH)_2 (10 mg/l) were mostly within the proposed zeta potential range required for good coagulation conditions (Figure 4.1 b).

Organic polymer (4-14 mg/l) treatment without low Ca(OH)_2 dosages (10 mg/l) caused no pH adjustments, since pH levels remained similar to the control (Figure 4.1a). The zeta potential value range was closer to 0 mV (-6.64 mV to +4.90 mV) than what was previously observed for Ca(OH)_2 -organic polymer range that was recorded between -13.57 mV and +1.00 mV as illustrated Figures 4.1b.

ANOVA results indicates that after treating source water containing *C. hirundinella* cells with various coagulant ranges, the average pH values ($p = < 0.0001$) and zeta potential values ($p = < 0.0001$) were significantly different (Figure 4.1 a-b).

An investigation into the zeta potential changes of *Ceratum hirundinella* cells when simulating conventional coagulation using inorganic and organic coagulants

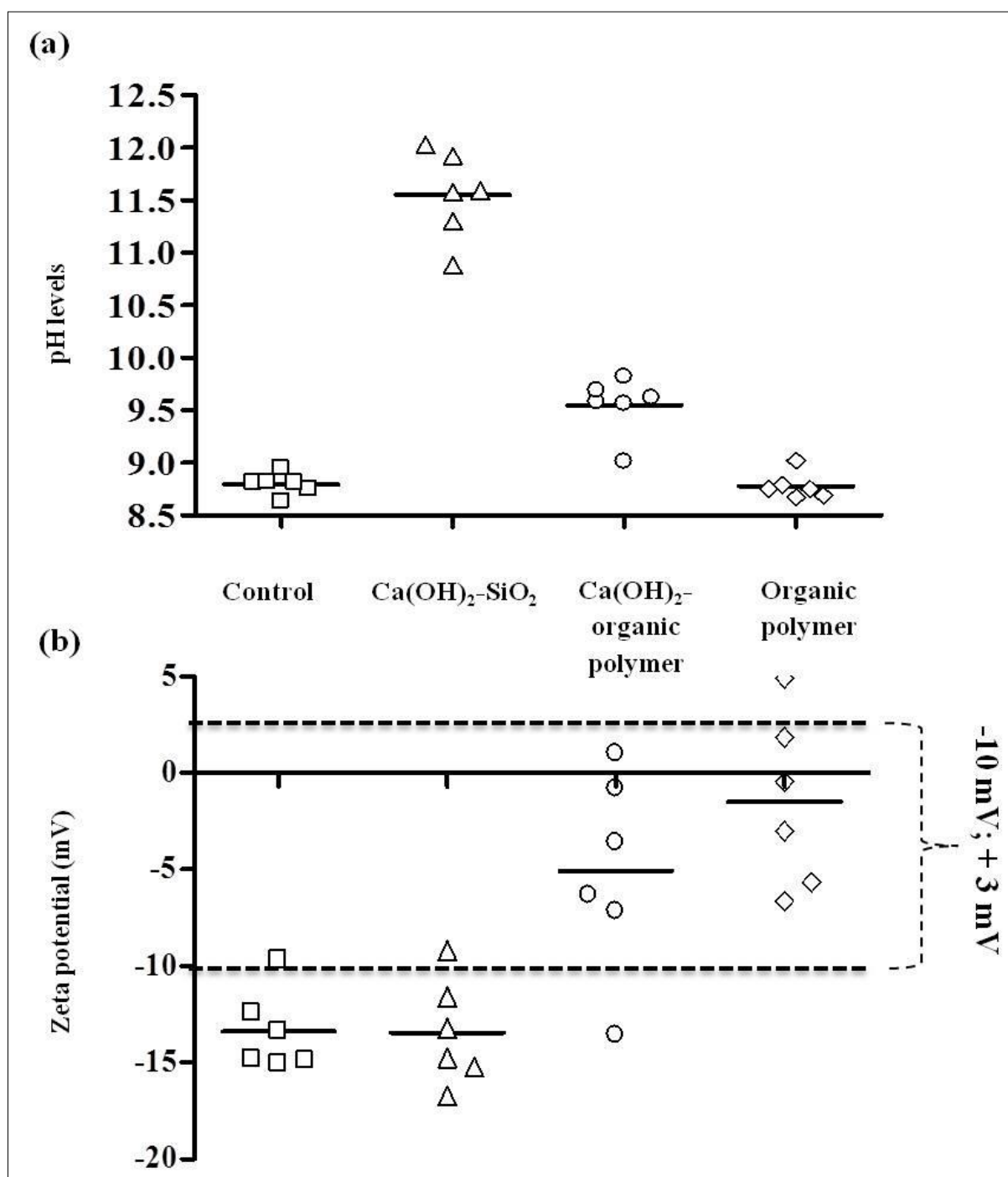


Figure 4.1

The comparison between pH levels and zeta potential values after treating the filtered same source water (control) containing *C. hirundinella* cells with the following coagulants: $\text{Ca(OH)}_2\text{-SiO}_2$, $\text{Ca(OH)}_2\text{-organic polymer}$ and organic polymer. Dotted lines indicate the zeta potential range required for good coagulation.

Chapter 4

An investigation into the zeta potential changes of Ceratium hirundinella cells when simulating conventional coagulation using inorganic and organic coagulants

When dosing $\text{Ca}(\text{OH})_2$ in combination with SiO_2 and $\text{Ca}(\text{OH})_2$ in combination organic polymer, the pH was increased from 8.79 to 11.5 and 9.5 respectively, while organic polymer alone was not able to change pH of the filtered source water (Figure 4.1 a). Although the pH levels were significantly increased by $\text{Ca}(\text{OH})_2$ - SiO_2 treatment, the zeta potential values remained constant when compared to the zeta potential values (mostly ≤ -10 mV) recorded in the source water (Figure 4.1 b). On the other hand, both $\text{Ca}(\text{OH})_2$ -organic polymer and organic polymer exhibited changes in the zeta potential values from ≤ -10 mV to ≥ -5 mV (Figure 4.1 b). It has become clear from the results that $\text{Ca}(\text{OH})_2$ - SiO_2 did not influence the zeta potential and organic polymer alone was not able to influence the pH, but $\text{Ca}(\text{OH})_2$ -organic polymer was able to influence both zeta potential and pH to achieve more effective coagulation conditions.

4.3.2 Using a constant established coagulant dose to treat source water enriched with different *Ceratium hirundinella* cell concentrations and with different settling times

The effects of different *C. hirundinella* cell concentrations and settling times on the use of established coagulant dosage (commonly used in a water treatment plant) were investigated. Table 4.2 gives the initial zeta potential of *C. hirundinella* cells and pH values of filtered source water containing different *C. hirundinella* cell concentrations. The initial zeta potential values varied between -13 mV and -21 mV, while the pH values varied between 6.97 and 7.34. Zeta potential values recorded in Table 4.2 for *C. hirundinella* cell concentrations (≥ 2000 cells/mL) in filtered source water were slightly more negative when compared to previous results Table 4.1. The zeta potential changes recorded in Tables 4.1 and 4.2 may be ascribed to the growth phase of *C. hirundinella* cells together with extracellular organic matter (EOM) in the filtered source water. The amount of organic polymer dose to be used available per single *C. hirundinella* cell was also determined and expressed as ng/cell (Table 4.2). For the different cell concentrations (2000-7000 cells/mL) the organic polymer dose per cell varied from 5.5 ng/cell to 1.5 ng/cell. Therefore, less coagulant becomes available as a result of increasing cell concentrations (Table 4.2).

Chapter 4

An investigation into the zeta potential changes of Ceratium hirundinella cells when simulating conventional coagulation using inorganic and organic coagulants

Table 4.2

The zeta potential and pH measurements ($n=5$) of the six pre-determined initial *C. hirundinella* concentrations that were used in simulated jar stirring test experiments with Ca(OH)_2 -organic polymer. The amount of organic polymer (0.011 mg/mL) per cell ranging from 5.5 ng/cell to 1.5 ng/cell is also reported.

Initial <i>C. hirundinella</i> concentrations (cells/mL)	2000	3000	4000	5000	6000	7000
Organic polymer available per cell (ng/cell)	5.5	3.6	2.7	2.2	1.8	1.5
zeta potential (mV)	-21	-21	-17	-13	-17	-19
pH	6.97	7.14	7.18	7.26	7.31	7.34

The percentage removal achieved after a settling time of 20 minutes (which is the appropriate settling time simulation of a treatment plant by means of a jar stirring test) varied from 82 % to 88 %, After 120 and 240 minutes the percentage removal was improved to percentages up to 93 % and 95 % respectively (Figure 4.2). The percentage removals recorded for different initial cell concentrations (2000-7000 cells/mL) after different settling times, especially after 240 minutes were similar (e.g. 94-95 %). Furthermore, irrespective of the initial cell concentrations or the organic polymer per cell, notable differences in percentage removal was only observed as a result of longer settling times. The difference in percentage removal recorded between 20 minutes and 240 minutes is more significant than what was recorded between 120 minutes and 240 minutes. Longer settling times were not used simulate plant settling times, but to evaluate what happen to the percentage removals when allowing longer settling times. A particle such as an immobilized *C. hirundinella* can spend up to 4 hours in a sedimentation tank.

Chapter 4

An investigation into the zeta potential changes of Ceratium hirundinella cells when simulating conventional coagulation using inorganic and organic coagulants

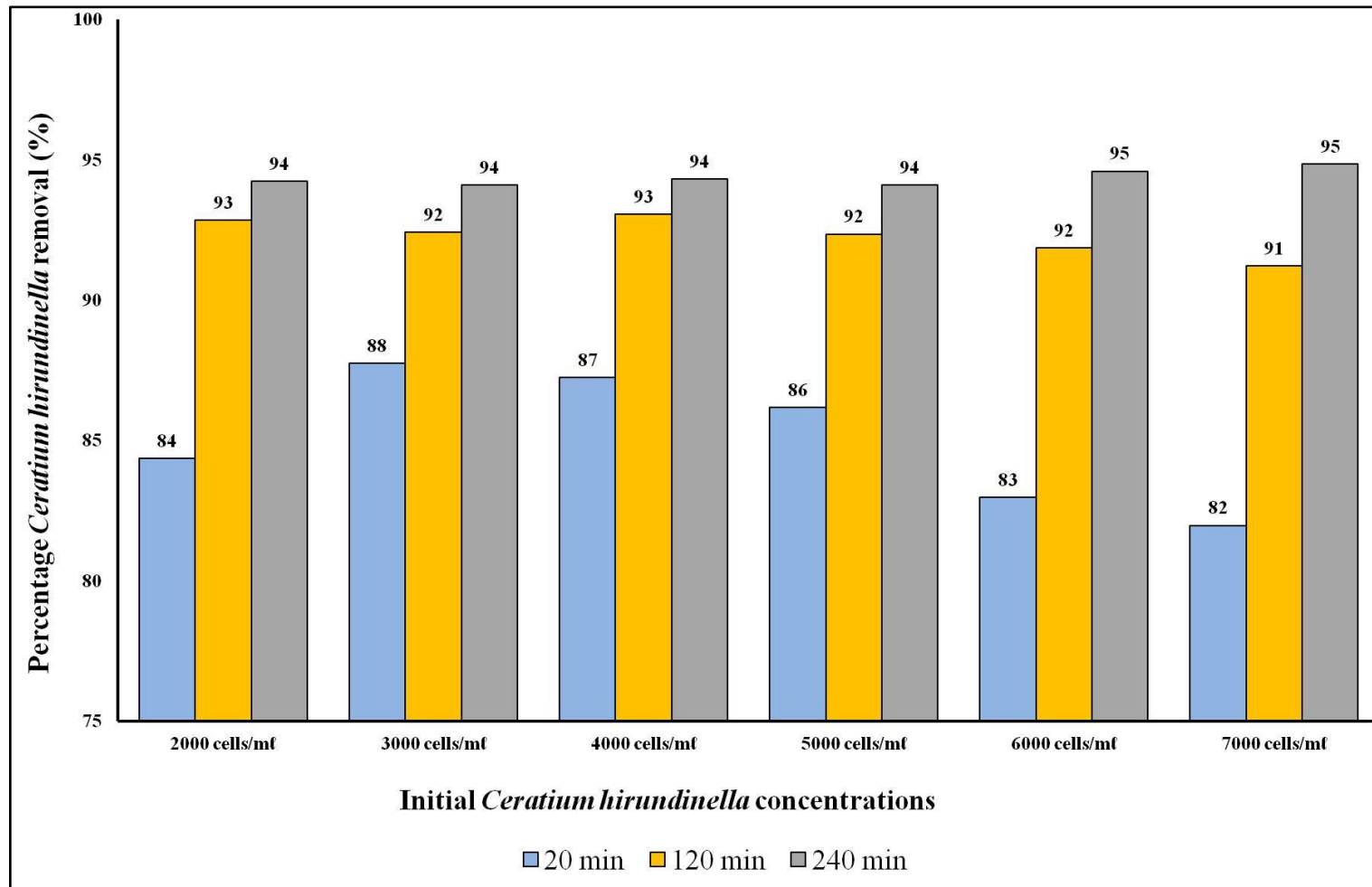


Figure 4.2

The number of *C. hirundinella* cells removed after settling times of 20 minutes (a), 2 hours (b) and 4 hours (c) with increasing organic polymer dosages (1-6) per cell concentrations (from 5.5 ng/cell to 1.5 ng/cell; $\text{Ca(OH)}_2 = 10 \text{ mg/l}$; Organic polymer = 11 mg/l).

Chapter 4

An investigation into the zeta potential changes of Ceratium hirundinella cells when simulating conventional coagulation using inorganic and organic coagulants

The following zeta potential values were recorded in source water containing different initial *C. hirundinella* cell concentrations from 2000 cells/ml to 7000 cells/ml (with increments of 1000): -21 mV, -21 mV, -17 mV, -13 mV, -17 mV and -19 mV respectively (Table 4.2). After a settling time of 20 minutes, the initial zeta potential values were increased to the proposed range of +3 mV to -10 mV required for good coagulation conditions. The best average zeta potential value (-3.28 mV) was recorded for source water with the highest initial *C. hirundinella* cell concentrations (Figure 4.3). After 240 minutes, the zeta potential values shown recovery trend, especially when higher initial *C. hirundinella* cell concentrations (6000-7000 cells/ml) occurred in the source water. An overall zeta potential recovery trend is illustrated as a result of longer settling times (Figure 4.3).

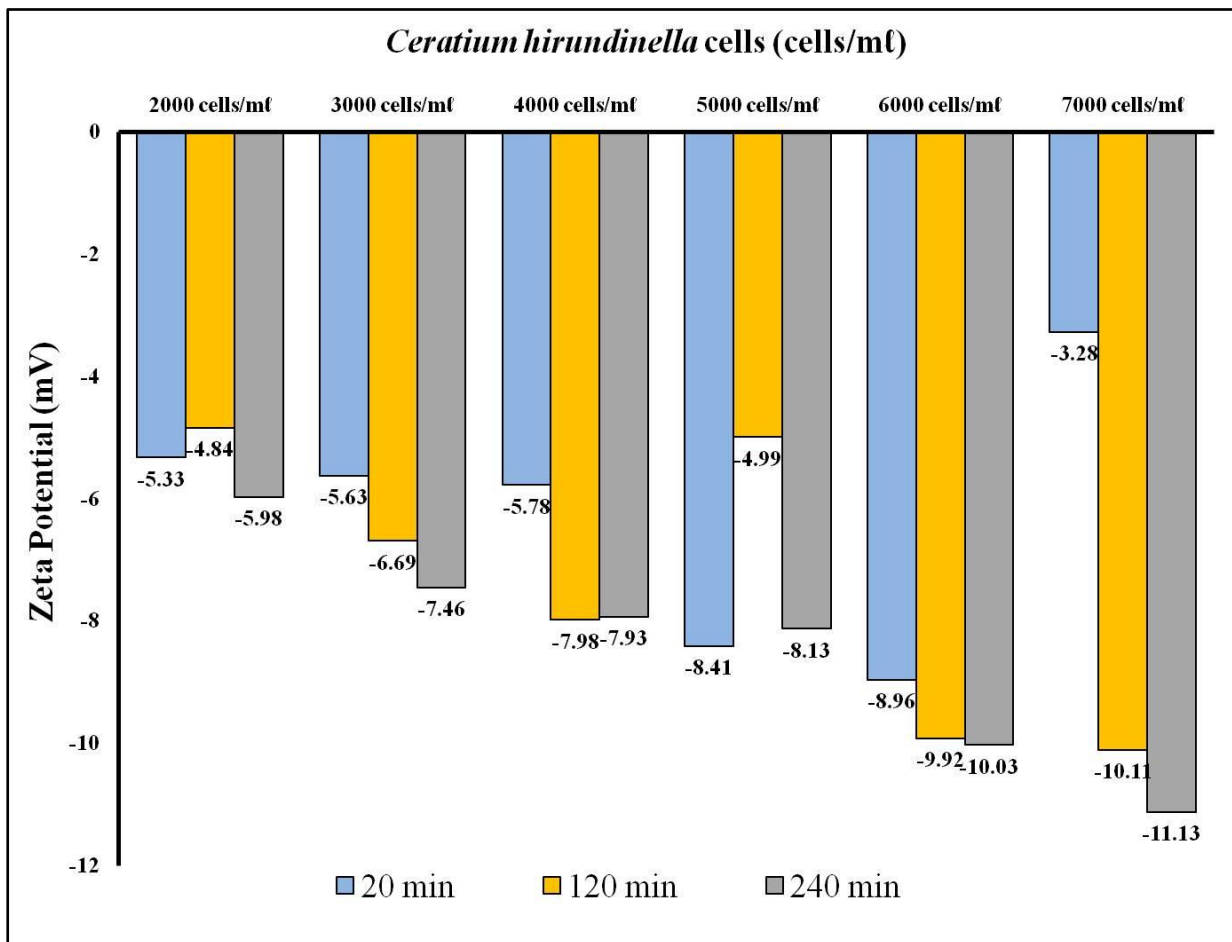


Figure 4.3

The changes in zeta potential after different settling times (20 minutes, 120 minutes and 240 minutes) when dosing the same coagulant dosages ($\text{Ca(OH)}_2 = 10 \text{ mg/l}$; Organic polymer = 11 mg/l) to treat water with increasing *C. hirundinella* cell concentrations

Chapter 4

An investigation into the zeta potential changes of Ceratium hirundinella cells when simulating conventional coagulation using inorganic and organic coagulants

Increasing *C. hirundinella* cell concentrations from 312 cells/mL to 1261 cells/mL were recorded in the supernatant with an increase in initial cell concentration and thus a decrease in the amount of flocculants per cell. The numbers of cells that remain in the supernatant after different settling times correspond with the percentage removal presented in Figure 4.2. However, when converting these percentages removed after 20 minutes to number of cells in the supernatant are high (close to 500 cells/mL). Figure 4.2 illustrates percentage removals of 93 % and 95 % after 120 minutes and 240 minutes respectively, which correspond with cell numbers below 500 cells/mL (Figure 4.4). The number of *C. hirundinella* cells in the supernatant is exposed to elevated pH levels (Figure 4.5) which will keep cells immobile. *C. hirundinella* cells in the supernatant may restore motility, depending on the chemical stabilization of water and the retention time of the sedimentation tanks. The retention time of horizontal sedimentation tanks of Rand Water's treatment plants is 4 hours.

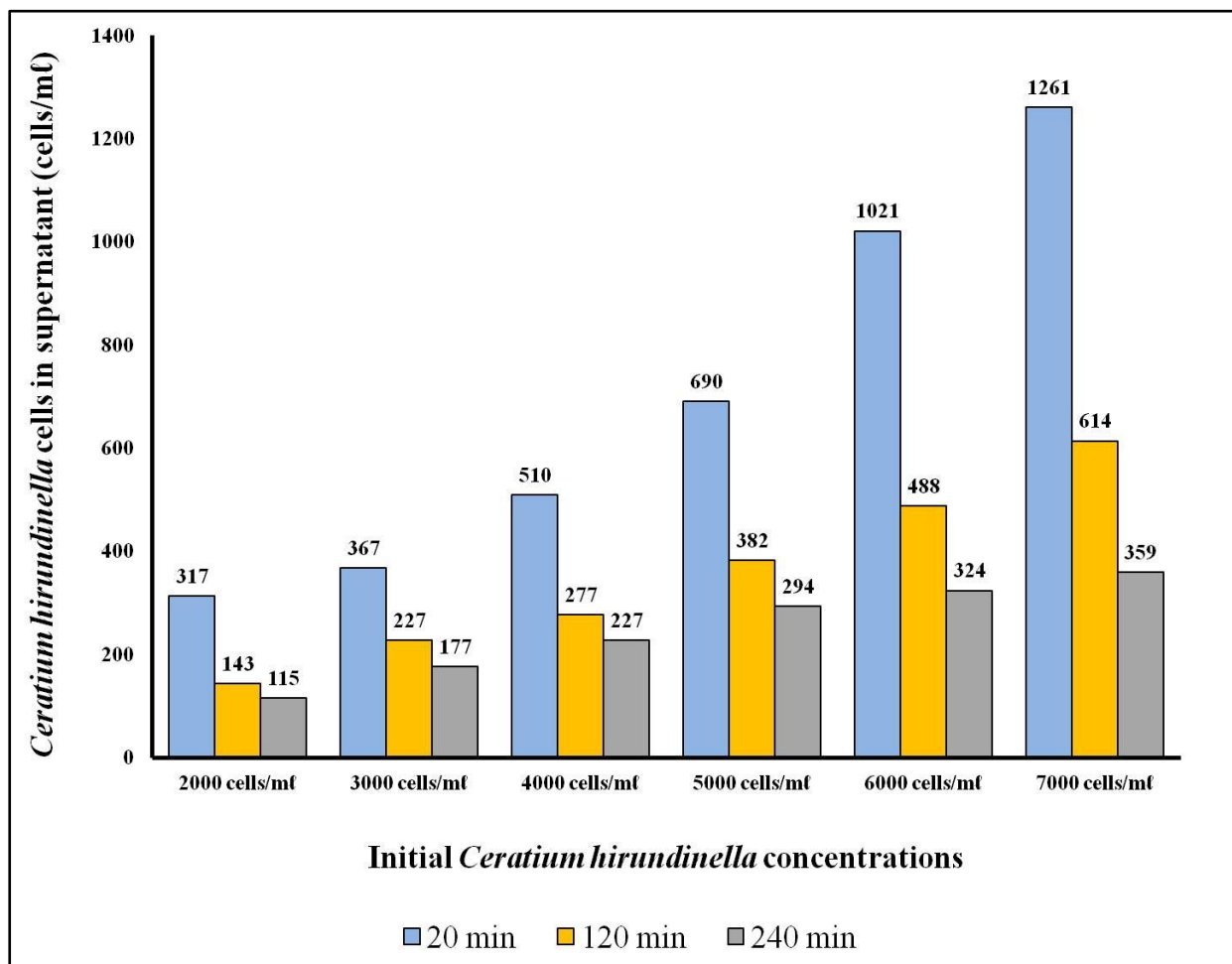


Figure 4.4

C. hirundinella cells in the supernatant after different settling times (20 minutes, 120 minutes and 240 minutes) when dosing the same coagulant dosages to treat water with increasing *C. hirundinella* cell concentrations

Chapter 4

*An investigation into the zeta potential changes of *Ceratium hirundinella* cells when simulating conventional coagulation using inorganic and organic coagulants*

The pH changing factor for source water with increasing initial cell concentration was constant at a dosage of 10 mg/l for $\text{Ca}(\text{OH})_2$. The pH levels recorded after a settling time of 20 minutes varied between pH 9.64 and pH 9.75, which were the highest pH levels. After 120 minutes and 240 minutes the following pH ranges were recorded respectively: pH 9.64–pH 9.70 and pH 9.59–pH 9.63. The pH changes measured after different settling times have an impact on the zeta potential changes on the surface area of *C. hirundinella* cells. The pH levels illustrated in Figure 4.5 below are showing a decreasing trend as a result of increasing *C. hirundinella* cell concentrations. Thus, it can be speculated that when pH is decreasing as a result of increasing initial *C. hirundinella* cell concentrations, the zeta potential measured on the surface of cells will become more negative or move out the proposed range required for good coagulation conditions (Figure 4.3). This speculation can be confirmed by the fact that less coagulant is available per cell at higher cell concentrations (e.g. 7000 cells/ml) when compared to coagulant availability at lower cell concentrations (e.g. 2000 cells/ml).

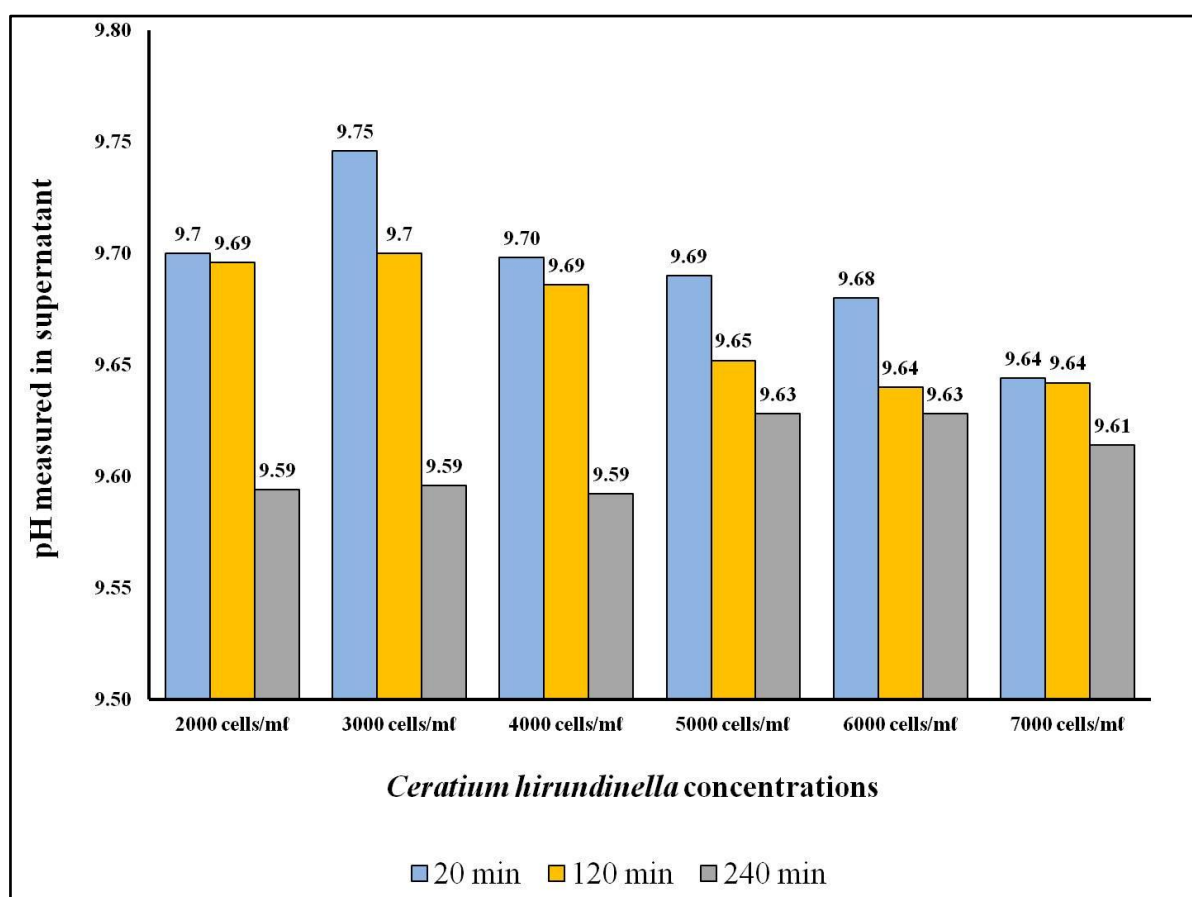


Figure 4.5

pH levels measured in the supernatant after different settling times (20 minutes, 120 minutes and 240 minutes) when dosing the same coagulant dosages ($\text{Ca}(\text{OH})_2 = 10 \text{ mg/l}$; Organic polymer = 11 mg/l) to treat water with increasing *C. hirundinella* cell concentrations

4.4 Discussion

During past decades the application of zeta potential, with specific emphasis on coagulation optimization strategies was successfully implemented (Jefferson *et al.*, 2004; Sharp *et al.*, 2005; Kwak *et al.*, 2006; Taki *et al.*, 2008). Blue-green algal species (Cyanophyceae) and green algae (Chlorophyceae) were mostly used during investigations to determine the effectiveness of coagulants and coagulant aids (Jun *et al.*, 2001; Yang *et al.*, 2005, Taki 2008). In this Chapter the focus was on *C. hirundinella* that belongs to the group Dinophyceae. Unlike cyanobacteria cells that are covered with membranes and in some cases slime sheaths, the relatively large sized *C. hirundinella* cells are covered with unique cell covering (theca plates) with different surface charge characteristics. In the light of these previous findings, there has been a renewed interest during this Chapter to investigate the coagulant dosages required for the removal of these relatively large algal cells, while taking into consideration the potential of these algal cells to restore their natural surface charge and then to break up flocs due to their ability to swim.

According to the author, in literature no records on investigations into the zeta potential or surface charge characteristics associated with freshwater dinoflagellates could be found. Previous studies on the occurrence of *C. hirundinella* in South African freshwater impoundments focussed mostly on ecological aspects and associated water quality parameters, giving limited knowledge on the impacts that *C. hirundinella* may have on the water treatment process. Therefore, little is known about the relationship between zeta potential of algae such as, *C. hirundinella* and optimization of coagulation conditions. When source water used for drinking water production contains relatively high concentrations (> 500 cells/mL) of algae, such as *C. hirundinella*, it is suggested that coagulants and dosages should be optimized. Coagulant optimization focuses on effective dosing strategies to enhance the removal of algal cells during coagulation and flocculation. Amongst other mechanisms, the surface charge of algae referred to as zeta potential plays an important role during coagulation and flocculation (Knappe *et al.*, 2004; Sharp *et al.*, 2005). This Chapter investigated the effects of coagulants (that were optimized in Chapter 3) and pH on the zeta potential of *C. hirundinella* cells.

The zeta potential values recorded for *C. hirundinella* in filtered source water varied between -9.67 mV and -15.05 mV with an average value of -13.38 mV. The zeta potential on algal cells of the same genus may vary in response to the growth stage and other environmental factors (Knappe *et al.*, 2004). However, the zeta potential and pH values recorded during this Chapter may also be different in comparison to natural values due to sample preparations under laboratory conditions.

Chapter 4

An investigation into the zeta potential changes of Ceratium hirundinella cells when simulating conventional coagulation using inorganic and organic coagulants

The best coagulation conditions based on zeta potential values were reported to range from -10 mV to + 3 mV (Jefferson *et al.*, 2004 and Eikkebrokk *et al.*, 2006). Sharp *et al.* (2005) also suggested an operating window of -10 mV to +5 mV for good coagulation conditions of algae. For the purpose of this Chapter, the zeta potential range for good coagulation was considered to be between -10 mV and + 3 mV. The range (-10 mV to + 3 mV), as opposed to a single value (e.g. 0 mV) suggests that in general, colloidal destabilization occurs before complete charge neutralization of surface charge takes place (Ratnaweera *et al.*, 1999). This means that good coagulation conditions are still possible although the zeta potential may not necessarily be zero (Ratnaweera *et al.*, 1999).

High Ca(OH)_2 dosages (e.g. ranging between 60-160 mg/l) have proved to increase the pH of source water significantly (> pH 11.5 after sedimentation), while lower constant dosages of Ca(OH)_2 in combination with organic polymer increased the pH to a more moderate level (> pH 9.5). The organic polymer alone did not have an impact on the pH levels. During the same treatments no changes in zeta potential occurred for $\text{Ca(OH)}_2\text{-SiO}_2$. By contrast the zeta potential values are increased when dosing either with low Ca(OH)_2 dosages in combination with organic polymer or organic polymer alone.

The adjustment of pH by adding among others Ca(OH)_2 , NaOH and Na_2CO_3 promotes alkalinity which will affect not only the buffer capacity of the solution but decreases the zeta potential values (Lohtaja, 2005). Although high dosages of Ca(OH)_2 treatments were not always beneficial to increase the zeta potential in this Chapter to meet the range required for good coagulation, the pH adjustments or alkalinity provided good conditions to address challenges with biological activity during water treatment (e.g. pH shock as described in Chapter 3). Algae, such as *C. hirundinella* exude large quantities of organic compounds that are collectively referred to as extracellular organic matter (EOM) (Knappe *et al.*, 2004). When dosing Ca(OH)_2 the interactions between Ca^{2+} ions and deprotonated EOM at high pH (e.g. > pH 11.5 in current Chapter) and can reduce the interference of EOM on coagulation by reducing negative charge density of EOM (Bernhardt *et al.*, 1986).

The organic polymer achieved a zeta potential range that was closer to 0 mV with no pH adjustments. A pre-determined appropriate Ca(OH)_2 -organic polymer dosage (10 mg/l-11 mg/l) based on turbidity (≤ 5 NTU) as indicator was used to further investigate the effect of different cell concentrations and settling times on zeta potential, pH changes and *C. hirundinella* cell removal. Coagulants, such as cationic organic polymer are not able to provide alkaline conditions (no pH adjustments) which are often required for good coagulation conditions, especially for coagulation of organic material (Knappe *et al.*, 2004). In Chapter 3 it was also found that the cationic organic polymer achieved good turbidity removal, but failed to remove *C. hirundinella* cells.

Chapter 4

An investigation into the zeta potential changes of Ceratium hirundinella cells when simulating conventional coagulation using inorganic and organic coagulants

The combination of low Ca(OH)_2 and organic polymer have an impact on both the pH (> 9.5) and zeta potential (-6.64 mV to $+4.90$ mV). Other studies have also shown that the use of Ca(OH)_2 -organic polymer is effective at simultaneously removing algae and turbidity after sedimentation (Van der Walt, 2012; Ewerts *et al.*, 2014). The changes in pH and zeta potential observed in this Chapter most likely explain why the Ca(OH)_2 -organic polymer treatment achieved the best coagulation conditions. Irrespective of the initial *C. hirundinella* concentrations in the source water, the pre-determined dosage was able to remove high percentages after 20 minutes (or 0 hours), 2 hours and 4 hours. Rand Water uses horizontal flow tanks with retention times of 4 hours and produces water with turbidity 5 NTU (Rand Water, 2015). Therefore, the time series simulated in this Chapter cover a range from 20 minutes, which simulate the plant retention under laboratory conditions. The time series of 2-4 hours was simulated to allow recovery of metabolic activity of *C. hirundinella* cells to subsequently assist cells in recovering its surface charge. The surface charge of cells remain unchanged or recover slowly if water is not chemically stable or elevated pH levels are not reduced.

Percentage removal of *C. hirundinella* cells can improve from 82-88 % after a settling time of 20 minutes to 91-93 % and 94-95 % after settling times of 120 minutes and 240 minutes, respectively. Chapter 3 reported similar percentage removals of *C. hirundinella* cells when using the aforementioned coagulant option. When percentage removal achieved after 20 minutes, especially at higher initial cell concentrations are converted to actual cell numbers, water treatment problems may occur due to relatively high cell concentrations carried over to sand filters. *C. hirundinella* concentrations in the supernatant decrease after a settling time of 120 minutes to concentrations below 500 cells/mL. Therefore, in water treatment plants where *C. hirundinella* cells are causing sand filter problems such as clogging or taste and odours, the settling times should be adjusted to allow effective settling of cells. A time series investigation (20 – 240 minutes) has also shown that zeta potential values has remained within the proposed range of -10 mV and $+3$ mV required for good coagulation. There appears to be a recovery in zeta potential observed over time when there are lower amounts of coagulant is available per cell. Changes in zeta potential of *C. hirundinella* cells can be ascribed to changes in cell concentrations when coagulant dose is not adjusted accordingly. It can therefore be expected that when high initial *C. hirundinella* concentrations occur in source water used for drinking water production, higher cell concentrations will occur in the supernatant after sedimentation.

The pH values measured after 20 minutes for increasing *C. hirundinella* cell concentrations are expected to be similar due to the constant Ca(OH)_2 dosages; however, the differences in zeta potential can be ascribed to charge density as a result of increasing cell concentrations. When dosing Ca(OH)_2 , elevated pH levels ($> \text{pH } 9$) occur after 20 minutes with a decreasing trend as a result of

Chapter 4

An investigation into the zeta potential changes of Ceratium hirundinella cells when simulating conventional coagulation using inorganic and organic coagulants

longer settling times. Negatively charged *C. hirundinella* cells in source water become more positive after dosing $\text{Ca}(\text{OH})_2$ in combination with the organic polymer. The appropriate zeta potential range for good coagulation conditions (+3 mV and -10 mV) may change as a result of long settling times and stabilisation. When *C. hirundinella* cells restore the surface charge, cells may break out from flocs. Rand Water uses carbonation bays where carbon dioxide gas is bubbled into the water to stabilize the water.

Bernhardt *et al.* (1986) stated that when algae concentrations in source water fluctuate, the concentrations of negative charges in the source water and the coagulant dose required neutralizing these charges can vary rapidly. An investigation conducted by Henderson *et al.* (2008) have shown that zeta potential control (during coagulation) of the following algae; *Asterionella formosa*, *Melosira* sp., *Microcystis aeruginosa*, and *Chlorella vulgaris* achieved optimum removal when the zeta potential on the surface of cells was reduced to between -8 mV and +2 mV, irrespective of the coagulant dosages or pH changes. Gerde *et al.* (2014) also investigated the zeta potential control of *Scenedesmus* spp., *Chlamydomonas reinhardtii* and *Schizochytrium limacinum* when dosing various cationic coagulants and stated that further research is required investigate more thoroughly how coagulants (or flocculants) changes pH and surface zeta potential of algal cells to obtain more insights on the flocculation mechanisms involved.

It has become evident from this research that good coagulation conditions can be achieved within a specific zeta potential range (as proposed previously) similar to the zeta potential values reported by Henderson *et al.* (2008). Furthermore, it was also shown during this Chapter that the same coagulant dosage can be used to control the zeta potential to achieve optimum coagulation conditions for wide algal cell concentration range; however, better coagulation occurred when sufficient coagulant is available per cell. Thus, the effective coagulant dosage provided sufficient adsorption neutralization mechanisms required to neutralize negative surface charges of *C. hirundinella* cells. The impacts of various coagulants on the morphology (including surface and structure) during coagulation and flocculation will be investigated in Chapter 5.

4.5 Conclusions

The application of zeta potential as a tool to determine coagulant dosages to improve coagulation conditions during water treatment is well established for the groups Cyanophyceae and Chlorophyceae. The genera *Micocystis*, *Synechococcus* and *Planktothrix* contain different surface characteristics when compared to *Ceratium* belonging to the group Dinophyceae. The freshwater dinoflagellate *C. hirundinella* used during this research possesses unique characteristics such as motile structures that contribute to removal difficulties. The surface charge on algae such as *C. hirundinella* cells (zeta potential) play a significant role during the water treatment processes coagulation. Irrespective of surface characteristics and subsequently the surface charge associated with different phytoplankton genera, the zeta potential operation range for good coagulation remain constant (proposed range of -10 mV to +3 mV) during water treatment. The cell concentrations in source water used for the production of drinking water have an impact on the efficacy coagulation conditions. When *C. hirundinella* cells are present in source water due for the production of drinking water, monitoring strategies from source water to drinking water should be implemented. Therefore, the coagulant used to achieve sufficient charge neutralization and destabilization should be optimized to ensure the formation of strong flocs, irrespective of the zeta potential (whether positively or negatively charged) on the suspended material and algae in the water. Algal cells, such as those of *C. hirundinella* are negatively charged in natural source water. The zeta potential of cells can vary between -13 mV and -21 mV depending on the growth stage. When treating source waters enriched with motile *C. hirundinella* cells, the objective is to render these cells immobile followed by coagulation of the cells. A combination of low Ca(OH)_2 dosages and the organic polymer achieved the best coagulation conditions based on zeta potential and pH requirements. When *C. hirundinella* concentrations increase and coagulant dosages are not adjusted accordingly, more metabolically active cells will remain in the supernatant that are able to restore their natural zeta potential after 2-4 hours after stabilization. The zeta potential of algae can therefore be investigated, especially during floc formation or after sedimentation to determine the effectiveness of coagulants.

Chapter 5

Physical and Chemical Impacts of Conventional Unit Processes and Coagulants on the Dinoflagellate, *Ceratium hirundinella*

5.1 Introduction

Dinoflagellates are algae, belonging to the phylum Pyrrophyta and the class Dinophyceae (Wetzel, 2001). Dinoflagellates are considered as an intriguing planktonic algal group for numerous reasons, including their ability to luminesce and produce neuro-toxins (Harland, 1988). Members of the group are often covered with armour-like cellulose plates within the cell membrane. The presence of these thecal plates differentiates dinoflagellates from other algal groups (Canter-Lund and Lund, 1995). The movement of dinoflagellates is accomplished by two flagella implanted in grooves on the surface (Canter-Lund and Lund, 1995; Janse van Vuuren *et al.*, 2006). Dinoflagellates are common in freshwater, but the greatest diversity are found in marine environments (Canter-Lund and Lund, 1995). In the marine environment, dinoflagellates (e.g. *Gymnodinium*, *Gonyaulax*) are responsible for the so called “toxic red tides” that may result in the death of marine life as well as humans that consume contaminated marine animals (Canter-Lund and Lund, 1995). Though no freshwater “toxic red tide blooms” have been reported, when freshwater genera (e.g. *Ceratium*, *Peridinium*) proliferate their blooms produce a brown discoloration of the affected water.

The structure of the freshwater dinoflagellate, *Ceratium hirundinella* is shown in Figure 5.1. Due to its distinct shape, the genus can be readily identified (Janse van Vuuren *et al.*, 2006). *C. hirundinella* cells are broadly or narrowly spindle-shaped, strongly dorsiventrally flattened with one anterior and three posterior horns when swimming (Wetzel, 2001; John *et al.*, 2002; Janse van Vuuren *et al.*, 2006). The anterior horn develops from the epitheca, while the posterior horns (two post-cingular horns and one antapical horn) develop from the hypotheca. Theca plates arrangements for the freshwater species *C. hirundinella*; therefore, the plate formulate is used for identification purposes (John *et al.*, 2002).

Morphological variability in dinoflagellates is described by Taylor (1987) and has been correlated to buoyancy and cell resistance to sinking (Wetzel, 2001 and Hamlaoui *et al.*, 1998). Members found in this group (especially *Ceratium* species) may undergo seasonal changes known as poly- or cyclomorphism to enlarge the surface area of cells (Wetzel, 2001, Hamlaoui *et al.*, 1998 and Gligora *et al.*, 2003). Poly- or cyclomorphism can be recognized by lengthening of the horns as the temperature increases during warmer summer months and shortening during winter (Wetzel, 2001).

These adaptive changes are significant to *C. hirundinella* as a mechanism to reduce the sinking rate from the euphotic zone (Wetzel, 2001).

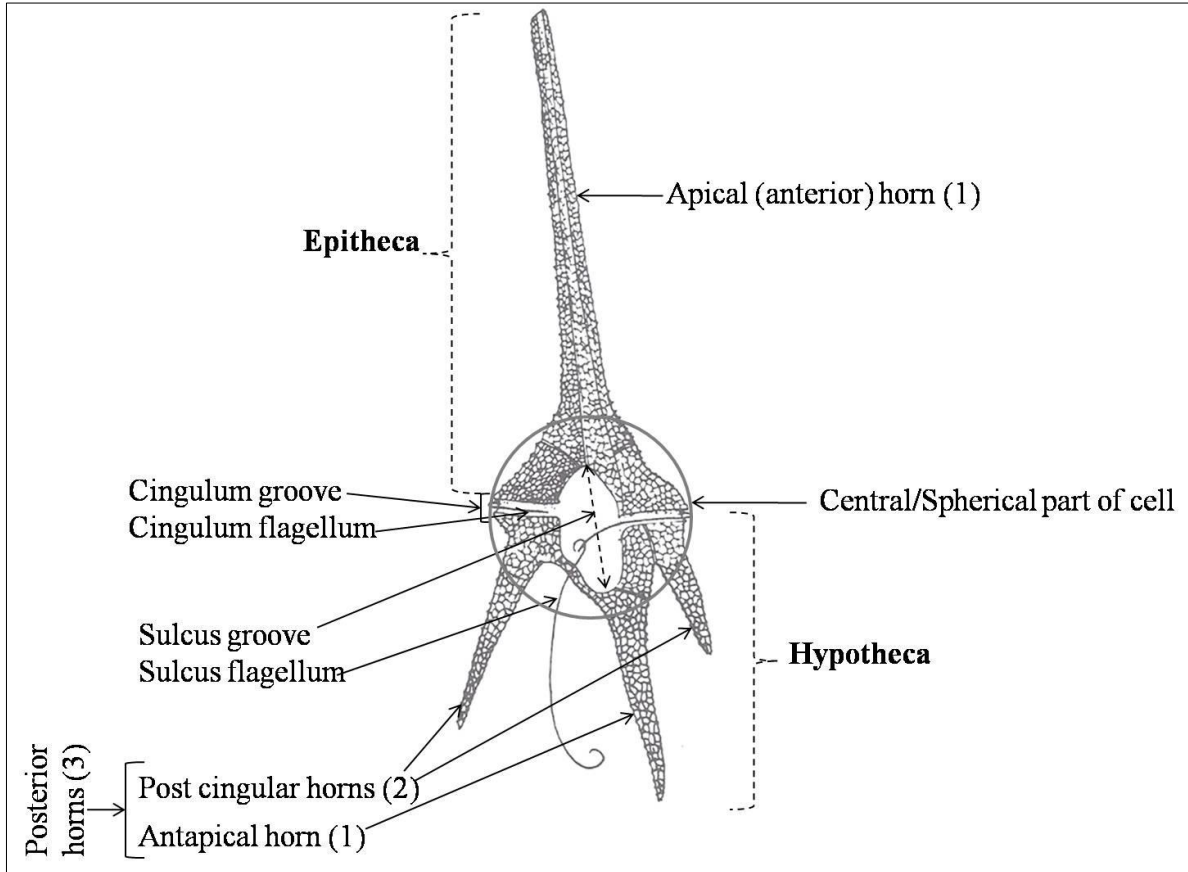


Figure 5.1

Line diagram representing the structure of the freshwater dinoflagellate, *C. hirundinella* (adapted from Prescott *et al.*, 1978)

The morphology and surface characteristics of *C. hirundinella* are complex and may interfere with coagulation. During the water treatment process, appendages to cells reduce contact between the cells, which is necessary for successful agglomeration during floc formation (Knappe *et al.*, 2004). Furthermore, dinoflagellate cells can clog sand filters and produce taste and odour compounds (Knappe *et al.*, 2004 and Swanepoel *et al.*, 2008 a). In addition, the presence of advanced motile structures causes several challenges during conventional water treatment, because of its ability to disrupt coagulation and flocculation and avoid sedimentation (Swanepoel *et al.*, 2008 a). As a result it has become evident that *C. hirundinella* cells may penetrate through potable water treatment systems where they are responsible for high concentrations of organic compounds (Swanepoel *et al.*, 2008 a).

Chapter 5

Physical and Chemical Impacts of Conventional unit Processes and Coagulants on the Dinoflagellate, Ceratium hirundinella

According to Palmer (1980) the control of such algae during water treatment requires optimized unit processes. However, well regulated coagulation with sedimentation will often remove 90 percent of algae and in some cases 95-96 percent removal has been reported (Palmer, 1980). *C. hirundinella* can avoid coagulation, flocculation and sedimentation when coagulants dosed are not appropriate to promote the removal of these robust cells (Swanepoel *et al.*, 2008 a and Van der Walt, 2012). Henderson *et al.* (2008) reported that the surface area of algal cells is a useful preliminary indicator of coagulant dose required for optimum (appropriate) cell removal. Furthermore, the physical and chemical aspects involved during conventional water treatment can cause major damage to cells, which can clearly be observed during scanning electron microscopy (SEM) investigations. The aims of this Chapter were to: 1) investigate the physical and chemical impacts of coagulation, flocculation and sedimentation on the morphology of *C. hirundinella* cells and 2) describe the physical appearance of flocs containing *C. hirundinella* cells.

5.2 Materials and Methods

5.2.1 Source water sampling and water treatment simulations

Source water samples enriched with *C. hirundinella* cells collected from Benoni Lake, South Africa (as described in Chapter 3) were used to simulate conventional water treatment processes using the jar test apparatus (Phipps and Bird Model) under laboratory conditions ($\pm 22^{\circ}\text{C}$).

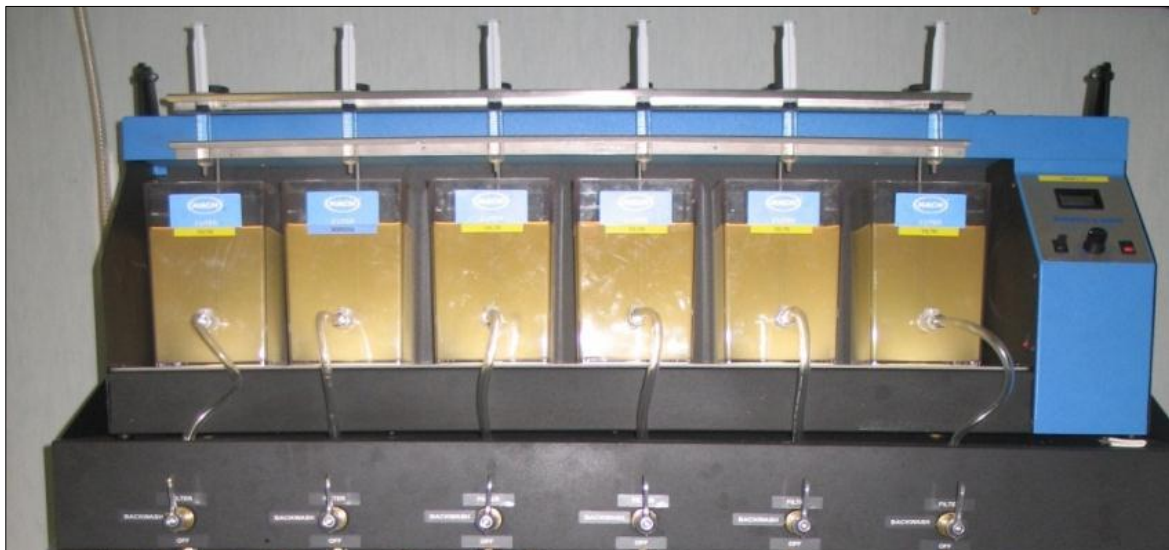


Figure 5.2

The six paddle Phipps and Bird jar stirring test apparatus (Model 7790-704) with six 2 ℓ beakers.

Hydrated lime ($\text{Ca}(\text{OH})_2$), activated silica (SiO_2) and organic polymer stock solutions were prepared (as described in Chapter 3) similar to what is used in a water treatment plant. The dosage ranges for various coagulants were as follows:

- $\text{Ca}(\text{OH})_2$ - SiO_2 treatment: $\text{Ca}(\text{OH})_2$ dosages ranged from 60 to 160 mg/l (with increments of 20). SiO_2 dosages of 4 mg/l were dosed as a coagulant aid;
- $\text{Ca}(\text{OH})_2$ -organic polymer treatment: organic polymer dosages ranged from 4 to 14 mg/l (with increments of 2). $\text{Ca}(\text{OH})_2$ dosages of 10 mg/l were added as a coagulants aid; and
- Organic polymer treatment: dosages ranged from 4 to 14 mg/l (with increments of 2).

5.2.2 Sampling and preparation of biological material for scanning electron microscopy investigations

After conducting jar stirring tests using various coagulants, flocs that settled for 20 minutes were collected to perform SEM investigations. *C. hirundinella* cells collected from source water and floc samples formed during jar stirring tests were primarily preserved with formaldehyde solution (35 %) by adding 200 μl of preservative to 10 ml of floc-sample. After washing in cacodilate buffer and distilled water, samples were dehydrated in an ethanol series of 80 %, 90 % and 2 x 100 % ethanol for 15 -30 minutes each. Samples were critical-point dried with CO_2 and mounted on SEM stubs with double-sided carbon tape (coated with copper).

A FEI QUANTA 250 FEG scanning electron microscope was used to investigate the morphology (characteristics and structure) of *C. hirundinella* cells in source water as well as in the flocs. The surface images (electron micrographs) were captured at 7.30kV and 10.00 kV.

5.3 Results

5.3.1 The physical appearance of *C. hirundinella* cells in source water and after flash mixing

SEM investigation revealed that *C. hirundinella* cells in source water were usually intact (Figure 5.3a). Living cells swim by means of two flagella that steer and rotate cells through the water column. These flagella are situated in the cingulum groove (Figure 5.3a) and sulcus groove (on the opposite side of the cell depicted in Figure 5.3a), respectively. The majority of the cell surface is covered with smooth thecal plates (Figure 5.3a), but spiny appendages were present on the anterior and posterior horns (Figures 5.3b and 5.3c), and the tips of the posterior horns were sharply pointed (Figure 5.3c). The physical appearance of *C. hirundinella* cells, as previously described, may be altered by both physical and chemical impacts.

Chapter 5

Physical and Chemical Impacts of Conventional unit Processes and Coagulants on the Dinoflagellate, Ceratium hirundinella

C. hirundinella cells were first exposed to the physical impacts of flash mixing, the stage when coagulants were added and uniformly dispersed through source water. High energy flash mixing alone that forms an integral part of the conventional water treatment process, caused damage to some of the *C. hirundinella* cells (Figures 5.4a and 5.4c). After flash mixing alone, it has become evident that the physical appearance of some *C. hirundinella* cells had changed. Organic material released from the vertical sulcus groove situated below the horizontal cingulum groove (Figures 5.4 and 5.4 b) was observed. It can, therefore be concluded that the connections between thecal plates in the sulcus groove are weaker than those on the rest of the cell surface, since most of the organic material was released through the sulcus groove. Organic material extruded by the cells remained attached to the cell body and it can therefore be considered as a minor impact on water quality if subsequent processes remove cells together with uncovered organic material. However, subsequent unit processes may cause major problems with respect to water treatment and water quality, when the disrupted cells release the uncovered organic material. It was also found that the process of flash mixing alone resulted in the breakage of several cells (illustrated in Figure 5.4c).

Chapter 5

Physical and Chemical Impacts of Conventional unit Processes and Coagulants on the Dinoflagellate, Ceratium hirundinella

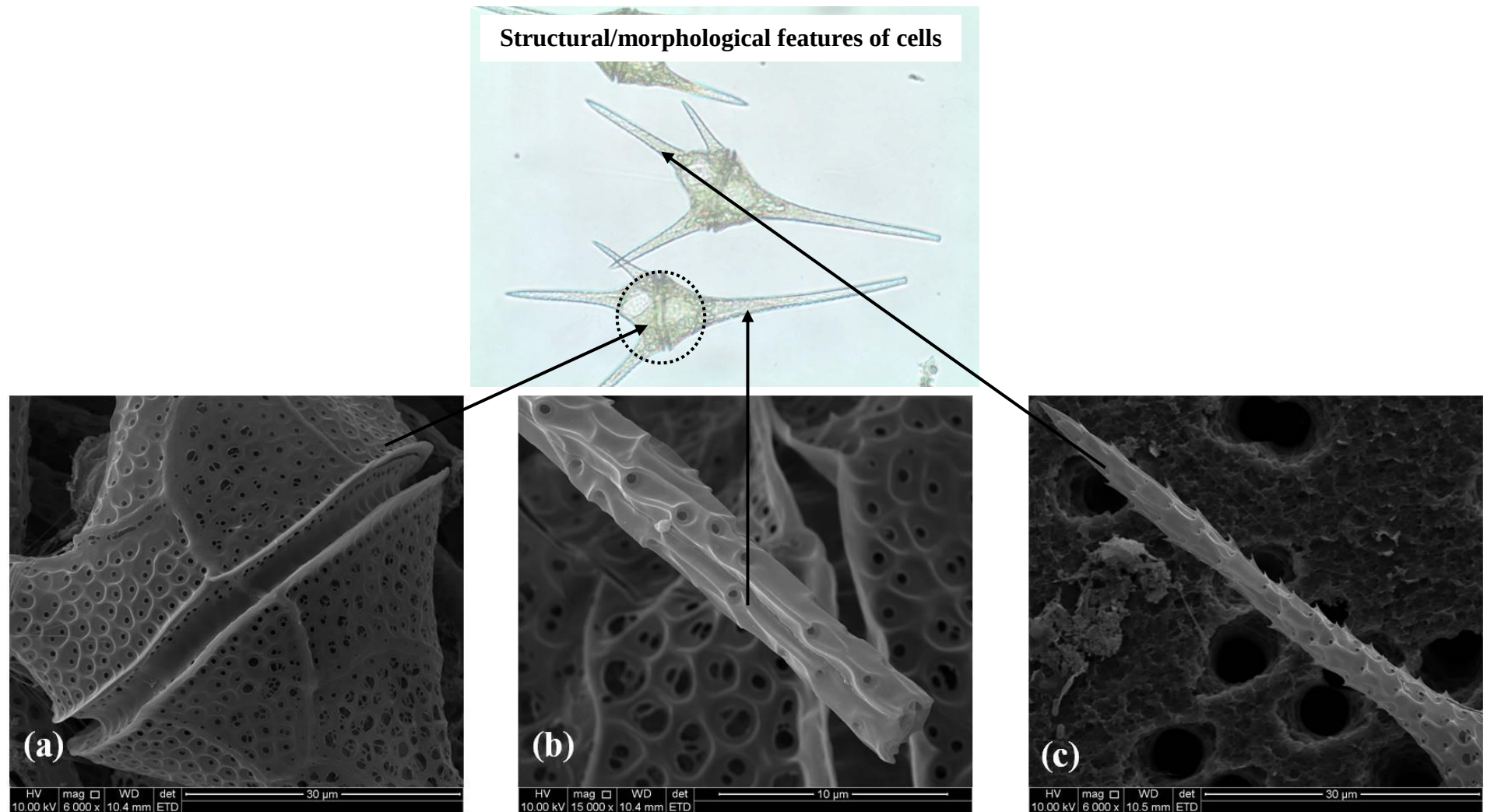


Figure 5.3

Dorsal view of *C. hirundinella* (a), the apical horn with flattened end (b) and antapical horn with pointed end (c).

Chapter 5

Physical and Chemical Impacts of Conventional unit Processes and Coagulants on the Dinoflagellate, Ceratium hirundinella

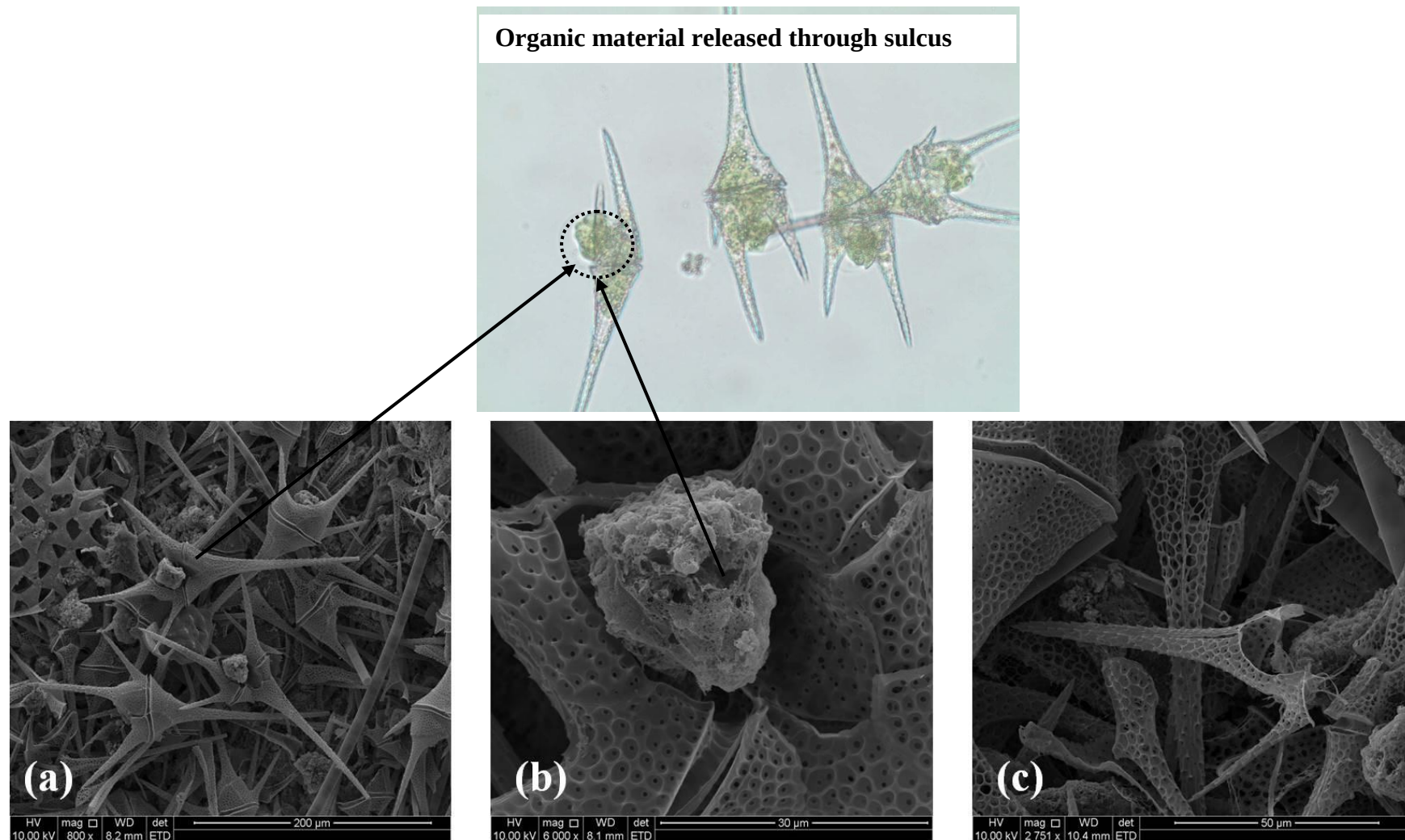


Figure 5.4

Ventral view of *C. hirundinella* (a) illustrating perforations in thecal plates and organic material released in the sulcus area (b) and broken cells observed after flash mixing (c).

5.3.2 Hydrated lime (Ca(OH)_2) in combination with activated silica (SiO_2) treatment

Broken cells, with uncovered organic material attached to the sulcus area, became more fragile after flash mixing and subsequently fragile to chemical treatment, notably when the pH levels were increased by dosing hydrated lime as the primary coagulant. Ca(OH)_2 , in combination with SiO_2 , increased the pH levels in the source water from 7-8 to levels of 11-12. Visually larger flocs were formed compared to the floc sizes when other coagulants were used.

The flocs also appear denser, compared to flocs formed by other coagulants. Major damage was observed to *C. hirundinella* cells imbedded in Ca(OH)_2 - SiO_2 flocs (Figure 5.5a), notably to the spherical (central) parts of cells (Figure 5.5b) and bending of horns (Figure 5.5c). Although Ca(OH)_2 - SiO_2 caused major damage to *C. hirundinella* by breaking up the cells, it removed the largest amount of cells which is confirmed by TPP removal (as observed in Chapter 3 and Chapter 4). Cell lysis that occurs during this stage of water treatment can be limited by dosing lower amounts of Ca(OH)_2 that slightly increase the pH to levels between 8-9.

5.3.3 Ca(OH)_2 in combination with organic polymer treatment

Damage to *C. hirundinella* cells was limited after dosing lower Ca(OH)_2 dosages, which can be ascribed to moderate pH changes. SEM images revealed less damage to *C. hirundinella* cells embedded in flocs and cells that were removed by coagulation, flocculation and sedimentation with no further damage, since the organic material was still attached to cells in floc aggregation (Figures 5.6a – 5.6c). SEM images illustrate moderate impacts on the *C. hirundinella* cells when compared to cells observed in flocs that formed after Ca(OH)_2 - SiO_2 treatment under the same laboratory and jar stirring conditions.

Medium sized flocs were formed during Ca(OH)_2 -organic polymer treatment, when compared to Ca(OH)_2 - SiO_2 treatment (when large flocs formed) and organic polymer alone treatment (when small flocs formed). The flocs formed during this treatment appeared smooth to slightly dense and contained cells with damage that occurred only from high energy flash mixing. This observation was reached since cells were only observed to release—organic material through the sulcus grooves (Figures 5.6 a – 5.6 c). Relatively good *C. hirundinella* removal was confirmed by TPP measured in the supernatant after sedimentation (Chapter 3 and Chapter 4).

5.3.4 Organic polymer treatment

No further damage to *C. hirundinella* cells occurred as a result of dosing organic polymer alone and fewer cells were embedded in the smooth flocs that formed (Figure 5.7 c). This confirms the fact *C. hirundinella* cells remained motile in the supernatant and avoided removal by disrupting organic polymer floc formation.

Less dense flocs were formed when compared to flocs formed with other coagulant options (Figures 5.5 – 5.7). Only a few *C. hirundinella* cells were observed in the flocs that appeared smooth with large open areas between individual cells in aggregation. Poor floc formation is also revealed in Figure 5.7 c where *C. hirundinella* cells are lying apart from flocs. The poor removal of *C. hirundinella* cells by organic polymer is also confirmed by the highest TPP concentrations measured in the supernatant after sedimentation (Chapter 3 and Chapter 4).

Chapter 5

Physical and Chemical Impacts of Conventional unit Processes and Coagulants on the Dinoflagellate, Ceratium hirundinella

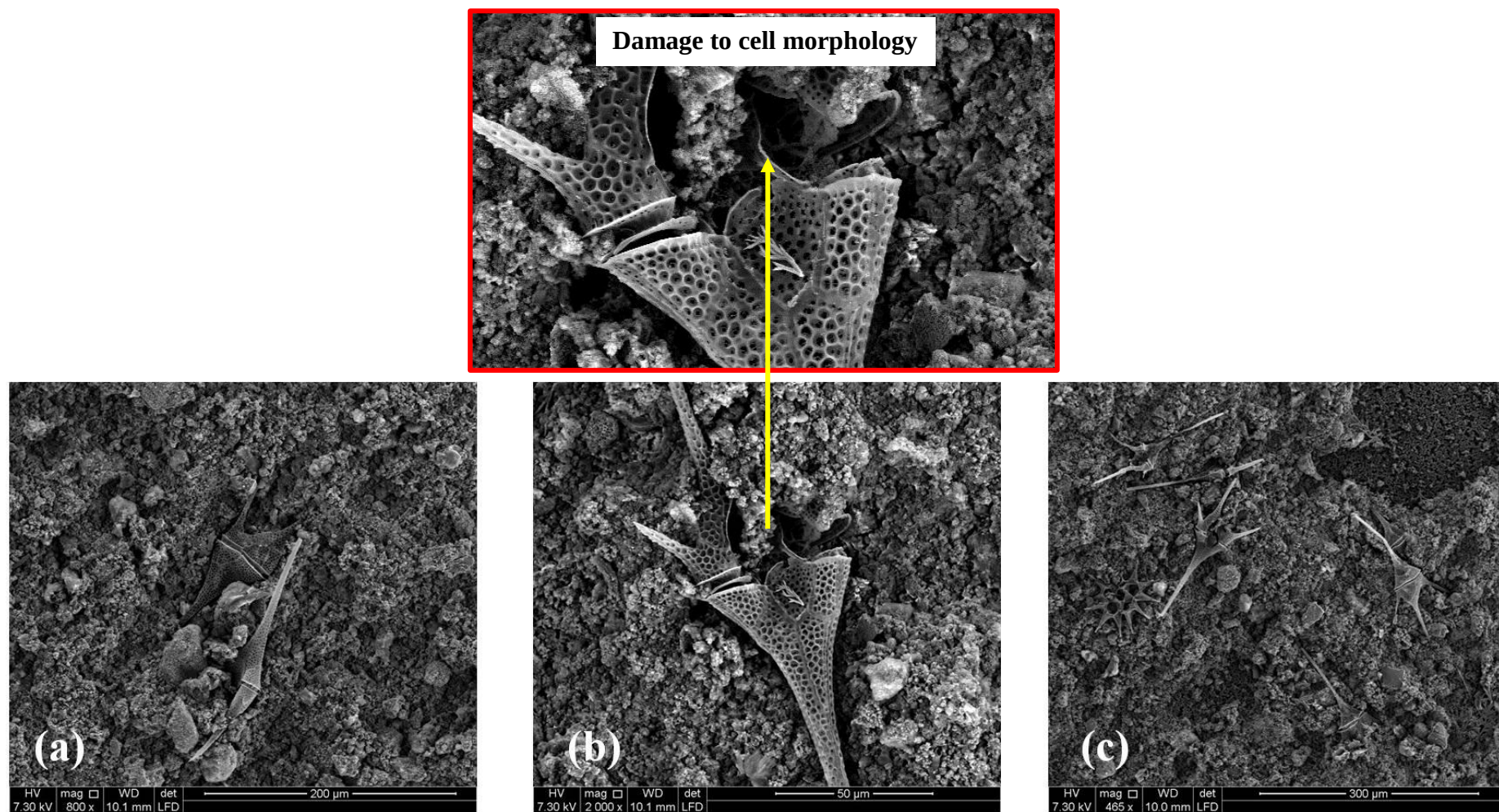


Figure 5.5

SEM images showing the morphology of *C. hirundinella* cells embedded in $\text{Ca(OH)}_2\text{-SiO}_2$ flocs (a) and damage to spherical part of cells (b) and bending of horns (c).

Chapter 5

Physical and Chemical Impacts of Conventional unit Processes and Coagulants on the Dinoflagellate, Ceratium hirundinella

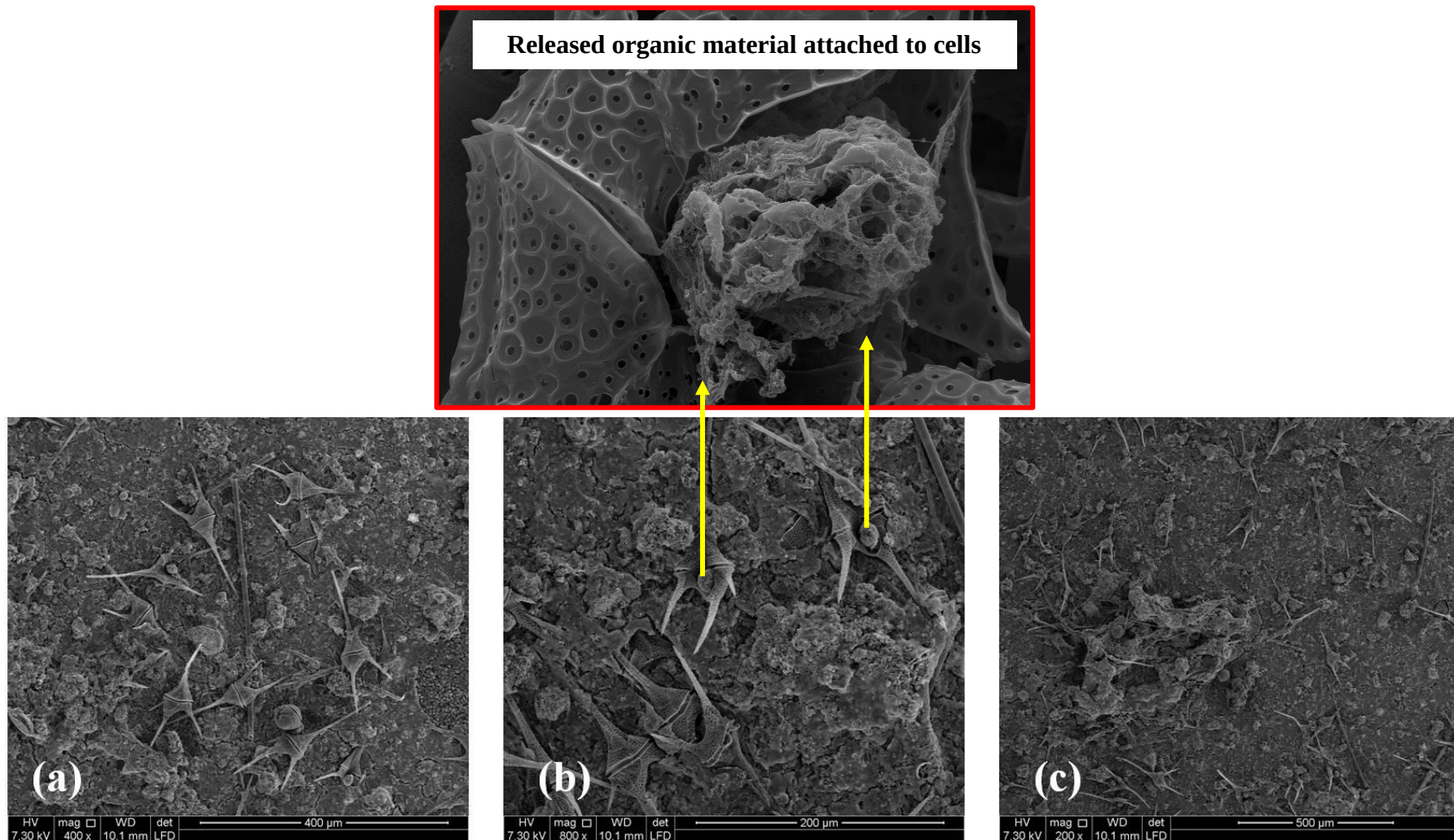


Figure 5.6

SEM images showing the morphology of *C. hirundinella* cells embedded in $\text{Ca}(\text{OH})_2$ -organic polymer flocs where organic material is released through the sulcus grooves (a-c).

Chapter 5

Physical and Chemical Impacts of Conventional unit Processes and Coagulants on the Dinoflagellate, Ceratium hirundinella

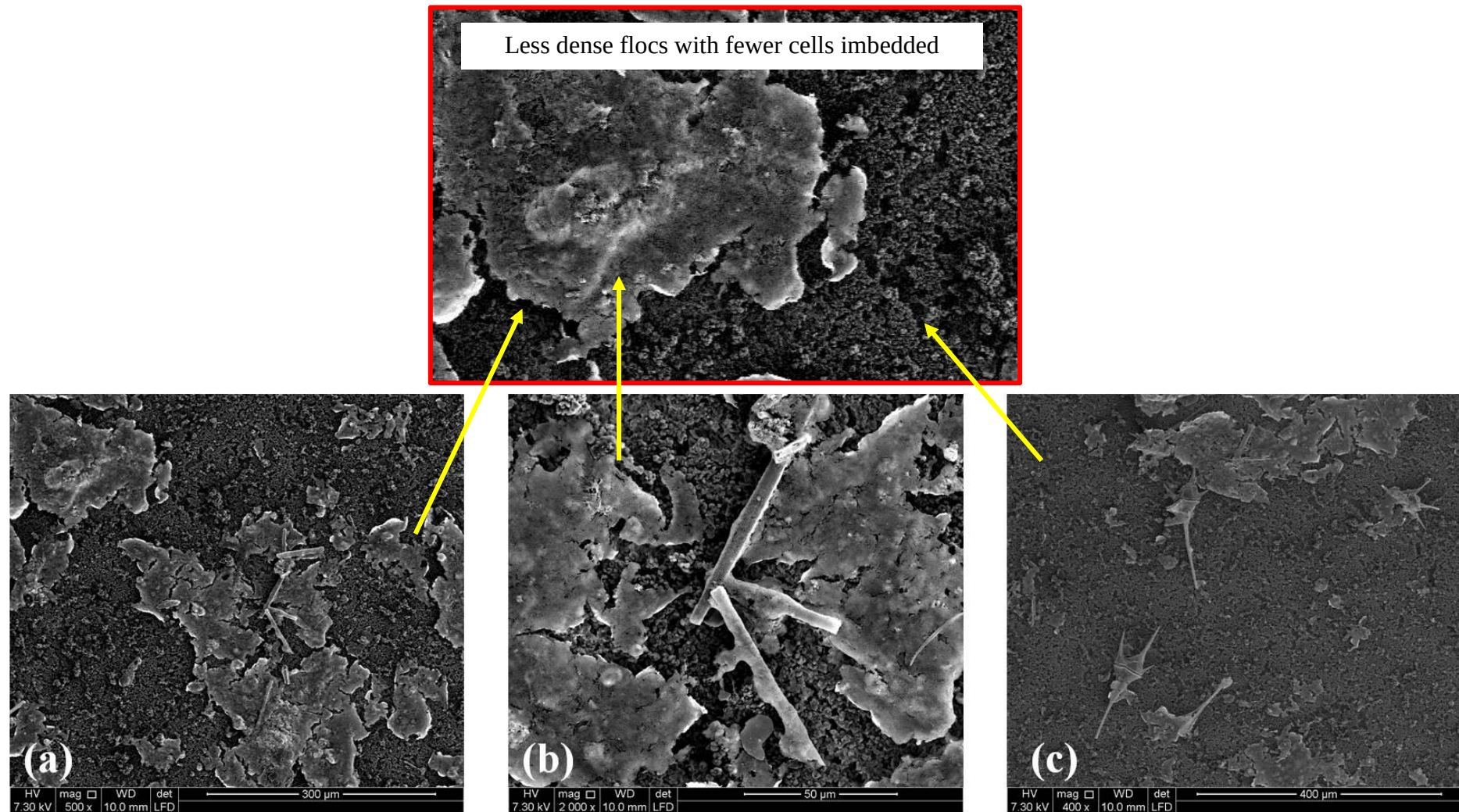


Figure 5.7

SEM images showing the morphology of *C. hirundinella* cells in organic polymer flocs, which are smaller (a) appear more smooth (b) with only a few intact cells (c) embedded.

Chapter 3 and Chapter 4 gives a comparisons of the average TPP concentrations removed by various coagulants dosages ($\text{Ca(OH)}_2\text{-SiO}_2$; $\text{Ca(OH)}_2\text{-organic polymer}$; organic polymer) during four occasions. The removal of *C. hirundinella* cells was recorded at various $\text{Ca(OH)}_2\text{-SiO}_2$ dosages (as indicated by TPP removal) and can be explained by the formation of denser flocs in aggregation containing relatively large pieces of broken cells. Organic polymer achieved poor *C. hirundinella* removal during all dosages which is explained by fewer cells imbedded in less dense flocs in aggregation (Figure 5.7). Relatively good *C. hirundinella* removal was achieved by $\text{Ca(OH)}_2\text{-organic polymer}$ which is confirmed by TPP removal. Flocs that formed after dosing $\text{Ca(OH)}_2\text{-organic polymer}$ were dense with no further damage after high energy flash mixing.

5.4 Discussion

Conventional water treatment includes a series of unit processes (coagulation, flocculation, sedimentation, sand filtration and disinfection) aimed at reducing the concentration of microorganisms and other suspended particles (Betancourt and Rose, 2004). When source water contains *C. hirundinella* cells, many water treatment challenges can occur (e.g. clogging of sand filters) and these may be compounded by water quality problems (e.g. tastes and odours) (Swanepoel *et al.*, 2008a). *Ceratium* cells penetrating into the treated water also contribute to large quantities of organic material due to their large cell size (Swanepoel *et al.*, 2008 a). Organic material entering water may result in the formation of harmful disinfection products, such as trihalomethanes, when chlorine is used as a disinfectant (Rositano *et al.*, 2001; Rodríguez *et al.*, 2007; Zamyadi *et al.*, 2011). Furthermore, organic material in a water distribution network also serves as a carbon source which promotes bacterial growth (Prévost *et al.*, 2005). This Chapter is therefore focused on addressing challenges experienced by conventional water treatment from a scanning electron microscopy perspective, since the objectives were set to investigate both the impacts on water treatment options on *C. hirundinella* cells and to analyse the flocs in aggregation.

These freshwater dinoflagellate cells are often described in literature to be “robust” and therefore it was expected that cells would remain intact after the physical high energy flash mixing step. The physical flash mixing procedure forms an integral part of the conventional coagulation unit process to disperse coagulants quickly and uniformly throughout the source water (Chow *et al.*, 1999). The impacts of flash mixing proved to cause disruption of *C. hirundinella* cells, which force the cells to release organic material (Figure 5.4). Organic material released from *C. hirundinella* cells into the water may create an increased coagulant demand (Sharp *et al.*, 2006). Increasing coagulant demand (e.g. Ca(OH)_2) may impact negatively on the physical integrity of *C. hirundinella* cells and causes major disruption to the cell morphology which will have subsequent adverse impacts on coagulation conditions (Henderson *et al.*, 2008).

Chapter 5

Physical and Chemical Impacts of Conventional unit Processes and Coagulants on the Dinoflagellate, Ceratium hirundinella

The structure and strength of the flocs play important roles in water containing high phytoplankton biomass, notably when unit processes, such as coagulation and flocculation are affected by the selection of coagulant chemicals and optimum dosages thereof (Yuheng *et al.*, 2011). It was observed that $\text{Ca(OH)}_2\text{-SiO}_2$ treatments formed larger, denser flocs than other coagulants used during this Chapter (Figure 5.5). The chemical impacts of $\text{Ca(OH)}_2\text{-SiO}_2$ treatment caused major damage to the cell morphology of *C. hirundinella*. It has become evident from SEM images that $\text{Ca(OH)}_2\text{-SiO}_2$ removed cells by causing serious cellular damage, as a result of high pH levels, instead of removing the intact cells (Figure 5.5). During a recent investigation where a bioflocculant was used to harvest microalgae, cell damage was also observed when flocculation was induced by decreasing pH (Nfikubwimana *et al.*, 2014). The influence of pH during water treatment is widely applied to achieve optimum coagulation and flocculation of suspended matter in water (Divakaran and Sivasankara Pillai, 2002, Knappe *et al.*, 2004, and Degremont, 2007). The impact of pH adjustments during flocculation is described as feasible (either by increasing or decreasing pH) to improve flocculation efficiency (Nfikubwimana *et al.*, 2014). However, major concerns have arisen by the impacts of high pH levels on *C. hirundinella* cells, notably with respect to aforementioned water quality problems.

Using organic polymer as a primary coagulant, in combination with Ca(OH)_2 , formed medium sized flocs that appeared smooth to in dense aggregation (Figure 5.6). *C. hirundinella* cells in flocs aggregations were more intact than what was previously observed when dosing $\text{Ca(OH)}_2\text{-SiO}_2$. Less cell damage to *C. hirundinella* cell morphology can be ascribed to adjusted pH levels of 9-10, which enhanced the effective removal of *C. hirundinella* cells. These pH levels were sufficient to render cells immobile, thereby assisting in the coagulation and flocculation processes, since *C. hirundinella* cells could no longer swim to disrupt floc formation. This finding was also confirmed by Van der Walt (2012) who noted that immobilization of *C. hirundinella* cells will assist coagulation and flocculation. The inhibition of flagellar movement is often the result of a “pH shock” as described by Ferreira and Du Preez (2012).

It has been reported that organic polymers resulted in stronger aggregates (flocs) than other coagulants and are beneficial for slow-settling flocs (Bolto and Gregory, 2007). However, during this investigation it was observed that flocs settled slowly, but were smaller than those formed with other coagulant options. The cells were separate from flocs in aggregation, giving an indication of poor coagulation and flocculation conditions. It has been reported that organic polymers as coagulants produce fragile loosely packed flocs (Wakeman and Tarleton, 1999) which can be observed in Figure 5.7. The mechanisms influencing floc formation, such as pH changes and charge neutralization, are different when using organic polymer and Ca(OH)_2 . Because *Ceratium* cells are highly mobile (Grigorszky *et al.*, 2003), they often disrupt flocs and are difficult to remove. Floc

stability is directly related to the strength and number of bonds holding the floc together (Jarvis *et al.*, 2005). It has become evident from this Chapter that organic polymer alone causes no further damaging effects to the morphology of the cells. No pH adjustments therefore result in water treatment problems, since most of the cells remain motile in the supernatant and have the ability to interfere with floc formation by disrupting flocs. These observations are confirmed by SEM images of flocs, where fewer cells were embedded in flocs or appear separate from aggregates (Figures 5.7a – 5.7c). Poor *C. hirundinella* cell removal during coagulation and flocculation puts major strain on the sand filters. Cell accumulation in the sand filters can result in taste and odour problems or penetration into the treated water. The organic compounds associated with *C. hirundinella*, those that are responsible for taste and odour problems will be discussed in the next Chapter (Chapter 6).

5.5 Conclusions

The freshwater dinoflagellate, *C. hirundinella*, with its complex morphology and robust thecal plate cell covering, is well-known to cause problems that can be divided into water treatment and water quality problems. Many water plants experience poor *C. hirundinella* removal, as well as taste and odour related problems when dosing certain coagulant chemicals. Scanning electron microscopy investigations revealed that both physical and chemical impacts on *C. hirundinella* cells that occur as a result of high energy flash mixing and coagulants. Flash mixing resulted in disruption to cell morphology and subsequently caused cells to release organic material through the sulcus groove. Released organic material remained attached to the sulcus groove after flash mixing, but the addition of certain coagulants, notably $\text{Ca(OH)}_2\text{-SiO}_2$, where pH was increased to levels above 10 resulted in major damage to cells. This coagulant option was recorded to achieve the best *C. hirundinella* cell removal as a result of breaking up cells and the formation of dense flocs. However, coagulant options associated with no pH adjusted (e.g. organic polymer) caused no further damage to cells after flash mixing, but poor *C. hirundinella* cell removal and loosely packed flocs. Ca(OH)_2 in combination with organic polymer as a primary coagulant caused no further major damage to cells after flash mixing, which can be observed in by intact cells with organic material attached to their sulcus grooves. This can be ascribed to adjusted pH levels below 10 that were able to assist coagulation conditions as observed from smooth and less dense to denser flocs that contain mostly intact cells. Water treatment plants that experience problems with phytoplankton in source waters can use SEM investigations to assist the coagulant selection process. Selecting the appropriate coagulant option can play a major role in addressing water-related problems caused by phytoplankton, such as *C. hirundinella*.

Chapter 6

Organic Compounds Associated with the Freshwater Dinoflagellate, *Ceratium hirundinella* and the Impact Thereof on Water Quality and Consumers

6.1 Introduction

Algae and cyanobacteria (phytoplankton) as well actinomycetes that occur naturally in surface waters used for drinking water production may be responsible for a variety of tastes and odours that arise from associated organic compounds. Most of the organic compounds (e.g. fatty acids) associated with phytoplankton are photosynthesis products that can be transformed into other organic compounds (Maazouzi *et al.*, 2008). The most commonly known taste and odour causing organic compounds in freshwaters are geosmin and 2-methylisoborneol, produced by cyanobacteria and certain actinomycetes (Davies *et al.*, 2004; Degrémont, 2007; Wu and Duirik, 2013). Geosmin and 2-methylisoborneol produce tastes and odours in drinking water, which may lead to unfavourable qualities of drinking water. This in turn leads to consumer complaints (Antonopoulou *et al.*, 2014). The effective removal of phytoplankton species that produce taste and odour problems is an important management objective of drinking water suppliers. (Davies *et al.*, 2004).

In recent decades, taste and odour compounds in addition to geosmin and 2-methylisoborneol were intensively investigated in order to identify organic compounds that associate with specific phytoplankton groups or genera. Amongst other studies undertaken, Knappe *et al.* (2004) also listed the most commonly produced organic compounds by the major phytoplankton groups and describe the taste and odours that may pose risks to drinking water. Some of the commonly produced organic compounds by phytoplankton included the following: 4c,7c-decatrienal and 2t,6c-nonadienal (produced by the genera *Synura* and *Dinobryon*); linoleic acid (produced by certain cyanobacteria genera and *Chlamydomonas*); isovaleric acid (produced by *Chlamydomonas*).

Almost all phytoplankton genera produce organic compounds that are responsible for specific taste and odour problems in both source water and drinking water when present in either moderate or abundant quantities (Degrémont, 2007). An investigation undertaken by Ewerts *et al.* (2013) at South Africa's largest conventional water treatment plant identified the cyanobacteria genus *Anabaena* and the freshwater dinoflagellate, *Ceratium hirundinella* (*C. hirundinella*) as problem-causing phytoplankton. In the light of these findings, *C. hirundinella* cells can be responsible for taste and odour problems due to the large quantities of organic material in water when these relatively large cells are present in water (Ewerts *et al.*, 2013).

Chapter 6

Organic Compounds Associated with the freshwater dinoflagellate, Ceratium hirundinella and the Impact Thereof on Water Quality and Consumers

It has been reported that *C. hirundinella* produces organic compounds that are responsible for cucumber-fishy odours when in moderate quantities and a rotten-medicinal odours when in abundant quantities (Degrémont, 2007). Previous studies have identified organic compounds during events of fishy odours as two unsaturated aldehydes (*trans*, *trans*-2-4-heptadienal and *trans*, 4-heptenal) and a ketone produced by dinoflagellates (Young and Suffet, 1999).

During sampling of this research, a fishy odour was detected in the source water collected from Benoni Lake as well as a brownish discolouration of the source water was observed. Both Degrémont (2007) and Knappe *et al.* (2004) confirmed a fishy odour when *C. hirundinella* cells occur in source water; however, little is known about the specific organic compounds that may be responsible for the tastes and odours. The removal of most taste and odour compounds from drinking water is a difficult task since the threshold of these odours is relatively low (usually in a range of nanogram per litre) (Antonopoulou *et al.*, 2014).

The identification of organic compounds that associate with *C. hirundinella* can add value (e.g. optimization of water treatment strategies) to conventional water treatment works where taste and odours problems are becoming a challenge. The aims of this Chapter were to identify the organic compounds excreted by *C. hirundinella* that may be responsible for taste and odour problems as well as to highlight the possible impacts on drinking quality and consumers.

6.2 Materials and Methods

6.2.1 Source water sample collection

Source water samples collected from Benoni Lake, were filtered through a 50 µm sieve to concentrate the *C. hirundinella* cells (in 50 ml Falcon tubes) and eliminate other smaller algae. The freshwater dinoflagellate *Peridinium* and the green algae genera *Pediastrum duplex* and *Pediastrum simplex* occurs occasionally in the source water and may be present in low concentrations in the concentrated samples due to the cell and colony sizes. Concentrated sampled was frozen at -80 °C for 24 hours and transported to the laboratory on dry ice, since the protocol requires that the sample does not defrost. All samples for organic compound analysis were prepared and analysed by scientists at the metabolic laboratories of the North-West University (Department for Biochemistry).

6.2.2 Chemicals and reagents

As an internal standard (IS), 3-OH-Butyric was used. The following reagent chemicals were used: hydrochloric acid (5 M), ethyl acetate, diethyl ether, sodium sulphate powder, BiS(trimethylsilyl)-TriFluoroAcetamide (BSTFA), TriMethylChloroSilane (TMCS) and pyridine.

6.2.3 Sample preparation and derivatization procedure

Sample (1 mL) was transferred into a kimax tube together with 6 drops of hydrochloric acid (5M) to adjust the pH of samples to 1. The internal standard (IS) of 100 µL was added to the kimax tube containing 1 mL sample. Ethyl acetate was added (6 mL) and mixed on rotor for 30 minutes. Samples were centrifuged for 3 minutes at 3000 rpm to allow organic- and water layers to separate. The organic phase on top of the ethyl acetate layer was transferred into a large kimax tube using a Pasteur pipette. Diethyl ether was added to the lower aqueous layer in the first kimax tube and mixed on the rotor for 10 minutes. The centrifugation step was repeated and the organic phase was separated into a second large kimax tube to remove water. A known amount of sodium sulphate was added to the second large kimax tube and vortexed.

Samples were then centrifuged for an additional 3 minutes at 3000 rpm in order to allow pellet formation. The organic phase of samples was poured into smaller kimax tubes to separate the sodium sulphate. The organic phase was evaporated under a steady stream of nitrogen (40 °C for 45 minutes).

The Hamilton syringe was used to add the derivatization (100 µL BSTFA, 20 µL TMCS and 20 µL Pyridine) reagents to the dry organic residue in the small kimax tube. The derivatization mixture was then incubated at 80 °C for 30 minutes and thereafter allowed to cool for 5 minutes. The extract was then transferred to a GC sample vial making sure that the cap is screwed tightly. Subsequently, the sample was loaded onto the Gas chromatography Mass Spectrophotometer (GC/MS).

6.2.4 Gas chromatography mass spectrometry (GC-MS) conditions

Organic compound analysis was carried out on the GC-MS from Agilent Technologies (Wilmington, DE, U.S.A) consisting of a 7890A GC system and a 5975C VL MSD MS system with Triple-Axis Detector. An Auto sampler (76936 model) from Agilent Technologies was used while the Inlet liner from Phenomenex (AG 0-4680) with the following properties was used: GC-liner for HP, S/SC, 1-Taper, w/Wool 4 mm ID x 78.5mm L x 6.3 mm OD Focus liner. A column from Agilent

Chapter 6

Organic Compounds Associated with the freshwater dinoflagellate, Ceratium hirundinella and the Impact Thereof on Water Quality and Consumers

Technologies (122-0132U, DB-1ms UI, 0.25 Micron, 30m x 0.250mm, -60 to 325/350C) was used. The analytical conditions for GC-MS are given in Table 6.1 below.

Table 6.1

Analytical conditions for the GC-MS analysis

Injection Volume	1 µl Split (12:1)
Pressure (PTV)	7.0337 psi
Total flow	15.29 ml/min
Septum purge flow (PTV)	3 ml/min
Temperature program (PTV)	280 °C
Quadruple temperature	150 °C maximum 200 °C
Oven temperature (GC)	50 °C for 1 min, then 20 °C/min to 60 °C for 0 min, then 5 °C/min to 120 °C for 0 min, then 7 °C/min to 280 °C for 4 min
Solvent delay	8 min
Carrier gas (back flush)	Helium, flow 1ml/min at pressure of 7.6522 psi
Run time	42.5 min
Post run (back flush)	1 min, 300 °C

6.2.5 Analyte quantification equations

The following equations were used for quantification of analytes (mg/l):

- 1) $\text{Analyte} = \frac{\text{Area (Analyte)}}{\text{Area (IS)}} \times (\text{IS}) \times \text{Rf}$
- 2) $\text{Rf} = \frac{\text{Area (IS)}}{\text{Area (Std)}} \times \frac{\text{Area (Std)}}{(\text{IS})}$

The Rf value during this investigation was always 1.

6.3 Results

6.3.1 Gas chromatography mass spectrometry (GC-MS) fragments

In freshwater environments such as the Benoni Lake, *C. hirundinella* is known for causing a fishy odour in water; therefore, a known organic compound that is characteristic of a fishy odour was used during the derivatisation process that is suitable for GC-MS analysis. The gas chromatography mass spectrometry (GC-MS) technique was performed to analyse for organic compounds in samples using

the trimethylamine/nitrogen-oxide ratio (TMA/N-oxide ratio). Table 6.2 gives the fragment results for mass/charge (m/z) at 60 and 76 for the samples analysed in four replicates. The fragments for the Blank samples were 190 and 7 at 60 m/z and 76 m/z respectively. In Table 6.2, the abundance of fragments (60 m/z and 76 m/z) of compounds that is similar to fragments in the known fishy odorous compound TMA/N-oxide is indicated. In all replicas, the 60 m/z fragments were more abundant; therefore, these abundant 60 m/z fragments (that are similar to TMA/N-oxide) may be responsible for the fishy odours in water when *C. hirundinella* cells occur. The structures of compounds is not indicated this Chapter, but the GC-MS results indicating the analyte separation and abundance over a retention period in Figure 6.1.

Table 6.2

The m/z 60 and m/z 76 fragments with the TMA-N-oxide ratio for GC-MS results in Figure 6.1

Samples	Abundance	m/z 60	m/z 76	TMA/N-oxide ratio
Replica a	52128	1282	7	183.14
Replica b	48823	1032	12	86.00
Replica c	31753	4423	14	315.93
Replica d	49261	6827	13	525.15
Blank	-	190	7	27.14

6.3.2 Organic acid abundance and identification

Figure 6.1 gives the abundance of the most significant analytes over a retention period of approximately 35 minutes. The organic compounds were succinic acid, propylpropionic acid, myristic acid, palmitoleic acid, palmitic acid, sebacic acid and 4,7,10,13,16,19-docosahexaenoic acid. Palmitic acid was the organic compound with the highest abundance, followed by succinic acid and oleic acid (Figure 6.1). The average quantified values for palmitic acid, succinic acid and oleic acid were 211.87 mg/ℓ, 128 mg/ℓ and 80.26 mg/ℓ respectively (Table 6.3).

6.3.3 The major organic compounds associated with *C. hirundinella*

The major organic compound groups associated with *C. hirundinella* were mostly fatty acids (FAs) and dicarboxylic acids (DAs). Palmitic acid, belonging to the FAs group contributed to the highest percentage abundance (34.53 %) of the organic compound composition. Other important contributions to the organic compound composition were made by succinic acid (20.98 %) and oleic acid (13.08 %) belonging to the DAs and FAs groups respectively.

Chapter 6

Organic Compounds Associated with the freshwater dinoflagellate, Ceratium hirundinella and the Impact Thereof on Water Quality and Consumers

The FAs group contribute to 66.15 % of the total organic compound composition, while contributed 26.04 % of the total organic compound composition. The remaining 7.8 % consisted of other organic compounds that contributed to less than 1 % of the total organic compound composition (Appendix Table A). Results in Table 6.3 indicate that DAs and FAs are mostly associated with *C. hirundinella*.

Chapter 6

Organic Compounds Associated with the freshwater dinoflagellate, *Ceratium hirundinella* and the Impact Thereof on Water Quality and Consumers

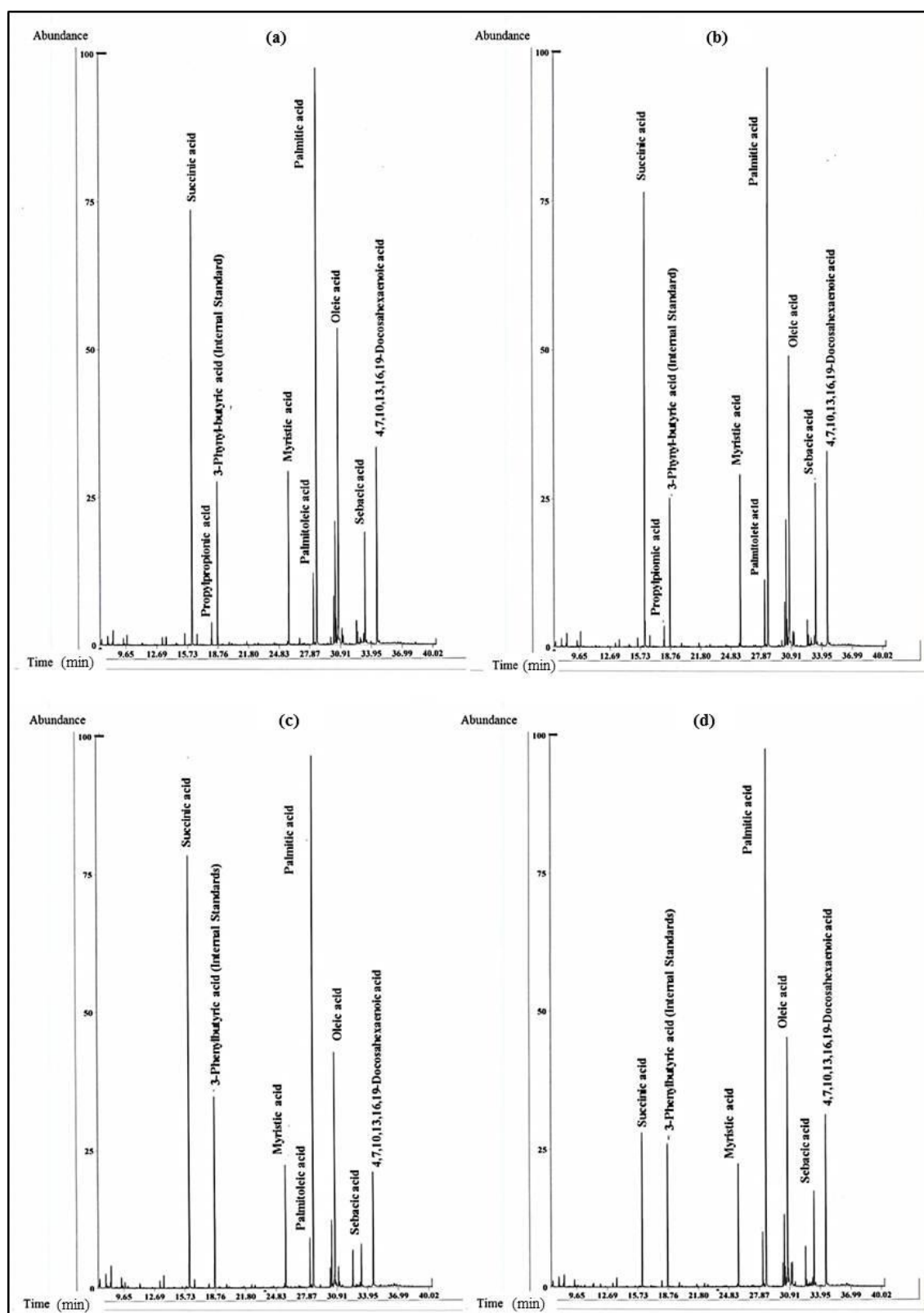


Figure 6.1

GC-MS results indicating the analyte separation and abundance over a retention period of approximately 40 minutes for four replicates (a-d).

Chapter 6

Organic Compounds Associated with the freshwater dinoflagellate, Ceratium hirundinella and the Impact Thereof on Water Quality and Consumers

Table 6.3

The organic compound values (mean, min and max) recorded for four replicates of an unknown concentrated *C. hirundinella* sample. The percentages (%) for organic compound of the total organic compound composition were determined and are classified into dicarboxylic acids (DAs) and fatty acids (FAs).

		Mean (mg/l)	Std. D	Min (mg/l)	Max (mg/l)	% Organic compound of total organic compound composition
DAs	Succinic acid	128.69	58.15	52.01	187.04	20.98
	Sebacic acid	31.02	17.71	10.16	53.48	5.06
Total % DAs						26.04
FAs	Myristic acid	44.22	11.42	27.88	54.09	7.21
	Palmitoleic acid	16.91	4.09	11.31	20.90	2.76
	Palmitic acid	211.87	44.46	149.66	253.89	34.53
	Oleic acid	80.26	15.82	57.99	94.61	13.08
	4,7,10,13,16,19- Docosaheptaenoic acid	52.54	17.57	27.09	67.33	8.57
Total % FAs						66.15
Other organic compounds (< 1 %, Appendix Table A)						7.81

6.4 Discussion

It has been reported that *C. hirundinella* cells in either moderate or abundant quantities are responsible for cucumber-fishy, rotten-medicinal tastes and odour problems in water (Degrémont, 2007). Palmer (1980) also stated that phytoplankton species are responsible for aromatic odours (e.g. fishy and cucumber) in abundant quantities. The presence of organic compounds also causes many problems during the production of drinking water. These problems include; 1) tastes and odours, 2) increased disinfection dose requirements, 3) formation harmful disinfection by-products as well as the 4) promotion of biological growth in distribution system (Matilainen and Sillanpää, 2010 and Jurado-Sánchez *et al.*, 2012).

Swanepoel *et al.* (2008 a) as well as Van der Walt (2012) who also investigated the occurrences of *C. hirundinella* in South African freshwater bodies also confirmed a fishy and rotten odour during their investigations¹. During sampling preparation (concentrating cells), before freezing samples an intensive rotten odour was detected in the samples. Biochemists and analysts at the North West University, South Africa also detected the intensive rotten odours in the concentrated *C. hirundinella* cells in samples after transportation in a cooler box.

The odours associated with phytoplankton genera detected by humans may vary as depending on the quantities present in water. Bolton and Halpern (2010) demonstrated that humans can smell fatty acids such as linoleic, oleic and stearic acids both orthonasally and retronasally. The presence of fatty acids associated with dinoflagellates, notably with *C. hirundinella* has been reported in various studies (Harrington *et al.*, 2007; Bolton and Halpern, 2010; Zhao *et al.*, 2013). During this research, the following FAs (and quantities) were identified in concentrated *C. hirundinella* samples: myristic acid (44.22 mg/l), palmitoleic acid (16.91 44.22 mg/l), palmitic acid (211.87 mg/l), oleic acid (80 mg/l) and 4,7,10,13,16,19-Docosahexaenoic acid (52.54 mg/l). The organic acid, palmitic acid was quantified with the largest value of 211.87 mg/l. Amongst other FAs, palmitic acid was identified as the most typical organic acid produced by dinoflagellates (e.g. *Peridinium* sp. and *C. hirundinella*) in abundant quantities (Harrington *et al.*, 2007) and the most common organic acid in natural water sources or in phytoplankton cultures (Garrett and Grisham, 2005).

¹ Personal observations and findings

Chapter 6

Organic Compounds Associated with the freshwater dinoflagellate, Ceratium hirundinella and the Impact Thereof on Water Quality and Consumers

Maazouzi *et al.* (2008) investigated the effects of the 2003 European heat wave on a freshwater plankton assemblage and its FAs composition. It was found that *C. hirundinella* dominated the plankton assemblage under high temperatures (Maazouzi *et al.*, 2008) with the occurrence of organic acids in similar proportion to what was identified in this Chapter. In Table 6.3 it is reported that FAs contributed to 66.15 % of the total percentage organic compounds detected in samples. The DAs (succinic and sebacic acid) contributed to 26.04 % of the total percentage organic compounds, while individual organic compounds (indicated in Appendix Table A) with less than 1 % contributed to the remaining percentage contribution (Table 6.3). The organic acids, palmitic acid (34.53 %) and succinic acid (20.98 %) contributed to the highest percentages in the FAs and DAs groups respectively (Table 6.3). Consequently, the tastes and odours associated with *C. hirundinella* in this Chapter were due to FAs and DAs, in particular palmitic and succinic acid. These findings are also supported by Zhao *et al.* (2013) and Maazouzi *et al.* (2013) who stated that *C. hirundinella* cells contain large quantities of FAs (photosynthesis storage products) that can be transformed into odours compounds.

Drinking water treatment plants, especially conventional water treatment plants such as the Zuikerbosch plant of Rand Water can control taste and odour compounds originating from phytoplankton as follows: 1) by using source water with limited phytoplankton growth or 2) by managing source water bodies such as the Vaal Dam which is used for the production of drinking water (Peter *et al.*, 2009). Furthermore, managers and operators of a drinking water treatment plant should be aware of taste and odour problems that may arise from sludge and sand filters when source water contains relatively high quantities of *C. hirundinella* cells.

When *C. hirundinella* cells penetrate into the final treated water and subsequently into distribution networks, odours will be detected in the drinking water as a result of microbial activities or volatile fatty acid degradation (Prévost *et al.*, 2005). When chlorine is used as a disinfectant, the formation of harmful organic compounds such as trihalomethanes (THMs) may pose a health risk to drinking water consumers (Want *et al.*, 2012). Both THMs and FAs have been reported to play an important role in cancer development (Beeharry *et al.*, 2003). Some FAs are described as the main target for lipid peroxidation, which can subsequently enhance the formation of mutagenic metabolites (Beeharry *et al.*, 2003). The importance of organic material as precursor material for the formation of THMs will be discussed in the next Chapter (Chapter 7).

6.5 Conclusions

Taste and odour problems in drinking water are directly linked to algae and cyanobacteria (phytoplankton); therefore, it is important to remove these organisms during the production of drinking water. When *C. hirundinella* occurs in either moderate or abundant quantities in source waters odours vary from cucumber, fishy to rotten. It has been reported that organic acids that associate with the presence of *C. hirundinella* are responsible for taste and odour problems and can be detected by drinking water consumers at a low threshold range. In this Chapter it has been confirmed that FAs are associated with *C. hirundinella* cells; therefore, FAs are responsible for taste and odours in source and drinking waters.

The control of tastes and odours in source water used for drinking water production has become a priority for drinking water utilities, as it has a direct impact on drinking water consumers. Therefore, when controlling *C. hirundinella* cells in source water as well as during different stages of the water treatment process will reduce the impacts of organic compounds on the aesthetic quality of water and in turn reduce health risks to consumers. When source water used for drinking water production contains *C. hirundinella* cells it is advisable to remove sludge frequently and increase sand filter backwash cycles to avoid accumulation of *C. hirundinella* cells.

Chapter 7

Investigating *Ceratium hirundinella* Cell Immobilization using Pre-Chlorination Prior to Conventional Coagulation and the Formation of Trihalomethanes

7.1 Introduction

Ceratium hirundinella (*C. hirundinella*) cells in source water used for drinking water production has become a concerning issue to water treatment utilities due to the fact that cells may cause water treatment related problems in relatively low concentrations (Swanepoel *et al.*, 2008a). Pieterse *et al.* (2000) stated that phytoplankton cells that contain flagella have the following abilities: 1) interfere with conventional coagulation and flocculation by disrupting flocs and 2) penetrate into the final water. Ewerts *et al.* (2013) also confirmed this species, amongst others, as a problem-causing alga in conventional water treatment plants. Water treatment problems caused by *C. hirundinella* cells can be ascribed to the fact that the dinoflagellate cells are relatively good swimmers, giving cells the advantage to migrate through the water column better than other algae (Gligora *et al.*, 2003). An investigation conducted by Van der Walt (2012) also proved that *C. hirundinella* cells can penetrate into the final water as a result of poor coagulant dosing practices.

A chlorophyll breakthrough event into the final water was recorded at South Africa's largest conventional water treatment plant during the summer of 2006, which was the result of *C. hirundinella* cells entering the final water (Swanepoel *et al.*, 2008a). Analytical laboratories accredited by the South African National Accreditation System (SANAS) assist the water treatment utility by monitoring phytoplankton species daily in source water used for drinking water production and confirmed that *C. hirundinella* only occur seasonally. During seasons when these highly motile dinoflagellate cells occur in source water, it is required by water treatment plants to optimize unit processes and coagulant dosages accordingly. However, not all coagulants used by water treatment plants are able to remove such motile algae; therefore, a pre-treatment (pre-oxidation) strategy to render cells immobile in order to assist coagulation and flocculation unit processes to improve removal efficiencies is investigated during this Chapter.

The implementation of pre-oxidation with various oxidants (e.g. potassium permanganate, ozone) is widely applied for various pre-treatment purposes during water treatment (Van der Walt *et al.*, 2009). These oxidants have biocidal properties; therefore, it can be used to control nuisance aquatic growths such phytoplankton (algae and cyanobacteria) and are also used as primary disinfectants (Van der Walt *et al.*, 2009). It was reported that chlorine inactivates microorganisms by crossing cell membranes and damaging the intracellular organelles

(Venkobacher *et al.*, 1977). 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).

In this Chapter, the effective use of pre-chlorination was evaluated by using the dosage response graphs (Hill slopes) showing the half maximal inhibitory concentration (IC₅₀-values) together with cell integrity. Therefore, the effective use of pre-chlorination dosages will render the alga *C. hirundinella* immobile in order assist coagulation to remove the problem-causing alga without causing damage that may result in cell lysis. When cell lysis occurs prior to coagulation, the following water-related problems may occur: 1) taste and odour problems and 2) formation of harmful chlorine by-products, such as trihalomethanes (THMs). In the light of these risks associated with pre-chlorination, this Chapter also focused on the use of effective chlorine concentrations required to render at least 50 % of the *C. hirundinella* cells immobile but still intact as well as monitoring the concentration of THMs that form as a result of pre-chlorination.

Unlike other algae, *C. hirundinella* cells are relatively large in cell size (up to 50 µm in width and 450 µm in length); therefore, these cells contribute significantly to high quantities of organic material (including photosynthetic pigments) in water. Organic material that originates from algae are important precursors for THMs (Abdullah *et al.*, 2009; Van der Walt *et al.*, 2009; Lui *et al.*, 2011; Zamyadi *et al.*, 2012). The formation THMs pose a health risk to drinking water consumers, since they are mutagens, carcinogens and toxic (Abdullah *et al.*, 2009; Hebert *et al.*, 2010).

The aims of this Chapter were to: 1) investigate the effect of pre-chlorination on the integrity of *C. hirundinella* cells, 2) evaluate the efficacy of *C. hirundinella* cell removal when various coagulants are assisted by pre-chlorination and 3) investigate the formation of THMs when using pre-chlorination to immobilize *C. hirundinella* cells.

7.2 Materials and Methods

7.2.1 Water samples: collection and preparation

Source water from Benoni Lake, South Africa containing relatively high *C. hirundinella* concentrations (ranging between 1000 - 4000 cells/mL) were collected during four sampling occasions. During periods of low *C. hirundinella* concentrations present in the source water, cells were concentrated using a 50 µm sieve to achieve initial cell concentrations above 500 cells/mL. The

C. hirundinella cells collected in water samples used for chlorine exposure experiments were at an advance growth stage, which may have contributed to the sensitivity of cells to relatively low chlorine dosages. All water samples were collected in pre-washed plastic containers and stored under laboratory conditions ($\pm 22^\circ\text{C}$).

7.2.2 Determination of pre-chlorination dosages using dose response fittings (half maximum inhibitory concentrations IC_{50}) and cell integrity (damage to cells).

Chlorine stock solutions were prepared to dose a chlorine dosage range starting 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 early stages of the *C. hirundinella* bloom lost their integrity (cell lysis occur) 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 TM II Analysis System, using the Diethyl-p-Phenylene-Diimine tests (APHA, 2013).

Filtered source water enriched with a known concentration of *C. hirundinella* cells were exposed for approximately 5 minutes to each chlorine dosage (0.05 mg/l, 0.10 mg/l, 0.15 mg/l, 0.20 mg/l, 0.25 mg/l, 0.30 mg/l, 0.35 mg/l, 0.40 mg/l, 0.45 mg/l). After 5 minutes exposure, an inverted light microscope was used to enumerate the number of immobile *C. hirundinella* cells using the phytoplankton identification and enumeration method described by Swanepoel *et al.* (2008b), which is based on the method from Lund *et al.* (1958). Microscopic observations were done to determine the immobility of the highly motile *C. hirundinella* cells. Chlorine exposure experiments with different chlorine dosages within the range were conducted in triplicate.

After chlorine exposure experiments, the average percentage immobile *C. hirundinella* (cells/ml) were plotted and fitted to a sigmoid regression (using CurveExpert Version 1.3, 1993). The half maximum inhibitory concentration (IC_{50}) that provokes a response halfway between the baseline (bottom) and maximum response (top) may not achieved due to the effect of chlorine on the integrity of cells. In order to plot sigmoidal curves, data were normalized to percentage of the maximum response, and subtracting a baseline. The actual pre-chlorination concentrations used during this Chapter may differ from the proposed IC_{50} -values on the graphs due to cell integrity considerations.

7.2.3 Pre-chlorination and jar stirring test experiments

Pre-determined chlorine dosages were dosed 5 minutes prior to flash mixing into 2000 ml source water samples containing 1360 cells/ml, 2583 cells/ml, 3746 cells/ml, and 1481 cells/ml during

sampling occasions a, b, c and d respectively (Table 7.1). Jar stirring tests were conducted to simulate conventional coagulation, flocculation and sedimentation. Stirrers were switched off to allow a settling period of 20 minutes, where-after the supernatant was withdrawn for subsequent analytical determinations. The complete jar stirring procedure is described in Chapter 3 (see Materials and Methods).

7.2.4 Coagulant dosages

Ca(OH)₂-SiO₂ - Ca(OH)₂ dosages ranged from 60 to 160 mg/ℓ (in increments of 20). SiO₂ dosages of 4 mg/ℓ were dosed as a coagulant aid. Ca(OH)₂-organic polymer - organic polymer dosages ranged from 4 to 14 mg/ℓ (in increments of 2). Ca(OH)₂ dosages of 10 mg/ℓ were added as a coagulants aid. Organic polymer - organic polymer dosages ranged from 4 to 14 mg/ℓ (in increments of 2).

7.2.5 Analytical determinations

Total photosynthetic pigments (TPP): Water samples (250 mℓ) were collected from the supernatant (after sedimentation) to perform total photosynthetic pigment (TPP) analyses. Water samples were filtered to trap phytoplankton cells and filter papers were placed in 10 mℓ methanol for the extraction of chlorophyll and other pigments. After samples were boiled for 1 hour the absorbance was determined at 650 and 750 nm using the Beckman Coulter (DU® 65i) spectrophotometer (Swanepoel *et al.*, 2008b).

7.2.6 Total organic carbon (TOC), dissolved organic carbon (DOC) and Trihalomethanes (THMs)

Organic carbon compounds were determined using a Fusion Ultraviolet detected (APHA, 2013). DOC and ultraviolet absorbance at a wavelength of 254 nm (UV₂₅₄) was measured to determine the overall organic loading in water samples. UV₂₅₄ was measured using a spectrophotometer and DOC was determined with a Shimadzu TOC-L analyser. THMs (bromochloroform, bromoform, chloroform and dibromochloroform) were determined using a gas chromatograph linked to an electron capture detector (GC-ECD). The GC was performed under split mode with an initial temperature of 250 °C using capillary column (HP-1 Methyl siloxane) and a front detector (μECD).

7.2.7 Dose response graphs (Hill slopes)

Dose responses (Hill slopes) with percentage (%) immobile *C. hirundinella* cells on y-axis and chlorine dosages on x-axis were determined using CurveExpert Pro version 2.0.3.

7.2.8 Statistical analysis

T-tests analyses were used to determine statistical 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.

7.3 Results

7.3.1 Effect of pre-chlorination on the integrity of *C. hirundinella* cells

The effects of pre-chlorination on the motility of cells were observed microscopically and the effects of pre-chlorination on the integrity of *C. hirundinella* cells are in Figure 7.1. The integrity of *C. hirundinella* cells in the source water is shown in Figure 7.1 a, while Figure 7.1 c displays *C. hirundinella* cell images with disruptions to the cell integrity after dosing chlorine concentrations > 0.20 mg/ℓ. From Figure 7.1 it is evident that chlorine dosages below 0.20 mg/ℓ should be used to avoid disruption of cell integrity by pre-chlorination.

Chapter 7

Investigating Ceratium hirundinella cell immobilization using pre-chlorination prior to conventional coagulation and the formation of trihalomethanes

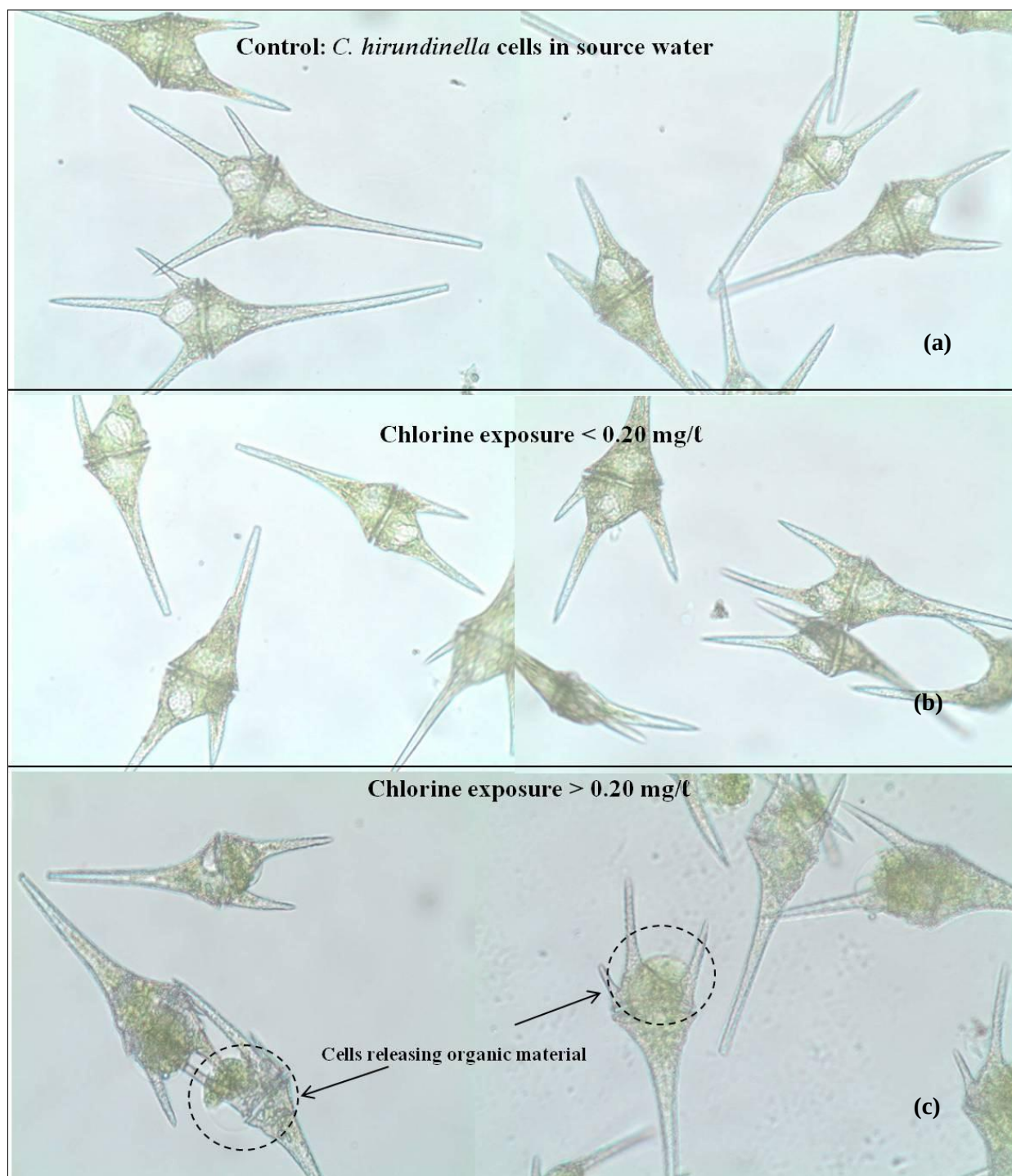


Figure 7.1

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

7.3.2 Pre-chlorination dosages required to immobilize *C. hirundinella* cells, while keeping the integrity of cells

Figure 7.2 shows the dose response models for chlorine exposure experiments when dosing the same chlorine dosage range (0.05 mg/l - 0.45 mg/l) to immobilize 50 % of the initial *C. hirundinella* concentrations. During the different chlorine exposure experiments, the IC₅₀ values and the impacts thereof on the integrity of cells were evaluated to determine the appropriate pre-chlorination dose (Table 7.1). Therefore, pre-chlorination values used during experiments may not necessarily correspond with the dose-response graphs due to cell integrity factors. 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 during advanced growth stages; however, the pre-chlorination dosage (0.121 mg/l) was similar to the dosage determined during occasion a.

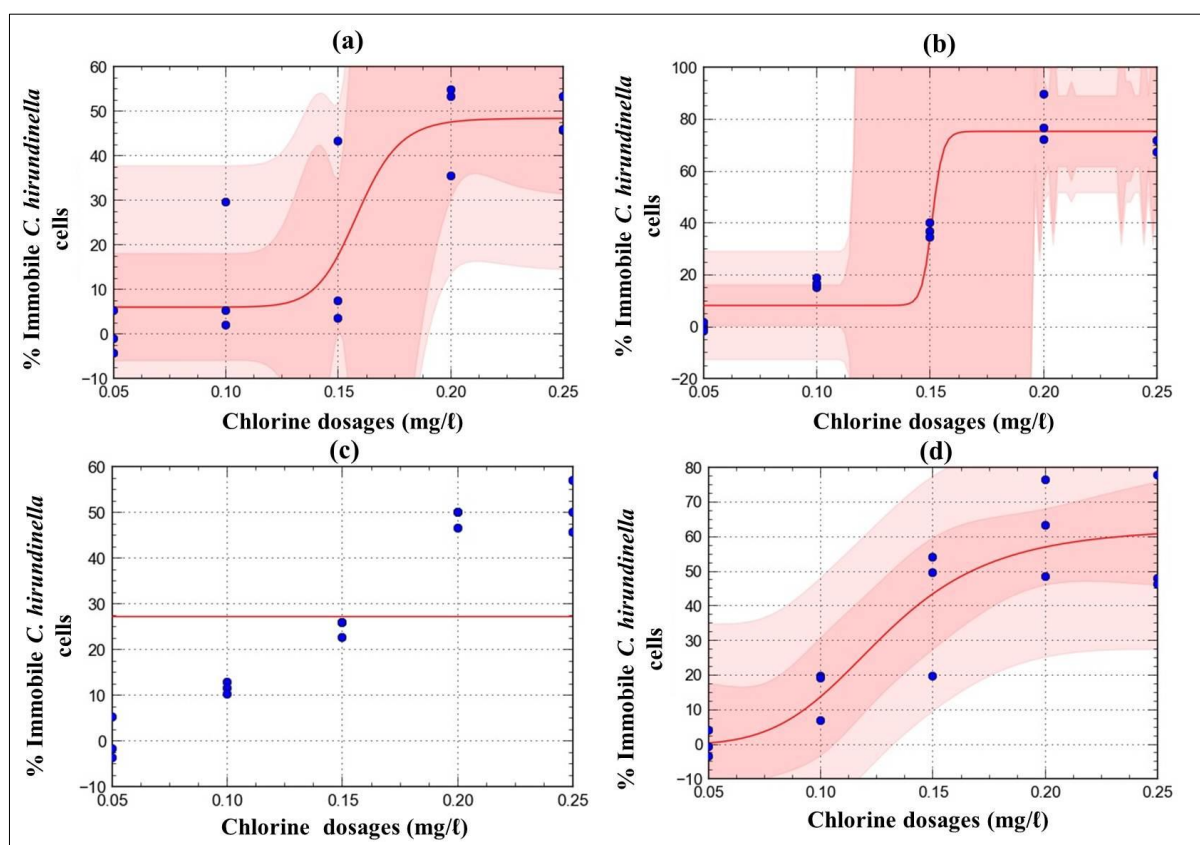


Figure 7.2

The dose response models (Hill slopes) for four chlorine exposure experiments and the relationships between the percentages (%) recorded for immobilized *C. hirundinella* cells and increasing chlorine dosages.

Table 7.1 gives the initial *C. hirundinella* concentrations in source water used during four chlorine exposure experiments together with pre-chlorination dosages. Although no specific correlations between the number of cells and pre-chlorination dosages can be observed, the pre-chlorination dosages used in this Chapter were below 0.20 mg/l.

Table 7.1

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

	Occasion a	Occasion b	Occasion c	Occasion D
Initial cell concentrations (cells/ml)	1360	2583	3746	1481
Pre-chlorination dosage (mg/l)	0.121	0.186	0.121	0.106

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

Pre-chlorination dosages as indicated in Table 7.1 (0.121 mg/l, 0.186 mg/l, 0.121 mg/l and 0.106 mg/l) were used to assist during coagulation and flocculation experiments. These results are indicated in Table 7.2, where the % TPP removal indicates the efficacy of *C. hirundinella* removal. Results obtained for pre-chlorination in combination with $\text{Ca(OH)}_2\text{-SiO}_2$, Ca(OH)_2 -organic polymer and organic polymer are compared to results obtained without pre-chlorination (control).

The pre-determined chlorine dosages used for pre-chlorination were below 0.20 mg/l (0.121 mg/l, 0.186 mg/l, 0.121 mg/l and 0.106 mg/l) during all treatments (Tables 7.2 and 7.3). The effects of pre-chlorination (chlorine dose of 0.121 mg/l) on % TPP removal when dosing $\text{Ca(OH)}_2\text{-SiO}_2$ and Ca(OH)_2 -organic polymer have shown improved % TPP removal for only the following dosages respectively; 60 mg/l and 8-10 mg/l. The effect of pre-chlorination on *C. hirundinella* removal was the best illustrated by the % TPP removal when organic polymer alone was used as a coagulant option (Table 7.2). These results have indicated that organic polymer may not be the best coagulant option to remove *C. hirundinella*, but the effect of *C. hirundinella* immobilization 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 pH adjustments render the cells immobile; therefore, the effects of pre-chlorination on cell removal as a result of immobilization could not be observed in the % TPP removal.

Chapter 7

Investigating Ceratium hirundinella cell immobilization using pre-chlorination prior to conventional coagulation and the formation of trihalomethanes

Results in Table 7.3 confirm the fact that immobilization as a result of pre-chlorination can assist organic polymer during coagulation and flocculation to improve *C. hirundinella* removal. The chlorine dosages 0.186 mg/ℓ, 0.121 mg/ℓ and 0.106 mg/ℓ have assisted the lowest organic coagulant dosage of 4 mg/ℓ to improve the percentage TPP removal with 9 %, 20 % and 2 % respectively, while the highest dosage of 14 mg/ℓ improved the TPP removal percentages with 20 %, 10 % and 2 % (Table 7.3).

Chapter 7

Investigating Ceratium hirundinella cell immobilization using pre-chlorination prior to conventional coagulation and the formation of trihalomethanes

Table 7.2

The % TPP removal by various coagulants ($\text{Ca}(\text{OH})_2\text{-SiO}_2$ (a), $\text{Ca}(\text{OH})_2\text{-organic polymer}$ (b) and organic polymer (c)) compared to % TPP removal achieved when the same dosages of were used in combination with pre-chlorination. (Pre-chlorination dosage of 0.121 mg/l as previously determined during occasion a Table 7.1).

$\text{Ca}(\text{OH})_2\text{-SiO}_2$			$\text{Ca}(\text{OH})_2\text{-Organic polymer}$			Organic Polymer		
Ca(OH) ₂ dosages (60 - 160 mg/l increments of 20)			Organic Polymer dosages (4 - 14 mg/l increments of 2)			Dosage (4 - 14 mg/l increments of 2)		
Pre-Cl ₂			Pre-Cl ₂			Pre-Cl ₂		
Control %	% TPP	Removal	Control %	% TPP	Removal	Control %	% TPP	Removal
TPP removal	removal	Success	TPP removal	removal	Success	TPP removal	removal	Success
58.90	60.27	✓	43.15	33.56	x	28.08	38.36	✓
74.66	63.01	x	50.68	45.89	x	30.14	34.25	✓
93.15	86.99	x	52.74	60.96	✓	34.93	43.15	✓
96.44	95.00	x	64.38	65.75	✓	50.00	55.48	✓
97.74	96.37	x	76.03	73.97	x	60.27	65.07	✓
98.29	97.67	x	78.77	76.71	x	61.64	68.49	✓
<i>p</i> -value		0.37	<i>p</i> -value		0.44	<i>p</i> -value		0.23

Removal success: The effect of pre-chlorination on % TPP removal is indicated with a tick (✓), while no significant %TPP removal is indicated with a cross (x) based on *p*-values and differences in percentages (%)

Chapter 7

Investigating Ceratium hirundinella cell immobilization using pre-chlorination prior to conventional coagulation and the formation of trihalomethanes

Table 7.3

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, c and d (Table 7.1).

Occasion b (Pre-chlorination dosage 0.186 mg/ℓ)			Occasion c (Pre-chlorination dosage 0.121 mg/ℓ)			Occasion d (Pre-chlorination dosage 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	✓
<i>p</i> -value		0.03	<i>p</i> -value		0.09	<i>p</i> -value		0.22

Removal success: The effect of pre-chlorination on % TPP removal is indicated with a tick (✓), while no significant %TPP removal is indicated with a cross (x) based on *p*-values and differences in percentages (%)

7.3.4 The formation of trihalomethanes (THMs) during pre-chlorination

Large algae such as, *C. hirundinella* cells contribute to significant quantities of the total organic carbon (TOC) in source water (Table 7.4). When chlorine reacts with TOC in the source water, it results in the formation by-products known as trihalomethanes which have potential chronic effects in humans. The TOC and DOC concentrations varied between 6.3 – 11 mg/ℓ and 5.4 – 9.9 mg/ℓ respectively during the different sampling occasions (a-d). The concentrations of organic carbon compounds measured during different sampling occasions of this Chapter were similar, while increased THM concentrations were recorded as a result of increasing chlorine dosages.

Four THMs were present after chlorine exposure namely; bromodichloroform, bromoform, chloroform and dibromochloroform. The THMs consisted mostly of bromodichloroform and chloroform, since bromoform and dibromochloroform were below the detection limits of the method used in this research. The minimum (min) and maximum (max) values recorded for bromodichloroform and chloroform is given in Table 7.4. These values were obtained after dosing a chlorine range of 0.05 to 0.45 mg/ℓ.

Chapter 7

Investigating Ceratium hirundinella cell immobilization using pre-chlorination prior to conventional coagulation and the formation of trihalomethanes

Table 7.4

The minimum (min) and maximum (max) values for THMs (bromodichloroform and chloroform) after chlorine exposure experiments and organic carbon (TOC and DOC) concentrations measured in samples collected during four sampling occasions (a-d).

	THMs		Organic Carbon			
	Bromodichloroform (µg/l)		Chloroform (µg/l)		TOC (mg/l)	DOC (mg/l)
	SANS 241 guideline for Bromo- dichloroform ≤ 100 µg/l		SANS 241 guideline for chloroform ≤ 300 µg/l		SANS 241 guideline for TOC ≤ 10 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)

7.4 Discussion

Poor phytoplankton removal during coagulation is often ascribed to the surface characteristics and the motility of certain genera (Pieterse *et al.*, 2000; and Ma *et al.*, 2012). The well-known freshwater dinoflagellate, *C. hirundinella* is known as a strong swimmer and when entering water treatment plants in either abundant or moderate quantities, cells can use their ability to cause water treatment problems in treatment plants, such as floc disruption, filter clogging, tastes and odours (Swanepoel *et al.*, 2008a; Ewerts *et al.*, 2013).

Water treatment plants facing challenges posed by flagellated phytoplankton should consider using pre-treatment options, such as pre-chlorination to immobilize cells and subsequently remove cells more effectively after sedimentation. When considering pre-chlorination as a pre-treatment step, cell integrity should be monitored, especially towards the final or advanced growth stage (end of the bloom) of cells to reduce damaging effects, such as cell lysis that can result in taste and odour problems as well as the formation of harmful chlorine by-products (e.g. THMs).

When implementing pre-chlorination, the objectives should be clear to avoid unnecessary problems, since pre-chlorination can be used to address various challenges during the water treatment process. Examples of water utilities in South Africa using pre-chlorination are Vaalkop and Rietvlei (Van der Walt *et al.*, 2009). At these locations pre-chlorination is used to either 1) reduce high iron and manganese levels, or 2) to control phytoplankton, colour, taste and odours (Van der Walt *et al.*, 2009). However, for the purpose of this Chapter, pre-chlorination is used to immobilize the highly motile *C. hirundinella* cells to subsequently assist coagulation and flocculation unit processes.

Steynberg *et al.* (1994) found that pre-chlorination dosages ($< 1 \text{ mg/l}$) improved the removal of flagellated species (*Chlamydomonas* sp. and *Euglena gracilis*) as a result of immobilization that assisted coagulation and flocculation. Steynberg *et al.* (1994) and Henderson *et al.* (2008) also stated that improvements in removal efficiencies of up to 95 % can be expected when immobilization assist coagulation and flocculation, depending of the chemical used. The removal success of *C. hirundinella* cells during this Chapter was influenced by various aspects such the growth phases (age of algae) as well as cell integrity.

Source water samples collected during four different occasions containing relatively high *C. hirundinella* concentrations ranging between 1000 – 4000 cells/ml were exposed to a chlorine range of 0.05 – 0.45 mg/l to determine pre-chlorination dosages required to assist coagulation and flocculation unit processes. The total organic carbon (TOC) concentrations measured in the source

water ranged between 4.70 – 11.00 mg/ℓ with an average value of 7.15 mg/ℓ (Appendix Table B). This TOC range is relatively low, which included the *C. hirundinella* concentrations in the water used for chlorine exposure experiments. Thus it can be speculated that the contribution of TOC to the chlorine demand was moderate. However, elevated levels of organic carbon in source water and the presence of algae in source water will increase the chlorine demand during pre-chlorination (Van der Walt et al., 2009). Furthermore, the cells used during this research were in an advance growth phase that contributed to the sensitivity of cells for chlorine exposure; therefore, relatively low pre-chlorination dosages were required to immobilize 50 % or more of the exposed cells. An investigation conducted by Van der Walt (2012) with relatively low initial *C. hirundinella* cells concentrations (<500 cells/mL) required approximately 10 times more chlorine as an effective dosage to immobilize 50 % of the cells, most probably because those cells were in another stage of the growth phase and therefore cannot be compared with this Chapter. In this Chapter, source water enriched with *C. hirundinella* cells (> 500 cells/mL) were used to investigate the effects of pre-chlorination to assist the following coagulants in removing phytoplankton cells more effectively: Ca(OH)-SiO₂, Ca(OH)₂-organic polymer and organic polymer. Pre-chlorination showed to be most effective as aid to organic polymer without pH adjustments to remove *C. hirundinella* cells. Another aspect or disadvantage of chlorine exposure method, it that the chlorine demands is dependent on the chlorine dose (Clesceri et al., 1998; Abdullah et al., 2009).

C. hirundinella cells used during this Chapter were not collected from a culture media, which give a representation of natural conditions of pre-treatment. Concentrations were mostly below 1000 cells/mL in the source water used to perform chlorine exposure experiments, which is lower than algae and cyanobacteria concentrations found in a culture media. Phytoplankton cell concentrations in cultures can have a cell density of up to 2.2×10^6 cells/mL (Ma et al., 2012). Zamyadi et al. (2012) also used cyanobacteria concentrations that varied between 50 000 – 200 000 cells/ℓ during pre-chlorination studies and dosed chlorine concentrations where the lowest dosage for chlorine decay experiments was 2 mg/ℓ. The use of low pre-chlorination dosages to immobilize *C. hirundinella* cells that occur in natural water with a low TOC content (Appendix Table B and C) can be considered as cost effective, to treat oligo- to mesotrophic (such as Vaal Dam and Benoni Lake source waters).

Algal and cyanobacteria culture media may also contain higher organic contents when compared to natural conditions. Source water containing *C. hirundinella* cells collected under natural conditions for chlorine exposure experiments will therefore require relatively low chlorine concentrations for immobilization as observed during this Chapter. An investigation conducted by Ma et al. (2012) where *Microcystis* cells were exposed to 4 mg/ℓ chlorine showed that no remarkable morphological

alteration were observed from SEM images even when cells were exposed for 20 minutes (Ma *et al.*, 2012).

Damage to cells was observed when dosing chlorine higher than 0.20 mg/l. Damaging effects caused to cell integrity by relatively low chlorine dosages (< 1 mg/l) gives an indication that cells are at a sensitive stage in the growth phase. Therefore, coagulants, such as Ca(OH)_2 in high dosages may have adverse effects on cell integrity during this growth phase. When considering pre-chlorination to immobilize phytoplankton cells, the primary coagulant dosed during coagulation also plays a major role, since coagulant options containing Ca(OH)_2 can also render motile cells immobilize. 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) refer to these impacts of pH on organisms as “pH shock”, which stopped flagellar movements of *C. hirundinella* cells. Pre-chlorination implemented to assist $\text{Ca(OH)}_2\text{-SiO}_2$ and Ca(OH)_2 -organic polymer was not effective in decreasing the %TPP removal. The pH increase or adjustment caused by Ca(OH)_2 was most probably sufficient to immobilize cells to ensure good removal.

When dosing organic polymer, no pH adjustments occur and subsequently removed less *C. hirundinella* cells when compared to other coagulant options. This means that *C. hirundinella* cells remain motile and disrupt the coagulation and flocculation processes. Flagellate species like the dinoflagellate *C. hirundinella* are able to liberate themselves from floc aggregates (Henderson *et al.*, 2008) when organic polymer is used as a coagulant. Therefore when pre-chlorination assists organic polymer, improvements in percentage TPP removal were observed. When source water used for drinking water production contains *C. hirundinella* cells, pre-chlorination can be considered as a pre-treatment step when dosing organic polymer alone. However, the effects of pre-chlorination on cell integrity should be monitored to prevent the release of organic material during cell lysis.

Organic material, 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). It is well-known that organic material arising from algae serves as precursor material for the formation of harmful chlorine by-products, such as THMs and may cause unpleasant tastes and odours in drinking water (Goslan *et al.*, 2009; Zamyadi *et al.*, 2012).

In open water bodies, THMs are detected at relatively low concentrations due to the fact that THMs are slowly lost to the atmosphere (DWAf, 1996). This statement was also confirmed during this Chapter, since no THMs were measured in the two controls (without chlorine) samples. The formation of THMs increased as a result of increasing chlorine concentrations dosed. The most

commonly found THM's (bromodichloroform, bromoform, chloroform and dibromochloroform) were identified; 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 explains the fact that chloroform was the most abundant THM.

The regulatory guidelines for THMs in drinking water and surface waters set by the United States Environmental Protection Agency (USEPA) and European Union (UE) are 80 µg/ℓ and 100 µg/ℓ respectively (USEPA, 2006; Agus *et al.*, 2009). The guidelines set for South African drinking water are similar to guidelines set by the WHO as indicated by SANS 241:2011 (SANS 241, 2011). THM concentrations formed during pre-chlorination of this research are not a concerning issue, since concentrations were well below 100 µg/ℓ (Table 7.4). However, higher THMs may form as a result of increased chlorine dosages (e.g. chlorine overdose, or during higher dosages for disinfection after sand filtration). THMs have significant vapour pressures at ambient temperature and are released into the atmosphere when water is either aerated or boiled (DWAF, 2009). Therefore, water treatment plants that use pre-chlorination can reduce the THMs entering treatment plant by using additional aeration step or allowing pre-chlorinated water to be exposed to ambient temperature in order to release THMs into the atmosphere.

7.5 Conclusions

Pre-chlorination can be used effectively prior to water treatment to assist coagulation and flocculation unit processes, especially in cases where organic polymers are used as primary coagulant. However, when implementing pre-chlorination, the integrity of cells should be monitored to avoid cell lysis which may lead to taste and odour problems in the drinking water. Depending on the growth phase (age) of the algal bloom and the organic content (DOC/TOC), the determination of an IC₅₀ chlorine concentration may be a challenge. A major aspect that may impact on pre-chlorination practices for algal immobilization purposes is organic material such as TOC which will subsequently increase the chlorine demand. Cell lysis that may occur as a result of pre-chlorination practices, not only result in taste and odour problems, but the formation of harmful chlorine by-products, such as THMs. The formation of THMs during this early stage of water treatment may not pose major risks due to low chlorine dosages. However, pre-chlorination can be performed in open aeration tanks to promote the release of the THMs that did form, reducing the effects of THMs further still.

Chapter 7

Investigating Ceratium hirundinella cell immobilization using pre-chlorination prior to conventional coagulation and the formation of trihalomethanes

Water treatment plants that dose organic polymer alone as the primary coagulant, should consider using pre-chlorination to immobilize cells prior to coagulation and flocculation. $\text{Ca}(\text{OH})_2$ treatment options not only increase the pH of water which is sufficient to render cells immobile, but can also cause major damage to cells and therefore pre-chlorination should not be considered when dosing $\text{Ca}(\text{OH})_2$ as coagulant.

Chapter 8

General Conclusions

This research proposes different strategies to address the challenges and problems that may occur when *C. hirundinella* cells enter a conventional water treatment plant. The application of chlorophyll-*a* analysis and turbidity measurements are well-known methods used to evaluate the effectiveness of coagulant dosage during water treatment. However, the application of TPP analyses, at laboratories associated with South Africa's largest conventional water treatment plant (Zuikerbosch Water Treatment Plant) was adopted as a better practice in order to evaluate the removal of phytoplankton. This study showed that removal of other impurities together with *C. hirundinella* cells was improved when using TPP as indicator of appropriate coagulant dosages. Thus when source water used for drinking water production contains highly motile nuisance algae, such as *C. hirundinella*, in either moderate or abundant quantities, it is advisable to conduct jar stirring test experiments using both turbidity and TPP analyses as indicators of appropriate coagulant choice and dosages.

The cell covering of phytoplankton cells are different depending on the specific group; therefore, the zeta potential on the surface of different genera is different. Unlike cyanobacteria cells that are only covered by cell membranes, the relatively large sized *C. hirundinella* cells are covered with theca plates that have different surface charges due to different surface characteristics. Algal cells are negatively charged in natural water. The zeta potential of *C. hirundinella* cells can range between -13 mV and -21 mV, depending on the growth stage of the algae. The application value of zeta potential as a tool to determine coagulant dosages to improve coagulation conditions during water treatment is well-known. This study showed that zeta potential measurements can be used as a tool to optimize coagulant dosages to effectively remove not only green algae and cyanobacteria, but also *C. hirundinella* cells during coagulation and flocculation. Time series experiments revealed that *C. hirundinella* cells were not able to reset their surface charge and break out of the formed flocs. However, after chemical stabilization of water (where elevated pH levels is reduced) metabolically active cells in the supernatant will be able restore its zeta potential within 2-4 hours. Therefore, when treating source water containing *C. hirundinella* cells, the primary objectives of coagulation should include; 1) cell immobilization, while *C. hirundinella* cells remain intact and 2) good coagulation conditions (e.g. zeta potential ranging between +3 mV and -10 mV) to ensure flocs imbedded with *C. hirundinella* cells are formed.

Chapter 8

General Conclusions

This research proved that a combination of low Ca(OH)_2 dosages and organic polymer achieved better coagulation conditions based on zeta potential and pH requirements. However, the impacts of coagulant treatments on *C. hirundinella* cell morphology should be taken into account due to water-related problems that occur when cells lysis takes place. Changes in pH not only impacts on the motility of cells, but on the cell integrity as well. This research showed that when adding coagulants, such as $\text{Ca(OH)}_2\text{-SiO}_2$, major damage to *C. hirundinella* cells can be expected, especially when the pH was increased to levels above 10. The damage to cells can be reduced when dosing organic polymer as primary coagulant in combination with low Ca(OH)_2 dosages (as a coagulant aid). Less damage to cells were observed when dosing organic polymer in combination with Ca(OH)_2 , which can be observed by the integrity of cells (with organic material attached to their sulcus grooves) after sedimentation. When dosing organic polymer alone no major damage to the integrity was observed, which can be ascribed the lack of pH adjustments. The lack pH adjustments or corrections may result in water treatment problems, since most of the cells remain motile in the supernatant and have the ability to interfere with aggregation processes by disrupting flocs that form.

Flash mixing which form part of coagulation is an integral part of the water treatment process to ensure coagulants are dispersed evenly through the water in order to destabilize all suspended particles. This study showed that prior to the addition of coagulants, flash mixing resulted in disruption of cell morphology. Cell disruptions result in the release of organic material from the sulcus grooves situated at the ventral side of *C. hirundinella* cells. Organic material released from the sulcus grooves of cells remained attached to the cells. However, further mixing may cause attached organic material to be released into the water, which make cells fragile and results in broken cell pieces.

Scanning electron microscopy investigations revealed that both physical and chemical impacts on *C. hirundinella* cells occurred as a result of; 1) high energy flash mixing (before coagulants were added) and 2) over-dosing of chemicals, such as chlorine and Ca(OH)_2 . Although *C. hirundinella* cells are covered with thecal plates, it was shown that cells are likely to release organic material through parts of cell where the links between plates are the weakest. Water treatment plants that are not able to effectively remove *C. hirundinella* may experience taste and odour problems, especially when the pH levels are increased above 10 during coagulation and flocculation with alkaline coagulants.

Chapter 8

General Conclusions

A single *C. hirundinella* cell is relatively large in size (up to 450 µm in length and 50 µm in width) and therefore it has the potential to contribute to large quantities of organic compounds in the water. Many organic compounds (see Appendix Table A) associated with *C. hirundinella* cells, such as palmitic acid (amongst other fatty acids) and succinic acid (amongst other dicarboxylic acid) were identified as notable organic compounds due to the percentage contribution to the total organic compound composition. The odours detected by drinking water consumers when *C. hirundinella* cells present in the water, most probably originated from the aforementioned organic acids.

Taste and odour problems, associated with the presence of *C. hirundinella*, can be controlled during conventional water treatment when intact cells are removed during coagulation and flocculation. When cells penetrate into the final water, it may not only have a negative impact on the aesthetic water quality, but may pose various risks to human health when entering the drinking water distribution network. Water treatment plants that use chlorine to disinfect water prior to treatment should also monitor the impacts on cell integrity and monitor the formation of potential harmful disinfection by-products, such as trihalomethanes (THMs). Organic material may also be a carbon source for heterotrophic bacteria and invertebrates which can be detrimental to human health.

Unlike practices where pre-chlorination is primarily used to oxidize organic material (including algae and cyanobacteria), in this research the purpose of pre-chlorination was to immobilize *C. hirundinella*. Pre-chlorination for immobilization purposes is not widely applied in the water treatment industry. This study showed that relatively low chlorine dosages were required to immobilize *C. hirundinella* cells when compared to dosages suggested by previous studies. The challenges for this pre-chlorination practice may be unique, and may vary due the presence of elevated levels of natural organic matter and TOC that increase the chlorine demand. Pre-chlorination should however be evaluated in combination with various coagulants, since certain coagulant options (e.g. $\text{Ca(OH)}_2\text{-SiO}_2$) increase the pH significantly, which is sufficient to render cells immobile. Pre-chlorination can be performed in open aeration tanks to promote the release of the THMs that did form, reducing the effects of THMs further.

Chapter 9

Recommendations and Operational Guidelines

9.1 Introduction

Many operational water treatment problems could arise at conventional water works that are related to poor design, changes in source water quality, lack of maintenance, unskilled operators, and lack of process monitoring or poor management of the treatment facility. This research can help the water treatment fraternity to address water-related problems associated with *Ceratium hirundinella* (*C. hirundinella*) or other related algae. Analyses and procedures are applicable to conventional water treatment works where the capacity exists. This Chapter will therefore focus on operational guidelines and recommendations resulting from the following research aspects of different Chapters:

- source water quality containing algae such as *C. hirundinella* (Chapters 1, 2 and 3),
- pre-chlorination to immobilize *C. hirundinella* cells (Chapter 7),
- coagulation, flocculation and sedimentation (Chapter 3),
- rapid sand filtration (Chapter 3),
- organic compounds associated with taste and odour produced by *C. hirundinella* (Chapter 6),
- The use of laboratory analyses to assist plant operators and managers with optimisation strategies
- Zeta potential as indicator of coagulation conditions which can improve *C. hirundinella* removal,
- Using scanning electron microscopy to evaluate the impact of water treatment on *C. hirundinella* to reduce taste and odour problems,
- Additional operational guidelines and recommendations for sludge removal and filter backwash will be discussed in this Chapter with emphasis on the treatment of source water enriched with algae such *C. hirundinella*.

9.2 Recommendations and Operational Guidelines: Source water quality containing algae such as *C. hirundinella*

The presence of algae as well as the concentration thereof can be measured by measuring the total photosynthetic pigments (TPP, which also includes chlorophyll-*a*) in the treated water. During bloom forming periods, algae cause a discolouration of water; e.g. cyanobacteria and green algae species are responsible for a greenish discolouration, while freshwater dinoflagellate species are known for causing a brownish discolouration in water. Algae species are known to produce characteristic odours in source water (or tastes when present in drinking water). Chapter 2 (Literature Review) describe various surface characteristic of algae and cyanobacteria that allow algae to maintain their position (buoyancy) in the water column. Surface characteristics, cell- colony- and filament sizes together with other appendages allow algae to occur either at the surface water (0 m) or below the surface (e.g. 5 m, 10 m). Therefore, when algae and cyanobacteria are present in source water used for the production of drinking water, it is recommended to:

- i. Draw up a depth profile of the algae present using algae identification and enumeration protocols.
- ii. Determine the TPP or chlorophyll-*a* concentrations of different levels from the surface to the bottom to confirm the concentrations of phytoplankton at different levels.
- iii. Avoid breaking up of algae and cyanobacteria cells during water treatment, since it may result in the release of taste- and odour-causing organic material and cyanotoxins (e.g. microcystins, nodularin or cylindrospermopsin)

Alert level: This is not a guideline, but rather a level used during routine monitoring. When alert levels (e.g. 2000 cells/mL; 30 of > µg/L) are exceeded in the source water, the plant operators and managers should have sufficient knowledge on to how optimize the water treatment. For source water entering Rand Water treatment facilities, the following operational alert levels are used for (Du Preez and Van Baalen, 2006):

- i. **Phytoplankton concentrations:** a guideline for cyanobacteria is set at 2000 cells/mL. When *C. hirundinella* cells exceed 500 cells/mL in the source water, water treatment plant should be alert and implement optimization strategies.
- ii. **TPP (which include chlorophyll-*a*) or Chlorophyll-*a*:** 30 of > µg/L alert level in source water and 1 µg/L specification for drinking water is used by Rand Water.
- iii. **Cyanotoxins (microcystins, nodularin or cylindrospermopsin):** a guideline of 1 µg/L (lifetime exposure in drinking water).

9.3 Recommendations and Operational Guidelines: Pre-chlorination to immobilize *C. hirundinella* cells

Pre-chlorination in pre-determined appropriate dosages can be used to render motile cells immobile. Therefore, it is recommended to:

- i. Use low chlorine dosages ($< 1 \text{ mg/l}$) for immobilisation of *C. hirundinella* cells and reduce the formation of trihalomethanes (THMs).
- ii. Monitor source water quality (e.g. organic compounds such as DOC and TOC, phytoplankton identification and enumeration) when employing pre-chlorination, since the organic material such as DOC and TOC will increase the chlorine demand.
- iii. Determine the concentration contact time (CT) guidelines for *C. hirundinella* cells, since the CT requirements to inactivate different target organisms may differ as described by Van der Walt *et al.* (2009). When using pre-chlorination to render *C. hirundinella* cells immobile, it is recommended to use a chlorine dosage below 1 mg/l for a contact time of between 5 to 10 minutes. When cells were exposed to concentrations higher than 1 mg/l for a contact time longer than 10 minutes, cell damage was observed.
- iv. Consider using pre-chlorination to render motile cells immobile in order to assist coagulation and flocculation in water treatment plants that dose organic polymer alone.
- v. Check the integrity of cells (using a light microscope) when dosing chlorine for immobilization purposes,
- vi. Check the stability of water daily, since operational problems may occur when chemical dosages or treatment are modified. Schutte (2006) suggest using the Stasoft program to calculate the calcium carbonate precipitation program (CCPP) to check the stability of water.

Chlorine dosages used by South African water treatment facilities may be relatively higher than what was determined during this research for immobilization of *C. hirundinella* cells. Du Preez and Van Baalen, 2006 proposed the following chlorine dosage oxidation during pre-chlorination: $3 \text{ to } 4 \text{ mg/l}$ but can be $> 4 \text{ mg/l}$ with a residual chlorine value of 0.5 mg/l after 30 minutes contact time. During practices of dosing high chlorine concentrations ($> 4 \text{ mg/l}$), the cell integrity was not taken into account.

9.4 Recommendations and Operational Guidelines: Coagulation, Flocculation and Sedimentation

To avoid operational problems during periods of high *C. hirundinella* or algae concentrations in source water, it is recommended to:

- i. Perform regular jar stirring tests or full-scale laboratory simulations to optimize unit processes, especially when significant source water quality changes occur. Adjust coagulant dosages and mixer speed accordingly.
- ii. Gently disperse coagulants to reduce damage to cells during flash mixing (only for plants that are able to adjust the mixing speed to approximately 200 rpm).
- iii. A low Ca(OH)_2 dosage (10 mg/l) in combination with organic polymer, is an effective option to remove *C. hirundinella* cells.
- iv. Frequently remove sludge from sedimentation tanks.
- v. Test turbidity and TPP of supernatant on regular basis after different treatment processes to ensure that your plant's production specifications are met.
- vi. A zeta potential range between +3 mV and -10 mV is suggested for good coagulation conditions.
- vii. Zeta potential can be used as a tool to evaluate or determine the effectiveness of coagulation (only water treatment facilities where the capacity exists).
- viii. The zeta potential of immobilized *C. hirundinella* cells that are metabolically active remain within range for good coagulation, but after chemical stabilization cells may restore its surface charge and motility.

9.5 Recommendations and Operational Guidelines: Rapid sand filtration (Chapter 3)

Sand filtration is the final process capable of removing particular matter from the supernatant after sedimentation. In Chapters 3, 4 and 5 of this study it were suggested to use a low dosage of Ca(OH)_2 in combination with organic polymer as the primary coagulant. The proposed coagulant option together with other impurities (algal particles and Ca(OH)_2 particles) may impact negatively on the optimal functioning of the sand filters. Therefore, it is recommended to:

- i. Check on a regular basis for mud ball formation, and
- ii. Perform regular backwash cycles to:
 - ii.2 Avoid the accumulation of *Ceratium* cells that may cause taste and odour problems in the drinking water;
 - ii.3 As well as to avoid filter clogging.

Guidelines for filtrate turbidity in South Africa: SANS 0241 requires turbidity value of less than 1 NTU (for Class 1 water) or less than 0.1 NTU (for Class 0 water). Most South African water supply authorities accepted an internal production specification of 0.5 NTU or below

9.6 Recommendations and Operational Guidelines: *C. hirundinella* organic compounds associated with taste and odour (Chapter 6)

The organic compounds associated with *C. hirundinella* were mostly dicarboxylic acids (DAs) and fatty acids (FAs) that are released into the supernatant or settle to the bottom of sedimentation tanks. To avoid taste and odour problems caused by these organic compounds in the drinking water, the following specific recommendations are applicable:

- i. Operators and managers should understand the nature of organic compounds associated with algae and how it can affect water quality and what treatment options are available (Van der Walt *et al.*, 2009),
- ii. When taste and odour problems occur, operators and managers should develop a practical taste and odour testing strategy for routine operations,
- iii. To implement treatment strategies such as powdered activated carbon to absorb taste and odour causing compounds, and
- iv. Good pre-chlorination practices should be performed to avoid algal cell lysis (or destroy), since the oxidation of organic compounds may result in taste and odour problems, and
- v. Frequent sludge removal and backwashing cycles.

The SANS 241 guideline for taste aesthetic requirement is < 5 FTN (Class 1 water) and 5-10 FTN (Class 2 water). The SANS 241 guideline for odour aesthetic requirement is < 5 TON (Class 1 water) and 5-10 TON (Class 2 water).

9.7 Recommendations and Operational Guidelines: The use of laboratory analyses to assist plant operators and managers with optimisation strategies (Chapter 4, 5).

- i. The use of total photosynthetic pigment analyses, together with turbidity measurements during jar stirring tests are recommended to ensure the simultaneous removal of algae and inorganic particles.
- ii. Regular jar stirring tests to optimize processes and coagulants.
- iii. Daily phytoplankton analysis to identify the species and concentrations thereof in source water and after different stages of water treatment.

Chapter 9

Recommendations and Operational Guidelines

- iv. Light- and electron microscopy investigations on the morphology of algae to investigate the impact of treatment on algal cells to avoid cell disruptions and damage that may result in taste and odour problems.

The largest water treatment facility in South Africa, Rand Water, uses the following production specifications for drinking water that are related to this research to comply with a minimum of 95 %.

- | | |
|-------------------------------------|---------------------|
| • Turbidity (NTU) | < 0.5 |
| • pH (pH units) | >7.6 to <8.8 |
| • CCPP (mg/ℓ as CaCO ₃) | -7 to +10 inclusive |
| • Taste (FTN) | <1 |
| • Odour (TON) | <1 |
| • DOC (mg/ℓ) | <7 |
| • TPP (µg/ℓ) | <1 |

Chapter 10

References

- Abdullah M.P., Yee I.F., Ata S., Abdullah A., Ishak B. and Abidin K.N.Z. (2009) The Study of Interrelationship Between Raw Water Quality Parameters, Chlorine Demand and the Formation of Disinfection By-products. *Physics and Chemistry of the Earth* **34** 806-811.
- Agus E., Voutchko N., Sedlak D.L. (2009) Disinfection By-Products and Their Potential Impact on the Quality of Water Produced by Desalination Systems: A Literature Review. *Desalination* **237** 214-237.
- American Public Health Association (APHA) (2013) Standard Methods for the Examination of Water and Wastewater. 22nd Edition. Washington D.C., United States of America.
- Anderson D.M. (1997) Turning back the harmful red tide. *Nature* **388** 513-514.
- Antonopoulou M., Evgenidou E., Lambropoulou D. and Konstantinou I. (2014) A review on advanced oxidation processes for the removal of taste and odor compounds from aqueous media. *Water Research* **53** 215-234.
- Beeharry N., Lowe J.E., Hernandez A.R., Chambers J.A., Fucassi F., Cragg P.J., Green M.H.L. and Green I.C. (2003) Linoleic Acid and Antioxidants Protect Against DNA Damage and Apoptosis Induced by Palmitic Acid. *Mutation Research* **530** 27-33.
- Bernhardt H. and Clasen J. (1991) Flocculation of Microorganisms. *Journal of Water Supply-Research and Technology-Aqua* **40** 76-87.
- Bernhardt H., Lüsse B. and Hoyer O. (1986) The Addition of Calcium to Reduce The Impairment Of Flocculation by Algogenic Organic Matter. *Z. Wasser-Abwasser-Forsch* **19** 219-228.
- Betancourt W.Q. and Rose J.B. (2004) Drinking water treatment processes for removal of *Cryptosporidium* and *Giardia*. *Vet Parasito* **126** (1-2) 219-234.
- Bolto B. (1995) Soluble Polymers in Water Purification. CSIRO Chemical and Polymers. *Prog. Polym. Sci* **20** 987-1041.
- Bolto B. and Gregory J. (2007) Review: Organic Polyelectrolytes in Water Treatment. *Water Research* **41** 2301-2324.
- Bolton B. and Halpern B.P. (2010) Orthonasal and Retronasal but not Oral-Cavity-Only Discrimination of Vapor-phase Fatty Acids. *Chemical Senses. Sensory Sciences: Research Development and Quality*, Glenview, USA. 1-38.

- Brookhaven Instruments Corporation (BIC) (2002) Instrument Manual for ZetaPlus Zeta Potential Analyzer. Holtsville, New York 11742.
- Canter-Lund H. and Lund W.G. (1995) Freshwater Algae, Their Microscopic World Explored. Biopress Ltd. 1-360.
- Cheng W.P. and Chi F. (2003) Influence of Eutrophication on Coagulation Efficiency in Reservoir Water. *Chemosphere* 53 773-778.
- Clesceri L.S., Greenberg A.E. and Eaton A.D. (1998) Standard Method for the Examination of Water and Waste Water, 20th Ed. American Public Health Association, Washington, DC.
- Chow C.W.K., Drikas M., House J., Burch M.D. and Velzeboer M.A. (1999) The Impact of Conventional Water Treatment Processes on the Cells of Cyanobacterium *Microcystis aeruginosa*. *Water Research* 33 (15) 3253-3262.
- Davies J.M., Roxborough M. and Mazumdera A. (2004) Origins and Implications of Drinking Water Odor in Lakes and Reservoirs of British Columbia, Canada. *Water Research* 38 1900-1910.
- Degrémont G. (2007) Water Treatment Handbook. Volume 1 and 2. 7th Edition. Lavoiser.
- Department of Water Affairs and Forestry (DWAf) (1996) South African Water Quality Guidelines (SAWQG): Domestic Water Use Volume 1. CSIR Environmental Services, Pretoria, South Africa.
- Divakaran R. and Sivasankara Pillai V.N. (2002) Flocculation of Algae Using Chitosan. *Journal of Applied Phycology* 14 419-422.
- Dodds L., King W., Allen A.C., Armson B.A., Fell D.B. and Nimrod C. (2004) Trihalomethanes in Public Water Supplies and Risk Of Stillbirth. *Epidemiology* 15 (2) 179-186.
- Du Preez H.H. and Van Baalen L. (2006) Generic Incident Management Framework for Toxic Blue-Green Algal Blooms, for the Application by Potable Water Suppliers. *Water Research Commission Report* TT 263/06.
- Duan J. and Gregory J. (2003) Coagulation by Hydrolysing Metal Salts. *Advances in Colloidal and Interface Science* 100-102 475-502.
- Ebeling J.M., Rishel K.L. and Sibrell P.L. (2005) Screening and Evaluation of Polymers as Flocculation Aids for the Treatment of Aquacultural Effluents. *Aquacultural Engineering* 33 235-249.
- Edzwald J.K., Pernitsky D.J. and Parmenter W.L. (2000) Polyaluminum Coagulants for Drinking Water Treatment: Chemistry and Selection. Article in Chemical Water and Wastewater Treatment VI. Hermann H. Hahn, Erhard Hoffmann and Hallvard Ødegard (Eds.). Springer, Berlin, Heidelberg, New York.
- Eikebrokk B., Juhna T. and Østerus S.W. (2006) Water Treatment by Enhanced Coagulation. Operational Status Optimization Issues. *TECHNEAU D* 5.3.1a.1-107.

- Ewerts H., Swanepoel A. and Du Preez H.H. (2013) Efficacy of conventional drinking water treatment processes in removing problem-causing phytoplankton and associated organic compounds. *Water SA* **39** (5) 739-750.
- Ewerts H., Swanepoel A., Du Preez H.H. and Van der Walt N (2014) Total photosynthetic pigments in addition to turbidity during the selection of coagulant treatments: drinking water treatment perspective. *Journal of Water Supply: Research and Technology – Aqua*. In Press, Uncorrected Proof. DOI: 10.2166/aqua.2014.020.
- Feki W., Hamza A., Frossard V., Abdennadher M., Hannachi I., Jacquot M., Belhassen M. and Aleya L. (2013) What are the potential drivers of blooms of the toxic dinoflagellate *Karenia selliformis*? A 10-year study in the Gulf of Gabes, Tunisia, southwestern Mediterranean Sea. *Harmful Algae* **23** 8-18.
- Fenchel T. (2001) How Dinoflagellates Swim. *Protist* **152** 329-338.
- Ferreira L. and Du Preez H.H. (2012) Investigation into the Occurrence of Aquatic Invertebrates Throughout Drinking Water Purification Plants. *Water Science & Technology: Water Supply* **12** (2) 250-257.
- Garrett R.H. and Grisham C.H. (2005) Biochemistry 3rd edition. *Thomson Brooks/Cole* 1-1086.
- Geldenshuys J.C., Giard E., Harmse M., Neveling K. and Potgieter M. (2000) The use of Ozonation in combination with Lime and Activated Silicate in Water Treatment. *Water Research Commission Report* 446/1/00. Pretoria, South Africa.
- Gerde J.A., Yao L., Lio J.Y., Wen Z. and Wang T. (2014) Microalgae flocculation: Impact of flocculant type, algae species and cell concentrations. *Algal Research* **3** 30-35.
- Gligora M., Plenковиć-Moraj A. and Ternjej I. (2003) Seasonal distribution and morphological changes of *Ceratium hirundinella* in two mediterranean shallow lakes. *Hydrobiologia* **506-509** 213-220.
- Goslan E.H., Krasner S.W., Bower N., Rocks S.A., Holmes, P., Levy L.S. and Parsons S.A. (2009) A Comparison of Disinfection By-Products Found In Chlorinated and Chloraminated Drinking Waters in Scotland. *Water Research* **43** 4698-4706.
- Graham M., Blignaut J., de Villiers L., Mostert D., Sibande X., Gebremedhin S., Harding W., Rossouw N., Freese N. S., Ferrer S. and Browne M. (2012) Development of a Generic Model to Assess the Costs Associated with Eutrophication. *Water Research Commission Report* 1568/1/12.
- Grigorszky I., Padisak J., Borics G., Schitchen C. and Borbely G. (2003) Deep Chlorophyll Maximum by *Ceratium hirundinella* (O. F. Müller) Bergh in a Shallow Oxbow in Hungary. *Hydrobiologia* **506-509** 209-212.
- Hamlaoui S., Couté A., Lacroix G. and Lescher-Moutoué F. (1998) Nutrient and Fish Effects on the Morphology of the Dinoflagellate. *Ecology*. Elsevier, Paris.
- Harland R. (1988) Dinoflagellates, Their Cyst and Quaternary Stratigraphy. *New Phytologist* **108** (1) 111-120.

- Harrington G.W., Beach D.H., Dunham J.E. and Holz G.G. (2007) The Polyunsaturated Fatty Acids of Marine Dinoflagellates. *The Journal of Eukaryotic Microbiology* **17** (2) 213-219.
- Hart R.C. and Wragg P.D. (2009) Recent Bloom of the Dinoflagellate *Ceratium* in Albert Falls Dam (Kzn): History, Causes, Spatial Features and Impacts on a Reservoir Ecosystem and its Zooplankton. *Water SA* **35** (4) 455-468.
- Hebert A., Forestier D., Lenés D., Benanou D., Jacob S., Arfi C., Lambolez L. and Levi Y. (2010) Innovative Method for Prioritizing Emerging Disinfection By-products (DBPs) in Drinking Water on the Basis of Their Potential Impact on Public Health. *Water Research* **44** 3147-3165.
- Hedger R.D., Olsen N.R.B., George D.G., Malthus T.J. and Atkinson P.M. (2004) Modeling Spatial Distributions of *Ceratium hirundinella* and *Microcystis* spp. in a Small Productive British Lake. *Hydrobiologia* **528** 217-227.
- Henderson R., Parsons S.A. and Jefferson B. (2007) The Impact Of Algal Properties and Pre-Oxidation on Solid Liquid Separation of Algae. Elsevier, Cranfield University, United Kingdom.
- Henderson R., Parsons S.A. and Jefferson B. (2008) The Impact of Algal Properties and Pre-Oxidation on Solid-Liquid Separation of Algae. *Water Research* **42** (8-9) 1827-1845.
- Hoko Z. and Makado P.K. (2011) Optimization Of Algal Removal Process At Morton Jaffray Water Works, Harare, Zimbabwe. *Physics and Chemistry of the Earth* **36** 1141-1150.
- Janse van Vuuren S. and Pieterse A.J.H. (2000) Seasonal and Spatial Aspects of Phytoplankton in the Vaal River, A Turbid and Polluted River in South Africa. *Verh. Internat. Verein. Limnol.* **27** 1946-1949.
- Janse van Vuuren S., Taylor J., Gerber A. and Van Ginkel C. (2006) Easy Identification of the Most Common Freshwater Algae- A Guide for the Identification of Microscopic Algae in South Africa ISBN 0-621-35471-6. *North-West University and Department of Water Affairs and Forestry* 1-200.
- Janse van Vuuren, S. (1996) Environmental Variables, Abundance and Seasonal Succession of Phytoplankton Populations in the Vaal River at Balkfontein. M.Sc., Potchefstroom, PU for CHE, South Africa 1-164.
- Jarvis P., Jefferson B. and Parsons S.A. (2005) Breakage, Regrowth and Fractal Nature of Flocs. *Environmental Science and Technology* **39** (7) 2307-2314.
- Jefferson B., Sharp E.L., Goslan E., Henderson R. and Parsons S.A. (2004) Application of Charge Measurement to Water Treatment Processes. *Water Science and Technology: Water Supply* **4** (5-6) 49-56.
- John D.M., Whitton B.A. and Brook A.J. (2002) The freshwater algal flora of the British Isles. *Cambridge University Press* 1-702.

- Joyce L.B., Pitcher G.C., Du Randt A. and Monteiro P.M.S. (2005) Dinoflagellate cysts from surface sediments of Saldanha Bay, South Africa: an indication of the potential risk of harmful algal blooms. *Harmful Algae* **4** 309-318.
- Jun H., Lee Y., Lee B and Knappe D.R.U. (2001) Effectiveness of coagulants and coagulant aids for the removal of filter-clogging *Synedra*. *Journal of Water Supply: Research and Technology - Aqua* **50** (3) 135-148.
- Jurado-Sánchez B., Ballesteros E. and Gallego M. (2012) Determination of Carboxylic Acids in Water by Gas Chromatography-Mass Spectrometry after Continuous Extraction and Derivatisation. *Talanta* **93** 224-232.
- Knappe D.R.U., Belk R.C., Briley D.S., Grandy S.R., Rastogi N. and Rike A.H. (2004) Algae Detection and Removal Strategies for Drinking Water Treatment Plants. *AWWA Research Foundation* 1-466.
- Kwak D.H., Kim S.J., Jung H.J. Won C.H., Kwon S.B., Ahn H.W. and Lee J.W. (2006) Removal of clay and blue-green algae particles through zeta potential and particle size distribution in the dissolved air flotation process. *Water Science and Technology: Water Supply* **6** (1) 95-103.
- LeChevallier M. and Au K. (2004) Water Treatment and Pathogen Control: Process Efficiency in Achieving Safe Drinking Water. Published by WHO and IWA, ISBN: 184339 069 8. London, United Kingdom.
- Leopold P. and Freese S.D. (2009) A Simple Guide To The Chemistry Selection and Use of Chemicals for Water and Wastewater Treatment. *Water Research Commission Report* TT 405/09.
- Linde J.J., Parsons W.J., Van Der Walt E. and Geldenhuys J.C. (2001) Rand Water Treatment Philosophy and Guide. *Rand Water, Water Treatment Technology Division Process Technology* 1-100.
- Liu H.L., Wang D.S., Shi B.Y., Wang M. and Zhang J.S. (2006) Enhanced Coagulation and NOM Fractionation Study of a Typical Southern Water. *Environmental Science* **27** (5) 909-912.
- Lohtaja H. (2005) Optimisation of flotation chemicals for effluent by-pass and tertiary treatment. *Bio-ja elintarviketekniikka*.
- Lui Y.S., Qiu J.W., Zhang Y.L., Wong M.H. and Liang Y. (2011) Algal-derived organic matter as precursors of disinfection by-products and mutagens upon chlorination. *Water Research* **45** 1454-1462.

- Lund J.W.G., Kipling C. and LeCren E.D. (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia*, 11, 143 – 170.
- Ma M., Liu R., Liu H. and Qu J. (2012) Chlorination of *Microcystis aeruginosa* Suspension: Cell Lyses, Toxin Release and Degradation. *Journal of Hazardous Materials* 217-128 279-285.
- Ma M., Liu R., Liu H., Qu J. and Jefferson W. (2012) Effects and Mechanisms of Pre-chlorination on *Microcystis aeruginosa* Removal by Alum Coagulation: Significance of the Released Intracellular Organic Matter. *Separation and Purification Technology* 86 19-25.
- Maazouzi C., Masson G., Izquierdo M.S. and Pihan J. (2008) Midsummer Heat Wave Effects on Lacustrine Plankton: Variation of Assemblage Structure and Fatty Acid Composition. *Journal of Thermal Biology* **33** 287-296.
- Matilainen A. and Sillanpää M. (2010) Removal of Natural Organic Matter from Drinking Water by Advanced Oxidation Processes. *Chemosphere* 80 351-365.
- Matsuyama Y. (2012) Impacts of the harmful dinoflagellate *Heterocapsa circularisquama* bloom on shellfish aquaculture in Japan and some experimental studies on invertebrates. *Harmful Algae* 14 144-155.
- Matthews M.W. (2014) Eutrophication and cyanobacterial blooms in South African inland waters: 10 years of MERIS observations. *Remote Sensing of Environment*. In Press, Corrected Proof. DOI 10.1016/j.rse.2014.08.010
- Matthews M.W., Bernard S. and Winter K. (2010) Remote sensing of cyanobacteria-dominant algal blooms and water quality parameters in Zeekoevlei, a small hypertrophic lake, using MERIS. *Remote Sensing of Environment*, **114** 2070-2087.
- McCormick N.J, Porter M. and Walsh M.E. (2010) Disinfection By-products in Filter Backwash Water: Implication to Water Quality in Recycle Designs. *Water Research* 44.
- Ministry of Health (2001) Public Health Risk Management Plan Guide. *Treatment Processes – Dissolved Air Flotation* Version 1, Ref P5.2
- Nakano S., Nakajima T. and Hayakawa K. (1999) Blooms of the Dinoflagellate *Ceratium hirundinella* in Large Enclosures Places in Lake Biwa. *Jpn. J. Limnol.* 60 495-505.
- Ndikubwimana T., Zeng X., Liu Y., Chang J. and Lu Y. (2014) Harvesting of microalgae *Desmodesmus* sp. F51 by bioflocculation with bacteria bioflocculant. *Algal Research* **6** 186-193.
- Palmer C.M. (1980) Algae and Water Pollution - The identification, significance, and control of algae in water supplies and in polluted water. *Castle House of Publications Ltd.*, England.
- Peter A., Köster O., Schildknecht A. and von Gunten U. (2009) Occurrence of dissolved and particle-bound taste and odor compounds in Swiss lake waters. *Water Research* 43

2191-2200.

- Pfiester L.A. (1971) Periodicity of *Ceratium hirundinella* (O.F.M) Dujardin and *Peridinium cinctum* (O.F.M) Ehrenberg in Relation to Certain Ecological Factors. *Castanea, Southern Appalachian* 36 (4) 247-257.
- Pieterse A.J.H., Basson N.D., Cloot A., Mienie L.J., Traut D.F. and Visser R. (2000) The occurrence, distribution and removal of algal species and related substances in a full-scale water purification plant. *Water Research Commission Report* 567/1/00.
- Prescott, G.W., Bamrick, J., Cawley, E. and Jaques, W.M., 1978. How to know the freshwater algae. 3rd Edition, University of Montana, United States of America.
- Prévost M., Laurent P., Servais P. and Joret J. (2005) Biodegradable Organic Matter in Drinking Water Treatment and Distribution. American Water Works Association ISBN – 58321-367-8. *Science and Technology* 1-300.
- Ratnaweera H., Hiller N. and Bunse U. (1999) Comparison of the coagulation behaviour of different Norwegian aquatic NOM sources. *Environment International* 25 (2-3) 347-355.
- Rand Water (2015) Rand Water Webpage.
<http://www.randwater.co.za/waterandinfrastructuremanagement/pages/waterpurification.aspx> (Accessed: 13 May 2015)
- Rodríguez E., Sordo A., Metcalf J.S. and Acero J.L. (2007) Kinetics of the oxidation of cylindrospermopsin and anatoxin-a with chlorine, monochloramine and permanganate. *Water Research* 41 2048-2056.
- Roos J.C. and Pieterse A.J.H. (1995) Salinity and Dissolved Substances in the Vaal River at Balkfontein, South Africa. *Hydrobiologia* 306 41-51 pp.
- Rositano J., Newcombe G., Nicolson B. and Sztajnbock P. (2001) Ozonation of NOM and algal toxins in four treated waters. *Water Research* 35 23-32.
- Schutte F. (2006) Handbook for the Operation of Water Treatment Works. The *Water Research Commission. The Water Institute of Southern Africa*. Water Utilisation Division, Department of Chemical Engineering, University of Pretoria. *Water Research Commission Report* TT 265/06.
- Sharp E.L., Banks J., Billica J.A. Gertig K.R., Henderson R., Parsons S.A., Wilson D. and Jefferson B. (2005) Application of zeta potential measurements for coagulation control: pilot-plant experiences from UK and US waters with elevated organics. *Water Science and Technology: Water Supply* 5 49-56.
- Sharp E.L., Jarvis P., Parsons S.A. and Jefferson B. (2006) Impact of Fractional Character on the Coagulation of NOM. *Physicochem Eng. Aspects* 286 104-111.
- Shears N.T. and Ross P.M. (2009) Blooms of benthic dinoflagellates of the genus *Ostreopsis*; an increasing and ecologically important phenomenon on temperate reefs in New Zealand and worldwide. *Harmful Algae* 8 916-925.
- Shehata S.A., Badr S.A. and Wahba S.Z. (2002) Drinking Water Treatment Options for Eliminating

- Freshwater Algae. *Int. J. Environ. Stud.* 59 679-688.
- Shen Q., Zhu J., Cheng L., Zhang J., Zhang Z. and Xu X. (2011) Enhanced Algae Removal by Drinking Water Treatment of Chlorination Coupled with Coagulation. *Desalination* 271 236-240.
- Silke J., O'Beirn F. and Cronin M. (2005) *Karenia mikimotoi*: An exceptional dinoflagellate bloom in western Irish Water, Summer 2005. *Marine Environment and Health Series* 21. ISSN NO: 1649-0053.
- Smayda T.J. (2010) Adaptations and Selection of Harmful and Other Dinoflagellate Species in Upwelling Systems. 2. Motility and Migratory Behaviour. *Progress in Oceanography* 85 71-91.
- South African National Standards 241-1 (SANS 214-1) (2011) Drinking Water. Part 1: Microbiological, Physical, Aesthetic and Chemical Determinants. Edition 1. ISBN 978-0-626-26115-3.
- Steynberg M.C., Geldenhuys J.C., Guclielmi M.M., Grobler S. and Maree B. (1994) The Influence of Water Quality on the Efficiency of Chlorine Dioxide as Pre-oxidant and Algicide in the Production of Potable Water. *Water Research Commission Report* 281/1/94.
- Swanepoel A. (2014) Early warning system for the prediction of algal-related impacts on drinking water purification. Thesis submitted for the degree Philosophiae Doctor in Botany at the Potchefstroom Campus of the North-West University, South Africa, pp. 145.
- Swanepoel A., Du Preez H.H., Dusrath I. and Rajele M. (2008a) *Ceratium hirundinella* reveals algal penetration into the potable water at Rand Water. Paper published as part of the conference proceedings of the *Water institute of Southern Africa* (WISA 2008), Sun City, South Africa.
- Swanepoel A., Du Preez H.H., Schoeman C., Janse van Vuuren S. and Sundrum A. (2008 b) Condensed Laboratory Methods for Monitoring Phytoplankton, Including Cyanobacteria, in South African freshwaters. *Water Research Commission. Report* TT323/08.
- Takaara T., Sanoa D., Konnob H. and Omuraa T. (2007) Cellular Proteins of *Microcystis aeruginosa* Inhibiting Coagulation with Polyaluminum Chloride. *Water Research* 41 1653-1658.
- Taki K., Seki T., Mononobe S. and Kato K. (2008) Zeta potential measurements on the surface of blue-green algae particles for micro-bubble process. *Water Science and Technology* 57 (1) 19-25.
- Taylor F.J.R. (1987) Dinoflagellate morphology. *The Biology of Dinoflagellates*. Blackwell, Oxford 24-91.
- Teixeira M.R. and Rosa M.J. (2006) Comparing Dissolved Air Flotation and Conventional Sedimentation to Remove Cyanobacterial Cells of *Microcystis aeruginosa*: Part 1: The Key Operating Conditions. *Separation and Purification Technology* 52 (1) 84-94.

- TerBraak C.J.F. (1988) CANACO – a FORTRAN program for canonical community ordination by [partial] [detrended] [canonical] correspondence analysis, principal component analysis and redundancy analysis (version 2.1). Report LWA-88-02, Agricultural Mathematics Group, Wageningen, The Netherlands, 1-95.
- United States Environmental Protection Agency (USEPA) 2006 Stage 2: Disinfectants and Disinfection By-products Rule. Washington DC, United States of America.
- Van der Walt M., Krüger M. and Van der Walt C. (2009) The South African oxidation and disinfection manual. WISA Oxidation and Disinfection Division. Water Research Commission, South Africa. *Water Research Commission Report* TT 406/09.
- Van der Walt N. (2012) Investigation Into the Occurrence of the Dinoflagellate, *Ceratium hirundinella* in source water and the impact thereof on drinking water purification. M.Sc. Dissertation, School for Environmental Sciences and Development, North-West University, Potchefstroom, South Africa.
- Van Ginkel C.E. and Conradie B. (2001) Potential toxic algal incident in the Orange River, Northern Cape, 2000. Report number: N/D801/12/EEQ/0800, Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria, South Africa. 1-47.
- Van Ginkel C.E. and Silberbauer M. (2007) Temporal trend in total phosphorus, temperature, oxygen, chlorophyll a and phytoplankton populations in Hartbeespoort Dam and Roodeplaat Dam, South Africa, between 1980 and 2000. *African Journal of Aquatic Science* **32** (1) 63-70.
- Van Ginkel C.E., Hohls B.C. and Vermaak E. (2001) A *Ceratium hirundinella* Bloom in Hartbeespoort Dam, South Africa. *Water S.A.* 27 (2) 269-276.
- Van Ginkel C.E., Hohls B.C. Belcher A., Vermaak E. and Gerber A. (2001b) Assessment of the trophic status project. Internal Report No N/0000/00/DEQ/1799. Institute for Water Quality Studies. Department of Water Affairs and Forestry (now Department of Water and Sanitation), Pretoria, South Africa.
- Van Ginkel C., Hongqing C., Recknagel F. and Du Plessis S. (2007) Forecasting of dinoflagellate blooms in warm-monomictic hypertrophic reservoirs in South Africa by means of rule-based agents. *Water SA* **33** (4) 531-538.
- Venkobachar C., Iyengar L., Rao A. (1977) Mechanism of disinfection: effect of chlorine on cell membrane functions. *Water Research* 11 727-729.
- Visser, R. & Pieterse, A.J.H. 1999 Algal species penetrating unit processes in the Balkfontein purification plant, in Pieterse, A.J.H. (Ed.) The occurrence, distribution and removal of algal species and related substances in a full-scale water purification plant. Part 2, detailed report. Report to the Water Research Commission, Pretoria, South Africa.
- Wakeman, R.J. and Tarleton, E.S. 1999. Filtration: Equipment selection, modeling, and process simulation. Elsevier Science Ltd., New York, 446.
- Wetzel R.D. (1983) Limnology (2nd edition). *Saunders College Publishing*. Fort Worth, TX.

- Wetzel R.G. 2001. Limnology. 3rd ed. San Diego. *Academic Press* (Elsevier) 1-1006.
- Williams P.J.L.B. and Laurens L.M.L. (2010) Microalgae as biodiesel and biomass feedstocks: review and analysis of the biochemistry, energetics and economics. *Energy Environmental Science* 3 554.
- World Health Organization (WHO) (2004) Drinking Water Series: Safe Piped Water. Managing Microbial Water in Piped Distribution Systems.
- Wu D. and Duirk S.E. (2013) Quantitative analysis of earthy and musty odors in drinking water sources impacted by wastewater and algal derived contaminants. *Chemosphere* 91 1495-1501.
- Yan Y. and Jameson G.J. (2004) Application of the Jameson Cell Technology for Algae and Phosphorus Removal from Maturation Ponds. *International Journal of Mineral Processing* 73 (1) 23-28.
- Yang T., Kwak D., Kim S., Lee J. and Jung H. (2005) Effects of zeta potential of clay and Blue-green algae particles on flotation efficiency in DAF process. Proceedings of Environmental Engineering Research Conference 2005 (4.28~4). *Water Sci. Technology.: Water Supply* 4 (28-4.30) 309-316.
- Young C.C. and Suffet I.H. (1999) Development of a standard method – Analysis of compounds causing taste and odor in drinking water. *Water Science and Technology* 40 (6) 279-285.
- Yuheng W., Shengguang Z., Na L. and Yixin Y. (2011) Influence of various aluminum coagulants on algae floc structure, strength and flotation effect. *Procedia Environmental Sciences* 8 75-80.
- Zamyadi A., Ho L., Newcombe G. and Bustamante H. (2011) Fate of toxic cyanobacterial cells and disinfection by-products formation of chlorination. *Water Research* 1-12.
- Zamyadi A., Ho L., Newcombe G., Bustamante H. and Prévost M. (2012) Fate of toxic caynobacterial cells and disinfection by-products after chlorination. *Water Research* 46 (5) 1524-1534.
- Zhao Y., Yu J., Su M., An W. and Yang M. (2013) A fishy odor episode in north China reservoir: Occurrence, origin, and possible odor causing compounds. *Journal of Environmental Science* 25 (12) 2361-2366.
- Zhu H., Smith D.W., Zhou H. and Stanley S.J. (1995) Improving removal of turbidity causing materials by using polymers as a filter aid. *Water Research*, 30, 103-114.

Appendix

Tables A-C

Appendix Tables A-C

Appendix Table A

Other organic compounds (< 1 %) Abundance analysed in concentrated *C. hirundinella* water samples that contribute to 7.81 % Abundance of the organic compound composition (Table 6.2).

	Mean	Std. D	Min	Max	(< 1 %) Abundance
1,2,4,5-Tetramethylbenzene	0.033	0.006	0.028	0.042	0.005
1-Hydroxycyclohexene	2.276	0.316	1.929	2.639	0.343
2,3-Dihydroxybutane	0.083	0.096	0.000	0.178	0.012
2-Ethyl-1,4-dimethyl-benzene	0.075	0.027	0.040	0.107	0.011
2-Hydroxy-3-methylvaleric acid	0.184	0.133	0.067	0.326	0.028
2-Hydroxybutyric acid	0.145	0.110	0.000	0.259	0.022
2-Hydroxyglutaric acid	0.126	0.123	0.022	0.303	0.019
2-Hydroxyisocaproic acid	0.491	0.286	0.246	0.831	0.074
2-Hydroxyisovaleric acid	0.098	0.114	0.000	0.200	0.015
2-Ketoisovaleric acid	0.032	0.048	0.000	0.102	0.005
3,4-Dihydroxybutyric acid	0.173	0.058	0.107	0.246	0.026
3-Hydroxybutyric acid	0.250	0.180	0.000	0.430	0.038
3-Hydroxycaproic acid	0.008	0.011	0.000	0.023	0.001
3-Hydroxyoctanoic acid	0.042	0.054	0.000	0.114	0.006
3-Hydroxypropionic acid	0.038	0.015	0.025	0.055	0.006
4-Ethyl-1,2-dimethyl-benzene	0.024	0.032	0.000	0.068	0.004
4-Hydroxybutyric acid	0.056	0.054	0.000	0.103	0.008
6-Hydroxynicotinic acid	0.042	0.058	0.000	0.124	0.006
Adipic acid	0.132	0.050	0.061	0.181	0.020
Benzamide	0.006	0.011	0.000	0.023	0.001
Benzoic acid	0.093	0.020	0.073	0.117	0.014
Boric acid	4.250	0.610	3.772	5.081	0.641
Capric acid	0.070	0.019	0.046	0.094	0.011
Caproic acid	0.007	0.011	0.000	0.024	0.001
Dihydrothymine	0.008	0.011	0.000	0.023	0.001
Dodecamethylpentasiloxane	2.961	0.399	2.531	3.373	0.446
Fumaric acid	0.275	0.172	0.124	0.501	0.041
Glutaric acid	0.860	0.574	0.437	1.652	0.130
Glycerol	0.294	0.131	0.207	0.485	0.044
Glycolic acid	0.354	0.131	0.191	0.512	0.053
Lactic acid	2.554	1.976	0.687	5.192	0.385
Malic acid	0.321	0.073	0.218	0.384	0.048
Methylsuccinic acid	0.044	0.032	0.000	0.075	0.007
N-Acetylleucine	0.004	0.004	0.000	0.008	0.001
Nonanoic acid	0.078	0.009	0.068	0.086	0.012
Octamethyltetrasiloxane	0.010	0.006	0.000	0.014	0.001
Octanoic acid	0.026	0.015	0.009	0.040	0.004
Octanol	0.008	0.002	0.006	0.010	0.001
Oxalic acid	0.521	0.471	0.236	1.225	0.079
Phenol	0.098	0.018	0.081	0.119	0.015
Phenylacetic acid	1.846	1.721	0.314	3.464	0.278
Phenylpropionic acid	3.355	3.516	0.000	6.588	0.506

Appendix

Tables A-C

Phosphoric acid	0.025	0.018	0.013	0.050	0.004
Pyrocatechol	0.173	0.026	0.140	0.202	0.026
Pyroglutamic acid	0.113	0.060	0.032	0.177	0.017
Pyruvic acid	0.210	0.273	0.025	0.616	0.032
Thymine	1.095	0.866	0.043	1.887	0.165
Tiglic acid	0.966	0.137	0.799	1.093	0.146
Undecanoic acid	0.030	0.007	0.025	0.041	0.005
Uracil	2.222	1.581	0.063	3.723	0.335
Urea	0.011	0.012	0.000	0.023	0.002

Appendix Table A continue

Other organic compounds (< 1 %) Abundance analysed in concentrated *C. hirundinella* water samples that contribute to 7.81 % Abundance of the organic compound composition (Table 6.2).

	Mean	Std. D	Min	Max	(< 1 %) Abundance
1,2,3-Trihydroxybutane	0.017	0.034	0.000	0.068	0.003
1,6-Anhydro-.beta.-d-glucose	0.054	0.032	0.025	0.100	0.008
1-Hexadecanol	0.094	0.073	0.026	0.164	0.014
1H-Indole-2-Acetic acid	0.009	0.010	0.000	0.018	0.001
1-Methyl-3-propyl-benzene	0.024	0.023	0.000	0.053	0.004
2-Ethylhydracrylic acid	0.030	0.061	0.000	0.121	0.005
2-Hydroxy-2-methylbutyric acid	0.016	0.029	0.000	0.059	0.002
2-Octenedioic acid	0.009	0.018	0.000	0.035	0.001
3-(3-Hydroxyphenyl)propanoic acid	0.332	0.257	0.033	0.567	0.050
3-(4-Hydroxy-2,5-Dioxoimidazolidin-4-yl)propanoic-acid	0.045	0.063	0.000	0.134	0.007
3,4-Dihydroxyphenylglycol	0.006	0.013	0.000	0.025	0.001
3-Hydroxy-2-butanone	0.054	0.072	0.000	0.160	0.008
3-Hydroxycapric acid	0.085	0.025	0.053	0.113	0.013
4-Hydroxybenzoic acid	0.048	0.031	0.023	0.091	0.007
5-Hydroxymethyluracil	0.025	0.031	0.000	0.062	0.004
7,10,13,16-Docosatetraenoic acid	0.752	1.504	0.000	3.009	0.113
Azelaic acid	0.031	0.029	0.000	0.069	0.005
Benzophenone	0.037	0.013	0.022	0.052	0.006
Benzyl butyl phthalate	0.087	0.150	0.007	0.312	0.013
Cholesta-2,4-diene	0.006	0.012	0.000	0.024	0.001
Cholesta-3,5-diene	0.164	0.289	0.005	0.597	0.025
Cholesta-4,6-dien-3-ol, (3β)-	0.011	0.022	0.000	0.043	0.002
Cinnamic acid	0.005	0.009	0.000	0.018	0.001
Citric acid	0.027	0.027	0.000	0.058	0.004
Dehydroabietic acid	0.055	0.093	0.000	0.193	0.008
Dibutyl phthalate	0.538	0.799	0.120	1.736	0.081
Dimethylmalonic acid	0.016	0.032	0.000	0.065	0.002
Docosanoic acid	0.012	0.015	0.002	0.034	0.002
Dodecanoic acid	0.579	0.118	0.410	0.670	0.087
Eicosanoic acid	0.127	0.094	0.037	0.234	0.019
Ethylmalonic acid	0.012	0.025	0.000	0.049	0.002
Gamma-Linolenic acid	5.488	2.856	1.388	7.888	0.827
Glyceraldehyde	0.277	0.321	0.000	0.589	0.042
Heptadecanoic acid	0.059	0.035	0.034	0.109	0.009

Appendix

Tables A-C

Linoleic acid	2.144	0.467	1.561	2.672	0.323
Malonic acid	0.041	0.060	0.000	0.130	0.006
Myristoleic acid	0.602	0.210	0.387	0.830	0.091
Octadecane	0.021	0.042	0.000	0.084	0.003
Octadecanol	0.045	0.041	0.011	0.104	0.007
Palmitelaidic acid	0.484	0.336	0.000	0.759	0.073
Pentadecanoic acid	0.184	0.112	0.083	0.335	0.028
Phthalic acid	0.097	0.030	0.070	0.128	0.015
p-Hydroxyphenylacetic acid	0.687	0.394	0.360	1.224	0.104
Phytol	0.787	0.458	0.266	1.323	0.119
Stearic acid	5.079	1.162	4.453	6.821	0.765
Suberic acid	0.017	0.020	0.000	0.035	0.003
Tetracosanoic acid	0.122	0.047	0.058	0.163	0.018
Tridecanoic acid	0.037	0.014	0.018	0.050	0.006
Tyrosol	0.041	0.055	0.000	0.116	0.006

Appendix Table A continue

Other organic compounds (< 1 %) Abundance analysed in concentrated *C. hirundinella* water samples that contribute to 7.81 % Abundance of the organic compound composition (Table 6.2).

	Mean	Std. D	Min	Max	(< 1 %) Abundance
1,2-Butanediol	0.007	0.013	0.000	0.027	0.001
2,4-Hexadienedioic acid	0.025	0.051	0.000	0.101	0.004
2-Hydroxyphenylacetic acid	0.023	0.047	0.000	0.094	0.004
3-Hydroxyglutaric acid	0.065	0.129	0.000	0.258	0.010
3-Hydroxyisobutyric acid	0.032	0.064	0.000	0.128	0.005
3-Hydroxyisovaleric acid	0.012	0.024	0.000	0.048	0.002
3-Methylglutaconic acid	0.009	0.019	0.000	0.038	0.001
Acetoacetic acid	0.005	0.009	0.000	0.018	0.001
Erucic acid	0.009	0.018	0.000	0.036	0.001
Heptadec-10-enoic acid	0.026	0.051	0.000	0.103	0.004
Mandelic acid	0.005	0.009	0.000	0.018	0.001
Pimelic acid	0.004	0.007	0.000	0.014	0.001
Vaccenic acid	0.288	0.576	0.000	1.153	0.043

Appendix

Tables A-C

Appendix Table B

The key parameters summarizing the quality of source water collected from Benoni Lake during the sampling period. Typical eutrophic conditions as per DWAF (now DWS) Guidelines (Van Ginkel *et al.*, 2001b).

		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

Appendix

Tables A-C

Appendix Table C						
A comparison between physical, chemical and biological parameters measured in Benoni Lake and Vaal Dam source water during different study periods from 2000 to 2012.						
	Benoni Lake 2011-2012 (current study) <i>n</i> = 10			Vaal Dam 2000-2012 (Swanepoel, 2014) <i>n</i> = > 160		
Measured Variables	Min	Max	Mean	Min	Max	Mean
Nitrate (mg/ℓ)	0.00	8.60	0.95	0.01	7.12	0.34
Total Phosphate (mg/ℓ)	0.00	0.09	0.01	0.00	3.09	0.12
Chemical Oxygen Demand (mg/ℓ)	16.00	33.00	22.00	0.01	34.00	14.02
Hardness as CaCO3 (mg/ℓ)	57.00	92.00	75.80	39.78	290.21	69.10
M Alkalinity as CaCO3 (mg/ℓ)	53.00	260.00	83.40	27.00	253.70	72.70
pH	6.24	6.99	6.59	5.83	13.52	7.88
Conductivity (mS/m)	21.00	39.00	26.30	13.90	318.50	21.69
Chlorophyll-a	Refer to TPP below (chlorophyll-665/TPP = 1.24*chlorophyll-a)			< 2	194.00	12.00
The presence (✓) or the absence (✕) of Dinophyceae and Cyanophyceae genera in Benoni Lake and Vaal Dam source waters. The percentage (%) contributions of Dinophyceae and Cyanophyceae genera recorded in Benoni Lake and Vaal Dam to the total phytoplankton composition were based on cell concentrations. Turbidity and TPP measurements (<i>n</i> = 7) is also given for the two water sources.						
	Benoni Lake (Ewerts <i>et al.</i> , 2014)			Vaal Dam (Ewerts <i>et al.</i> , 2014)		
Dinophyceae (dinoflagellates)						
<i>Ceratium</i> *	✓	62 %		✓	-	
<i>Peridinium</i>	✓	4 %		✕	-	
Cyanophyceae (cyanobacteria)						
<i>Anabaena</i>	✓	-		✓	32 %	
<i>Merismopedia</i>	✓	-		✕	-	
<i>Microcystis</i>	✓	-		✓	19 %	
<i>Oscillatoria</i>	✓	-		✓	-	
Turbidity (NTU)	2.10	7.00	4.29	69.00	85.00	80.28
TPP (µg/ℓ)	6.86	100.00	34.94	1.70	7.70	2.29