COAGULATION AND SEDIMENTATION OF ALGAL CELLS AND ASSOCIATED MATERIAL IN VAAL RIVER WATER

DANIE TRAUT
B. Sc. Honours (UOFS)

Dissertation submitted in partial fulfillment of the requiremenmts for the degree
Magister Scientiae
in the Division Botany, School of Enviromental Sciences and Development at the
Potchefstroomse Universiteit vir Christelike Hoër Onderwys

Supervisor: Professor Dr. A.J.H. Pieterse

POTCHEFSTROOM

February 2002

COAGULATION AND SEDIMENTATION OF ALGAL CELLS AND ASSOCIATED MATERIAL IN VAAL RIVER WATER

ABSTRACT

In the research of drinking water treatment by various researchers, attention was given to the coagulation of inorganic particles (Gregory, 1989). Processes of coagulation and the removal of natural organic matter and suspended organisms received less research attention. Experience at Sedibeng Water showed that the lack of applicable information on removal requirements of organic matter, results in costly treatment processes (Basson and Pieterse, 1993).

Experiments were therefore, conducted to determine the optimum Fe^{3+} concentration for removal of dissolved organic matter, suspended algae and suspended particles, the removal efficiency by different iron salts, the pH adjustment chemical most suitable for the coagulation, and the effect of different algal concentrations as well as associated organic substances, on removal efficiencies. Water from the Vaal River at Balkfontein (Sedibeng Water) were used in the experiments. Jar tests were conducted to optimise chemical dosage and to determine removal efficiencies of dissolved organic matter and suspended particles and algal cells and colonies.

The three algal species used in this investigation were *Monoraphidium minitum*, *Cyclotella meneghiniana* and *Pandorina morum*.

Results showed that it is recommended to use lime instead of NaOH, in accordance with practice, because lime is a cheaper alternative and more effective than NaOH. When $FeCl_3$ and $Fe_2(SO_4)_3$ was compared, no significant differences were observed.

$FeCl_3$ concentrations between 8 and 10 mg l^{-1} were determined to be the optimum concentration for the removal of dissolved organic matter and suspended particles. High pH conditions (pH 11.5) also improved removal efficiencies. *C. meneghiniana* cells and *P. morum* colonies were effectively removed at pH 11.5. *C. meneghiniana* cells apparently enhanced the removal of suspended matter at pH 6.4. Organic substances excreted by *M. minutum* improved the removal of cells at pH 11.5, but algal biomass removal was inhibited at pH 6.4 by the organic substances. Organic matter excreted by *M. minutum* apparently improved the removal of suspended matter present in Vaal River water, but reduced the removal of dissolved organic carbon (DOC). In addition, results showed that algal cells, especially of *M. minutum* and *C. meneghiniana* (at pH 11.5) and *P. morum* colonies themselves, apparently assist in the removal of DOC. It can, in general, be concluded that increases in algal biomass together with the organic substances excreted by the algal cells, affected the removal of biomass, suspended matter and dissolved organic carbon.

KEYWORDS: Removal of algal cells; algal colonies; associated extracellular substances

KOAGULERING EN SEDIMENTERING VAN ALGSELLE EN GEASSOSIEERDE MATERIAAL IN VAALRIVIERWATER

OPSOMMING

Bestaande navorsing deur verskillende navorsers oor drinkwatersuiwering gee meestal aandag aan koagulasie van anorganiese deelttjies (Gregory, 1989). Die proses van koagulasie en verwydering van organiese materiaal, wat natuurlik voorkom, is min bestudeer. Ondervinding by die Balkfontein watersuiweringswerke het getoon dat 'n gebrek aan toepaslike inligting om organiese materiaal te verwyder kan lei tot duur suiweringsprosesse (Basson en Pieterse, 1993).

Eksperimente is derhalwe uitgevoer om die optimum Fe^{3+} konsentrasie vir die verwydering van organiese materiaal, gesuspendeerde alge en ander deeltjies te bepaal, sowel as om verwydering deur verskillende ystersoute met mekaar te vergelyk. Die chemikalieë wat die beste presteer in pH-verstelling is ondersoek, en die invloed van alge en geassosieerde organiese materiaal op suiweringsprosesse is bestudeer. Vaalrivierwater vanaf Balkfontein (Sedibeng Water) is in die eksperimente gebruik. Roertoetse is gedoen om optimum koagulantkonsentrasies te bepaal en om die verwyderingeffektiwiteit van organiese materiaal en gesuspendeerde deeltjies te ondersoek.

Die drie algspesies wat in die ondersoek gebruik is, is *Monoraphidium minitum*, *Cyclotella meneghiniana* en *Pandorina morum*.

Resultate toon dat dit aanbeveel kan word om kalk eerder as NaOH te gebruik vir pH-verstelling, omdat volgens praktyk, kalk goedkoper en meer effektief as NaOH is. 'n Vergelyking tussen $FeCl_3$ en $Fe_2(SO_4)_3$ toon dat daar nie 'n merkwaardige verkil tussen die twee ystersoute is nie.

$FeCl_3$ konsentrasies tussen 8 en 10 mg l^{-1} was as die optimum konsentrasie vir die verwydering van organiese materiaal en gesuspendeerde deeltjies bevind. Hoë pH's (pH 11.5) het verwydering verbeter en *C. meneghiniana* en *P. morum* is doeltreffend by hierdie pH verwyder. *C. meneghiniana* selle het moontlik bygedra tot die verwydering van gesuspendeerde deeltjies by pH 6.4. Organiese materiaal wat deur *M. minutum* uitgeskei is, het by pH 11.5 bygedra tot die verwydering van algselle maar die omgekeerde is gevind by pH 6.4. Dieselfde organiese materiaal het ook bygedra tot die verwydering van gesuspendeerde deeltjies maar het die verwydering van organiese materiaal geïnhibeer. Die resultate het getoon dat *M. minutum*, *C. meneghiniana* en *P. morum* by pH 11.5 bygedra het tot die verwydering van opgeloste organiese materiaal. 'n Algemene afleiding wat dus gemaak kan word, is dat algselle saam met die organiese materiaal wat deur alge uitgeskei word, die verwydering van biomassa, gesuspendeerde deeltjies en organiese materiaal beïnvloed.

SLEUTELWOORDE: Verwydering van algselle, algkolonies; geassosieerde organiese materiaal

CONTENTS

ABSTRACT i
OPSOMMING iii
CONTENTS v

CHAPTER 1: INTRODUCTION 1

1.1 Components of water treatment practices 2
1.1.1 Double-layer compression 5
1.1.2 Enmeshment in a precipitate 6
1.1.3 Adsorption and interparticle bridging 6

1.2 NaOH and Lime as pH adjustment chemicals 7
1.2.1 Lime as coagulant or pH adjustment chemical 7
1.2.2 Effect of high pH conditions pH > 11 8

1.3 Ferric chloride as primary coagulant 9
1.3.1 Ferric (III) salts as coagulants 9
1.3.2 Ferric chloride coagulation for removal of dissolved organic carbon 10

1.4 Coagulation and settling of different algae 12
1.4.1 Phytoplankton diversity in the Vaal River at Balkfontein 12
1.4.2 Removal of micro-organisms, especially algae 12

1.5 Motivation for research 14

CHAPTER 2: STUDY AREA, MATERIALS AND METHODS 15

2.1 Study area 15

2.1.1 Sampling locality 15
2.1.2 Phytoplankton diversity in the Vaal River at Balkfontein 16

2.2 Materials and methods 19
2.2.1 Jar Test apparatus 19
2.2.2 Chlorophyll-*a* determination 22
2.2.3 SAC 254 determination 23
2.2.4 Turbidity determination 23
2.2.5 Addition of algal cells to increase chlorophyll-*a* concentration 24
2.2.6 Composition of GBG 11 25
2.2.7 Analysis of extracellular organic substances 26
2.2.7.1 Identification and quantification of organic acids 27
2.2.7.2 Identification and quantification of amino acids and amines 28
2.2.7.3 Protein concentration determination 28
2.2.7.4 Oligosaccharide determination 30
2.2.8 Interpretation of results 30
2.2.8.1 The effect of algal cells only 31
2.2.8.2 The effect of organic and inorganic substances 31
2.2.8.3 The effect of organic substances only 32
2.2.8.4 The effect of inorganic substances only 32

CHAPTER 3: EFFECT OF DIFFERENT FLOCCULANTS AND pH ADJUSTMENT CHEMICALS ON COAGULATION AND SEDIMENTATION PROCESSES **33**

3.1 The effect of increased iron concentration on the removal of total suspended solids and dissolved organic carbon 33

3.2 The effect of NaOH and lime as pH adjustment chemicals 37

3.3 The effect of $FeCl_3$ and $Fe_2(SO_4)_3$ 44

3.4 The effect of pH > 11 on the removal of suspended material 48

3.5 The effect of the Ca2+ ions present in lime on the flocculation processes 51

CHAPTER 4: EFFECT OF EXCRETED ORGANIC SUBSTANCES BY THREE ALGAL SPECIES ON THE COAGULATION PROCESSES **54**

4.1 Introduction 54

4.2 Organic substances excreted by algal cells 57

4.3 The effect of *Monoraphidium minutum* cells on coagulation and flocculation 61
4.3.1 Removal of biomass (i.e. chlorophyll-*a*) 62
4.3.2 Removal of suspended solids 69
4.3.3 Removal of dissolved organic carbon 75

4.4 The effect of *Cyclotella meneghiniana* cells on coagulation and flocculation 79
4.4.1 Removal of biomass (i.e. chlorophyll-*a*) 80
4.4.2 Removal of suspended solids 85
4.4.3 Removal of dissolved organic carbon 89

4.5 The effect of *Pandorina morum* colonies on coagulation and flocculation 94
4.5.1 Removal of biomass (i.e. chlorophyll-*a*) 95
4.5.2 Removal of suspended solids 99
4.5.3 Removal of dissolved organic carbon 104

CHAPTER 5: CONCLUSIONS **112**

ACKNOWLEDGEMENTS **117**

REFERENCES **118**

CHAPTER 1: INTRODUCTION

Water is the most critical resource for any socio-economic development. Rivers of the Vaal River catchment that drain the Gauteng area produce a mere 4300 million cubic meters per annum (DWA, 1986). Some 42 % of the country's urban population lives in the Gauteng area and, moreover, the number of people living there will have increased from 5.9 million in 1985 to 8.5 million in the year 2000 (DWA, 1986). Corresponding with the increase in population, the water demand will increase by 294 %. This tendency of increase in human population is not only problematic to South Africa, it is a world-wide problem and, therefore, because of the extensive utilisation of water through household, mining and industrial activities as well as agriculture practices, the water in the Vaal River, and other fresh water systems, is highly polluted, mineralised, salinised and eutrophied.

The Balkfontein plant, near Bothaville, of Sedibeng Water (formerly Goudveld Water) treats water from the middle Vaal River. The water in this part of the river consists of a large fraction of recycled water and thus a decrease in water quality is experienced. Therefore, increase in water demand for potable purposes and deterioration of water quality calls for extensive research on coagulation and sedimentation of suspended material in polluted freshwater systems especially at the Sedibeng Water purification works.

In the research of drinking water treatment by various researchers, attention was paid to the coagulation of inorganic particles, i.e. turbidity. Thus, most theories were developed with this type of pollution in mind (Gregory, 1989). Processes of coagulation and the removal of natural organic matter and organisms attracted less attention, and experience at Sedibeng Water showed that this calls for enhanced and costly treatment (Basson and Pieterse, 1993).

There is not much information available about the influence of several important environmental variables on coagulation of dissolved organic matter, especially in Vaal River water. Bernhardt and co-workers published a number of papers dealing with the influence of extracellular organic substances on coagulation about research performed at the Wahnbach Reservoir in Germany (Bernhardt *et al.* 1985a, b, Hoyer *et al.* 1985). Bernhardt and Wilhelms (1972) published the first paper in this field from the Wahnbach laboratory. At that time (early 1970's) only bioflocculation (Tenney and Stumm, 1965) in wastewater treatment had been mentioned in a water treatment monograph like that of Weber (1972). Over the course of the last twenty years, the Wahnbach group published at least 20 papers, which dealt almost entirely with the influence of organic substances excreted by algal cells on drinking water treatment. The most important publications are probably those of Lüsse *et al.* (1985), Bernhardt *et al.* (1986), Kunikane *et al.* (1986), Hoyer *et al.* (1987) and Lüsse (1988). The present study presents the results of the influence of *Monoraphidium minutum*, *Cyclotella meneghiniana* and *Pandorina morum* and its extracellular organic substances on the results of coagulation experiments with Vaal River Water.

1.1 Components of water treatment practices

Particles in natural water vary widely in origin, concentration and size (Fig. 1.1). Some particles are constituents of land-based or atmospheric sources (i.e. suspended particles, such as clays, silts, and other terrestrial detritus) and some are produced by chemical and biological processes within the water source (e.g. algae, bacteria, viruses, precipitates of $CaCO_3$, etc; Amirtharajah *et al.*, 1990). The sizes and nature of these waterborne particles are illustrated in Fig. 1.1. The unit processes used to remove waterborne particles from the water are illustrated in Fig. 1.2. Substances in true solution need processes like oxidation, chemical precipitation and gas transfer for removal. Colloids in suspensions are removed through chemical coagulation. Suspended and floating solids, together with the flocs formed during coagulation, are removed through sedimentation and flotation, which are followed by filtration. In the present study, coagulation and flocculation processes received special attention.

Coagulation is an important process for combining small particles into larger aggregates. Coagulation is an essential component of water treatment practice in which coagulation, flocculation, sedimentation and filtration are combined to remove particles from water.

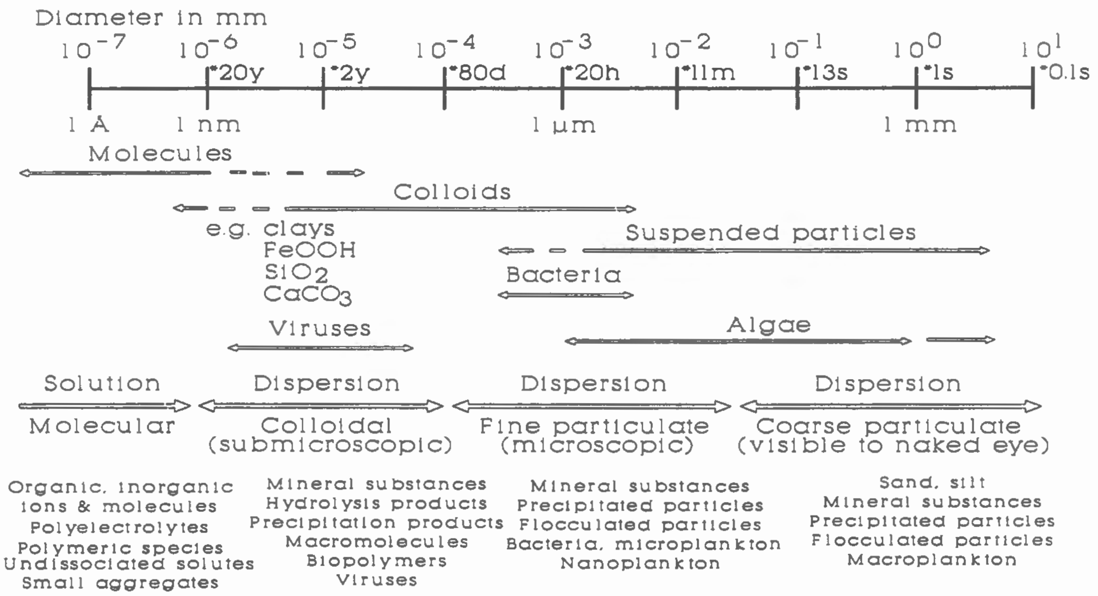


Figure 1.1 Size spectrum of waterborne particles (modified from Stumm and Morgan, 1981).

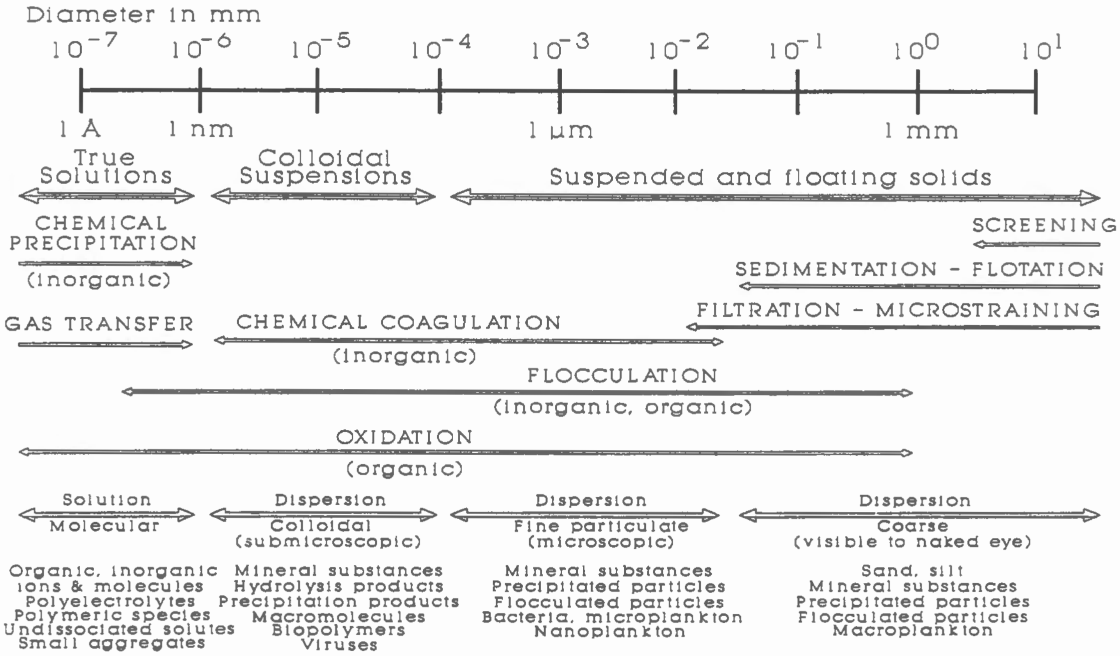


Figure 1.2 Diagram to illustrate the removal of waterborne particles with the application of unit processes (modified from Stumm and Morgan, 1981).

The theory of coagulation, which includes an understanding of the chemistry of the coagulant in the water, serves as the primary tool in explaining the behaviour of coagulants under certain conditions. Coagulation is, however, complex so that no easy and definitive explanation exists. Often terminology is defined to simplify and clarify discussion; at times the definitions have seemed to complicate communication (Gregory, 1989). Concepts such as coagulation and flocculation have different meanings to different people. Although they are used in this review, no unique, correct and universal definition for such concepts exist.

A number of applicable concepts and processes are described in the following paragraphs. Coagulation is considered to include all reactions and mechanisms which result in the overall process of particle aggregation within the treated water, as well as in chemical particle destabilisation and physical interparticle contact (Gregory, 1989).

Particle destabilisation occur in rapid mixing tanks and interparticle contact occurs in flocculation or slow mixing tanks, therefore the physical process of producing contact is termed flocculation (Amirtharajah *et al.*, 1990). Flocculation is an important step in many solid-liquid separation processes and is widely used in water and waste-water treatment. Unit processes such as sedimentation and filtration become more effective as the size of the particles are increased. If the particles to be removed, for example algal cells, bacteria, clays, silts and other terrestrial detritus, are too small for effective separation, their size can be increased substantially by causing them to form aggregates. The aggregation process is known as coagulation in the present study.

Particles which are to be flocculated may initially be very small and fall in the colloidal size range, which is conventionally assumed to cover sizes between 1 nm and 1 µm (Figs 1.1 and 1.2; Amirtharajah *et al.*, 1990). Because of the small size, colloidal particles are subject to diffusion and may settle very slowly under gravity. Furthermore, interparticle forces can become significant and play a significant role in the stability of colloids. Colloidal particles are said to be unstable if aggregation occurs readily. For particles larger than 1 µm, the laws of gravity play an important role, because they may outweigh colloidal interactions (Amirtharajah *et al.*, 1990). Nevertheless, many of the processes apply to larger particles and, in water and wastewater treatment, particles of up to ± 1 mm may be involved in flocculation processes (Figs 1.1 and 1.2; Gregory, 1989).

Two classes, amongst others, of materials occurring in aqueous system are known as hydrophilic and hydrophobic colloids (Amirtharajah *et al.*, 1990). Hydrophilic colloids consist of water soluble macro-molecules may be in true solution and are thermodynamically stable. It is possible that they can be influenced to aggregate by changing the solvency conditions, by adding for instance, large quantities of inorganic salts.

Hydrophobic colloids (with low solubility), by contrast, may be kinetically stable by virtue of interparticle repulsion (Niehof *et al.*, 1972; Hunter *et al.*, 1979). In most cases, the repulsion is electrical in nature because the majority of aqueous colloids are charged and the sign of the charge is usually negative (Amirtharajah *et al.*, 1990). These hydrophobic colloids include virtually all solid particles present in natural waters and include, for example, clays, metal oxides and micro-organisms. Some hydrophobic colloids coagulate slowly and others may coagulate more rapidly.

In water treatment, the coagulation process is used to increase the rate at which the particles aggregate, i.e. to transform a stable suspension into an unstable one. The design and operation of a coagulation process requires changing and controlling the kinetics of particle aggregation. Physical and chemical aspects are involved, therefore coagulation can be described a physico-chemical process (Amirtharajah *et al.*, 1990).

Different chemical coagulants can bring about the destabilisation of suspensions in different ways. Three distinct methods or mechanisms are presented here, namely compression of the double layer, enmeshment in a precipitate, and adsorption to permit interparticle bridging (Amirtharajah *et al.*,1990).

1.1.1 Double-layer compression

The ions in solution produced by sodium chloride act as point charges and have no chemical characteristics such as hydrolysis and adsorption reactions in coagulation.

These ions are sometimes called indifferent coagulants. The interactions between such indifferent coagulants and colloidal particles are purely electrostatic. Ions of similar charge to the primary charge of the colloid are repelled and counter ions are attracted. Destabilisation by counterions occurs by compression of the diffuse layer surrounding the particle. High concentration of point charges produce high concentration of counter ions in the diffuse layer, thus the diffuse layer is compressed to maintain electroneutrality. This causes the effective thickness of the diffuse layer to be reduced. The repulsive interaction between similar colloidal particles decreases. Then it is possible for Van der Waals interactions to dominate all separations, the activation energy barrier can disappear, and electrostatic stabilisation can be eliminated (Amirtharajah *et al.*, 1990).

1.1.2 Enmeshment in a precipitate

When a metal salt such as $FeCl_3$ or $Ca(OH)_2$ is added to water in concentrations sufficiently high to cause precipitation of a metal hydroxide (i.e. $Fe(OH)_3$), colloidal particles can be enmeshed in these precipitates as they are formed and also collide with them afterwards (Amirtharajah *et al.*, 1990). This has been termed "sweep-floc" coagulation by Packham (1965). The process, a combination of destabilisation and transport, is extensively used in the treatment of waters of variable turbidity and dissolved organic carbon (DOC) in conjunction with rapid mixing, slow mixing, sedimentation and filtration facilities in series.

1.1.3 Adsorption and interparticle bridging

Destabilisation by bridging occurs when segments of a polymer chain adsorb on more than one particle, thereby binding the particles together (Gregory, 1989). When a polymer molecule comes into contact with a colloidal particle, some of the reactive groups on the polymer adsorb at the particle surface, leaving other portions of the molecule extending into the solution. If a second particle with vacant adsorption sites contacts these extended loops and tails, attachment can occur. A particle-polymer-particle aggregate is formed in which the polymer serves as a bridge.

1.2 NaOH and Lime as pH-adjustment chemicals

NaOH and lime are two pH-adjustment chemicals. In this study, lime was compared with NaOH to determine the efficiency of the pH-adjustment chemicals as coagulant aids.

1.2.1 Lime as coagulant or pH adjustment chemical

Lime, CaO mixed with water in a lime slaker to form $Ca(OH)_2$, clarification is not widely applied in water treatment. In general, lime treatment has the following effects on the treated effluent: removal of suspended and colloidal matter and partial removal of DOC, precipitation of phosphorus (as calcium phosphate compounds) and heavy metals (as their hydroxides), and elevation of the pH to enable ammonia removal in subsequent stages (Ronen, 1981).

In coagulation, rapid mixing disperses the lime homogeneously to enable good contact between it and the liquid in order to affect destabilisation and aggregation of the dispersed phase present in sewage or secondary effluent. This phase consist mainly of a large amount of negatively charged, non-settleable, colloidal material, usually ranging in size from 10^{-3} to 102 µm. The following chemical reactions (providing sufficient reaction time is allowed) are involved:

$$Ca(OH)_2 + Ca(HCO_3)_2 \longrightarrow 2\ CaCO_3 + 2\ H_2O$$

$$5\ Ca^{2+} + 3\ HPO_4 + 4\ OH^- \longrightarrow Ca_5OH(PO_4)_3 + 3\ H_2O$$

$$Mg^{2+} + 2\ OH^- \longrightarrow Mg(OH)_2$$

During the application of lime, the pH of the treated effluent is raised to the desired level, depending on water quality, where coagulation occurs the best (Ronen, 1981).

The addition of lime to the water in the pH range 10.5 to 11.5 has the following effect on its inorganic composition. The alkalinity is increased due to the addition of hydroxide ions (OH^-), but is subsequently reduced by the precipitation of carbonate as calcium carbonate and hydroxide as magnesium hydroxide. Therefore, the calcium concentration is initially increased owing to the lime addition, but subsequently reduced. The magnesium concentration is not affected by the lime addition, but it is reduced as a result of the precipitation of magnesium hydroxide when pH levels increase to above 10.5.

Although some removal of micro-organisms takes place during the lime-treatment stage, this process is not aimed at the removal of micro-organisms from untreated water. Bacteria and viruses are generally removed together with the suspended and colloidal matter and could be killed as a result of the high pH levels (Grabow *et al.*, 1969).

There is evidence in the literature that lime treatment affects the molecular mass distribution of the organic matter, which means that hydrolysis of organic matter occurs during this stage (Ronen, 1981). This phenomenon was not observed or studied during the present investigation.

1.2.2 Effect of high pH conditions (pH > 11).

Apart from coagulation by $FeCl_3$ and $Fe_2(SO_4)_3$, lime-magnesium coagulation are also being employed in certain purification plants. A number of investigations on treatment technologies, involving an alkaline medium, have studied the contribution of calcium carbonate and magnesium hydroxide to coagulation (both precipitates in lime coagulation). The contributing effect of the two substances was first reported on in the late twenties (Flentje, 1927). The study led to the following findings, namely that lime increases clarification and that the relationship between lime dosage and clarifying effects should be attributed to the properties of magnesium hydroxide formed from magnesium salts present in the water. Since the Flentje study, a number of experimental studies have made use of the coagulating and absorbing properties of magnesium hydroxide. These approaches yielded a water treatment method in which magnesium carbonate (precipitated to magnesium hydroxide in the presence of lime) acted as a coagulant (Black and Thompson, 1971, 1975). Two other investigators (Oldham and Rush, 1978) showed the following: magnesium hydroxide results in the removal of coloured matter and turbidity which is comparable to that achieved by alum, while the flocs produced via this route are easier to settle. Experimental results on municipal sewage samples (Leentvaar and Rebhun, 1982) have supported the findings on the contribution of calcium carbonate and magnesium hydroxide to the removal of organic matter. Taking these findings into account, it can be concluded that the effect of water treatment at high pH is a joint contribution of the calcium carbonate and magnesium hydroxide components.

The primary objective of high-lime coagulation (Parker *et al.*, 1975; Dziubek and Kowal, 1984) is an alkalisation of the water or waste water to achieve a pH level of pH 11.5, thus making the precipitation of magnesium hydroxide quicker. The process, however, has two inherent limitations set by economic factors, namely the high consumption of lime (specifically when the influent stream displays high alkalinity levels), and the overalkalisation of the water under treatment, which calls for a two-stage recarbonation in order to decrease the levels of added salts. There is an important disadvantage of applying high-lime coagulation, namely the removal of magnesium, an important microelement for humans (Dziubek and Kowal, 1989).

In this study, high-lime experiments will be used to investigate the effect of pH between 10.5 and 12 on the removal of dissolved organic carbon substances. Experience at the Sedibeng Water purification plant showed that when the pH was raised to above 11.4, DOC concentrations were effectively decreased.

1.3 Ferric Chloride as primary coagulant

1.3.1 Ferric (III) salts as coagulants

$FeCl_3$ and $Fe_2(SO_4)_3$ are chemical coagulants that can bring about the destabilisation of suspensions in natural waters. Leprince *et al.* (1984) reported that partially neutralised "polymeric" $FeCl_3$ solutions were superior to untreated solutions in removing turbidity from synthetic suspensions at low temperatures.

When the optimum pH for coagulation is determined, it is possible to compare $FeCl_3$ with other iron salts, therefore it is possible to conduct a study where ferric chloride is compared with ferric sulphate. The ferric ions of $FeCl_3$ and $Fe_2(SO_4)_3$ have identical charges, but the chemicals are not identical as coagulants.

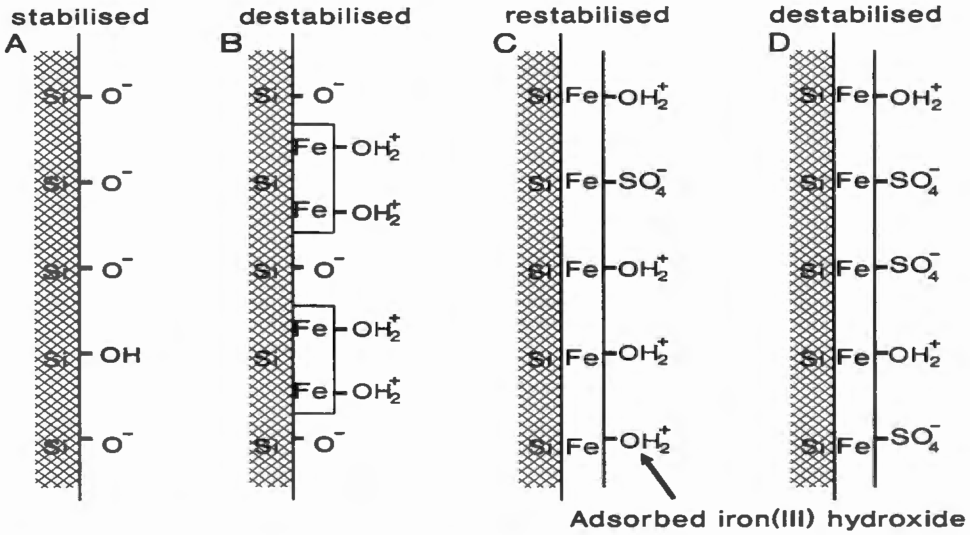


Figure 1.3 Schematic diagram of destabilisation and restabilisation of Ferric-treated particles; (A) surface of negatively charged particle (stabilised), (B) particle destabilised by charge neutralisation, (C) particle restabilised by excess ferric hydrolysis species and (D) destabilisation by adsorption of sulphate ions (Letterman and Vanderbrook, 1983).

The $(SO_4)^{2-}$ ions in solution assist in destabilisation of negatively charged particles, as illustrated in Fig. 1.3. The negatively charged particles are destabilised by Fe^{3+} through the process of charge neutralisation. The destabilised particles in the water have the ability to restabilise by excess iron hydrolysis species. The restabilised particle can be stabilised by adsorption of sulphate ions. This is illustrated in Fig. 1.3 (D).

1.3.2 Ferric chloride coagulation for removal of dissolved organic carbon

The need for optimising the removal of dissolved organic carbon (DOC) during drinking water treatment has become important because of the health risks associated with disinfection by-products. Coagulation often provides substantial removal of DOC. Numerous detailed investigations have focused on the treatment of natural waters containing DOC (Black and Christman, 1963; Hall and Packman, 1965; Dempsey *et al.*, 1984; Edwards and Amirtharajah, 1985; Sinsabaugh *et al.*, 1986). These investigations showed that coagulation of DOC was dependent on the pH conditions; the coagulant dose and the concentration of DOC. Effective removal of DOC occurred at lower pH conditions than turbidity removal. The optimum pH conditions for coagulation of dissolved organic matter were around pH 4 for Fe(III) salts. Except at low pH values, the optimum coagulant dose was proportional to the concentration of organic matter (Dempsey *et al.*, 1984).

The removal of DOC by coagulation was influenced by its charge, solubility, and molecular size characteristics (Sinsabaugh *et al.*, 1986). The efficiency of removal of DOC was found to be proportional to molecular size, with larger molecular weight components being more effectively removed. Sinsabaugh *et al.* (1986) determined that acidic and basic components of the DOC in natural waters were twice as likely to be removed by coagulation than nonpolar, neutral compounds. The removal of neutral compounds was dependent on their polarity and the number of available low-polarity adsorption sites on the flocs.

The removal of DOC was thought to occur by both adsorption onto the solid hydroxide precipitate and complexation between coagulant and DOC. Edwards and Amirtharajah (1985) proposed two mechanisms by which DOC is removed from solution during coagulation with metal salts, namely precipitation by cationic species and adsorption on organic and inorganic solids.

Recently researchers (Gray, 1988; Gregory, 1989; Dempsey, 1989) concluded that the removal of DOC by coagulation with metal salts involves a combination of both adsorption to solid hydroxide and chemical precipitation (i.e. coprecipitation of metal-DOC and metal-hydroxide). Both Gray (1988) and Gregory (1989) regarded the removal of DOC by coagulation using metal salts as a combination of precipitation of a metal-DOC complex and adsorption onto the surface of solid hydroxide precipitate. Gray (1988) reported that below pH 6, removal occurs by coprecipitation of ferric-organic matter or ferric hydroxide precipitate and concluded that effective removal of DOC with iron(III) occurs primarily by the formation of an iron-organic precipitate rather than the formation of ferric hydroxide.

In this study special attention has been given to removal of DOC, because the objective of this investigation was to study the influence of extracellular material of different algae on the coagulation process. It is also important to investigate the effect of DOC on the removal of suspended particles including algal cells in Vaal River water.

1.4 Coagulation and settling of different algae

1.4.1 Phytoplankton diversity in the Vaal River at Balkfontein

As a consequence of eutrophication and mineralisation, massive developments of planktonic algae are sometimes observed in the Vaal River (Janse van Vuuren, 1996), especially in sections of low flow, resulting in the interferences with treatment processes and problems in distribution systems (Bruwer *et al.*, 1985). Therefore, the phytoplankton species diversity at Balkfontein is important, and algae used in this study may have an influence on the purification processes at Sedibeng Water.

1.4.2 Removal of micro-organisms, especially algae

Many problems in water supply are caused by algae in eutrophicated water sources (AWWA, 1990) like the Vaal River. There are problems of tastes and odours, toxicity, obstruction to coagulation and sand filter clogging (Palmer, 1980).

Research on, and practical experience in, the processes of removal of various organisms from surface waters in drinking water treatment is still relatively scarce. Several earlier papers, which addressed this problem, were publised by Orlita (1960), Bernhardt (1967), Bernhardt and Clasen (1967) and Svorcova (1970). Only recently, have *Legionella*, *Giardia* and *Cryptosporidium* been considered as organisms of general interest (Dolejs, 1993). Publications on drinking water treatment, rarely contains useful information on the removal of different organisms from raw water. Because eutrophication is still a problem in many water sources, data is usually not available, about the numbers of organisms, like algae, in treated water. Corresponding to the great variety of planktonic species, organisms differ in a number of features that are important from a water treatment point of view.

The effect of algae in water treatment processes is experienced in general water treatment processes such as coagulation, sedimentation and filtration. A rapid sand filtration system is generally not suitable to remove the algae, because it has been developed for treatment of high density inorganic substances (Konno, 1993). Because the density of algae is generally low (Reynolds, 1975), algogenic substances cause obstruction to coagulation and sedimentation of inorganic matter such as clay (Bernhardt, 1982; Magara *et al.*, 1986). The effect of algae on the conditions for coagulation is to unstabalise the flocs, while the size of algae is larger than colloidal matter (Konno, 1993).

In this investigation three types of algae which may cause obstruction to coagulation or filter clogging were studied. Two of the algae belong to the Chlorophyta or green algae, namely *Monoraphidium minutum* and *Pandorina morum* and one, *Cyclotella meneghiniana*, a diatom, belongs to the Chrysophyta, sub phylum Bacillariophyceae. According to Steynberg (1994), microscopic observations of algal cultures showed that *Cyclotella meneghiniana* can change its shape, namely from approximately spherical to cylindrical, which possibly could influence the efficiency of coagulation-flocculation and sedimentation processes. *Pandorina morum* colonies showed that these were colonies removed without difficulty, and a 94 % removal may be obtained by sedimentation. Results from Steynberg (1994) also showed that in the case of *Monoraphidium minitum*, higher biomass concentrations resulted in decreases in the relative percentage removal efficiency by sedimentation.

Differential removal, according to Visser (1996), of algae occurred in the processes employed at the Balkfontein purification plant. The Bacillariophyceae units were reduced to a larger extent than those of the Chlorophyceae. Therefore, the Bacillariophyceae were removed more efficiently in relation to the Chlorophyceae under the prevailing conditions of flow, coagulation-flocculation and filtration. This observation confirmed that of Bernhardt & Clasen (1995), who showed that large diatom cells are removed efficiently by using flocculation and filtration.

Bacillariophyceae cells are probably denser because of the frustules containing silicate. Chlorophyceae cells, on the other hand, showed different shapes. The denser diatoms cells are expected to settle out easier than green algal cells, which are possibly less dense (Visser, 1996). Therefore, as indicated previously, it is important to investigate morphological characteristics of algal cells in relation to their ability or inability to be removed during purification processes. Previous studies (Pieterse *et al.*, 1993) on the effect of $FeCl_3$ and lime treatment indicated that the green algae were removed more efficiently by $FeCl_3$, while the diatoms were removed more efficiently by lime.

1.5 Motivation for research

At present various water treatment plants experience algal-related problems during treatment and are using, amongst others, iron (III) salts as coagulants. A project was, therefore, developed to investigate the effect of different coagulants at various concentrations, using different pH adjustment chemicals in a pH range of 5 to 11. The first phase of the study involved only water taken from the Vaal River. The effect of different coagulation and sedimentation conditions on naturally occurring suspended particles (i.e. colloidal and algal) were investigated. In the second phase of the study different algal species grown in culture and representing selected morphological characteristics were added to Vaal River water. The effect of comparable coagulation and sedimentation conditions on these suspensions was investigated. The following aspects received special attention:

a) To determine the optimum Fe^{3+} concentration for the effective removal of dissolved organic matter and other suspended particles.

b) To determine the removal efficiency of $FeCl_3$ and $Fe_2(SO_4)_3$ as coagulants.

c) To determine the pH adjustment chemical (i.e. HCl, NaOH and lime) most suitable for the coagulation processes.

d) To determine the effect of different algal concentrations and the organic substances excreted by them, on removal efficiencies.

CHAPTER 2: STUDY AREA, MATERIALS AND METHODS

2.1 STUDY AREA

2.1.1 Sampling locality

For the purposes of this study observations were made at the Sedibeng Water treatment plant at Balkfontein (near Bothaville; Fig 2.1). This treatment plant was chosen, because it is one of the important localities where water is withdrawn from the Vaal River for purification purposes. Sedibeng Water supplies water to the Welkom, Virginia and Bothaville area (DWA, 1986), as well as the Leeudoringstad, Wolmaransstad, and Makwassie areas. Irrigation farmers along the river and on the Vaalharts Government Water Scheme are also supplied with water from the Vaal River.

At Balkfontein (± 20 km by road from Bothaville) a sampling locality in the river (C2H022) is situated near the pumping station of the purification plant (27°23'45"N, 26°30'30"E Fig. 2.1) of Sedibeng Water. The width of the river at the sampling locality is about 77 m, with a maximum depth of 5 m and a average depth of about 4 m (Pieterse, 1986).

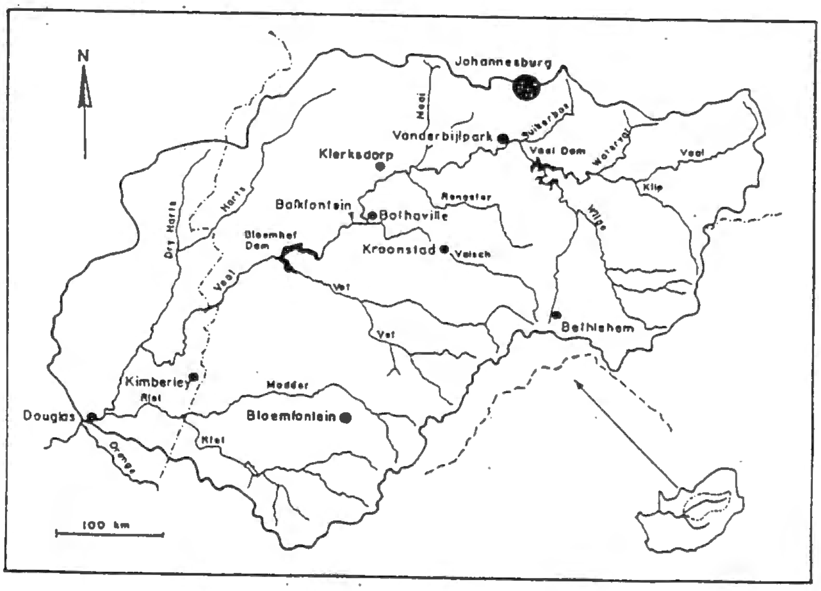


Figure 2.1 Map of the middle Vaal River region showing the sampling locality (C2H022) at Sedibeng Water near Bothaville.

2.1.2 Phytoplankton diversity in the Vaal River at Balkfontein

As a consequence of eutrophication and mineralisation, massive developments of planktonic algae are sometimes observed, especially in sections of low flow, resulting in the interferences with treatment processes and problems in distribution systems (Bruwer *et al.*, 1985). Therefore, the phytoplankton diversity at Balkfontein is important, because algae may influence the purification processes at Sedibeng Water. Individual algal species from the Vaal River will be used separately as experimental organisms.

Ninety nine algal species, belonging to seven major algal groups, were identified at Balkfontein (Janse van Vuuren, 1996). The diatoms and green algae were the main algal groups and succeeded each other rapidly. Morphological and physiological characteristics of the green algae and diatoms may play an important role in the purification of Vaal River water at Balkfontein.

In this investigation three types of algae which may cause obstruction to coagulation or filter clogging were studied. Two of the algae belong to the Chlorophyta or greenalgae, namely *Monoraphidium minutum* and *Pandorina morum* and one, *Cyclotella meneghiniana*, a diatom, belongs to the Chrysophyta, sub phylum Bacillariophyceae.

Monoraphidium minutum (Näg.) Kom.-Legn.

M. minutum cells are small (11 – 27.5 µm in length and 3 – 5 µm broad; Visser 1996), elongated and crescent shaped which can be responsible for the possibility that the compactness of flocs formed will be low and that sedimentation would be ineffective. According to Mouchet and Bonnélye (1998) the smallest species are the most difficult to remove (sometimes only 10 %) whereas they account for the most coagulant demand. Coagulation is a phenomenon concerning surface area, and the smallest organisms represent the highest ratio of developed surface area to volume. Due to the size of *M. minutum* cells, it may be possible for the cells to penetrate rapid sand filters. A study done by Visser (1996) showed that, although a dissolved air flotation unit was installed on top of a rapid sand filter, *M. minutum* still penetrated the filtration process. At Sedibeng Water *M. minutum* is one of the most problematic algal species when dominant in the Vaal River. Visser (1996) also indicated that *M. minutum* occurred in all the purification phases of Sedibeng Water and that these cells were one of the dominant species in the filter effluent. Experience at Sedibeng Water showed that pre-chlorination is essential when *M. minutum* is dominant in the raw water. It is important to remove *M. minutum* cells from the water because Steynberg (1994) showed that *M. minutum* cells is the second most resistant algal species to chlorine dosing.

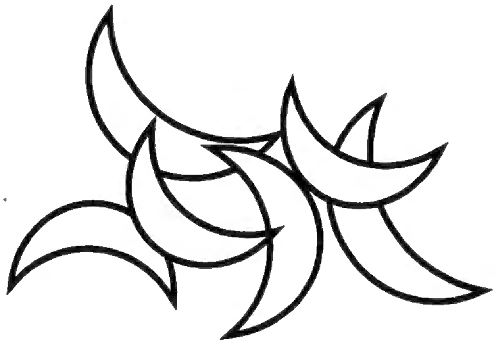


Figure 2.2 Schematic diagram of *Monoraphidium minutum* cells

Pandorina morum (Müller) Bory

P. morum colonies 63 – 73 µm in length and 55 – 64 µm broad (Visser (1996). *P. morum* colonies are motile by means of flagella and the movement of these flagella may break up the flocs in which the colonies were concentrated or may "swim" out of flocs. At Sedibeng Water *P. morum* colonies have shown that when dominant in Vaal River water may cause odour problems in the water. The grassy smell created by *P. morum* colonies are sometimes prominently in the filter house and difficult to remove with the ordinary processes available at the Sedibeng Water purification plant. Often chlorophyll-*a* concentrations are high in the final water when *P. morum* colonies are dominant. The study by Visser (1996) showed that *P. morum* colonies were present in most of the purification processes except for the final effluent. This may be an indication that *P. morum* colonies are not resistant to post – chlorination. A study done by Steynberg (1994) showed that Rand Water do not experience problem in removing *P. morum* colonies to chlorophyll-*a* values of less than 1 µg l^{-1}. The study also showed that when high concentrations of *P. morum* colonies were present in the water that less than 40 per cent of the algal concentration present in the water after sedimentation was removed by filtration.

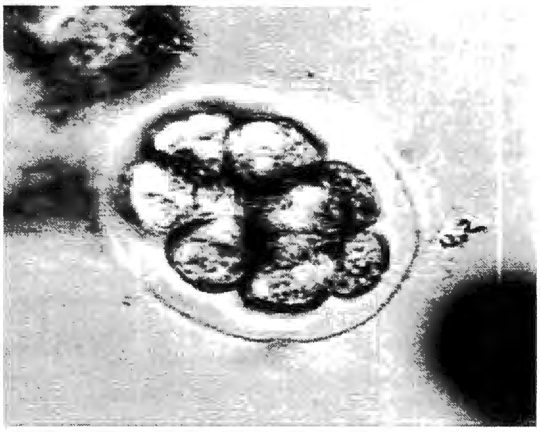


Figure 2.3 Light microscope picture of a *Pandorina morum* colony

Cyclotella meneghiniana (Kützing)

C. meneghiniana cells are circular in valvar view and narrowly rectangular in the girdle view. The cells consist of radiate costae with striae between them, which consist of areolae. (Costae: ribs; enlongated, solid thickinings of the valve of a diatom frustule; Frustule: the shell or cell covering of diatoms; Striae: delicate, long, narrow markings, streaks, bands, groove or channel, a row of pores, areolae, or an elongate chamber in the frustule of a diatom; Areolae: the regularly repeated perforation through the siliceous layer of a frustule usually covered on one side by a velum; Velum: a membrane or structure similar to a veil.). These areolae can capture oxygen during photosinthesis which could apparently be responsible for poorer sedimentation of flocs in which the cells are concentrated.

According to Visser (1996), Bacillariophyceae was removed more efficiently in relation to the Chlorophyceae in ordinary purification processes. Visser (1996) also indicated that the diatoms were the best removed by sedimentation when the dosed polymer concentration was high (approximately 11 mg l^{-1}) or when the prechlorination (above 4 mg l^{-1}) and pre-lime (above 20 mg l^{-1}) dosages were high. According to Mouchet and Bonnélye (1998) removal rates for *C. meneghiniana* can be estimated at an average of between 10 to 70 percent. This indicates the importance of optimisation for the removal of diatom cells.

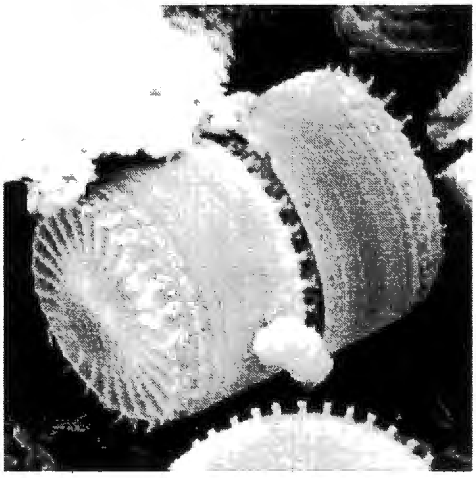


Figure 2.4 Electron micrograph of *Cyclotella meneghiniana* cells

2.2 MATERIALS AND METHODS

2.2.1 The Jar Test apparatus

The efficiency of coagulants can be determined with the aid of the jar test apparatus. The jar test is universally recognised as a tool for coagulation control, and may be used for coagulant selection, dosage selection, coagulant aid selection, dosage selection and the determination of optimum pH. In addition, the jar test can be used in the determination of the addition point of pH adjustment chemicals and coagulant aid, the optimisation of mixing energy and duration time for rapid mixing and slow mixing, as well as for the determination of the dilution of coagulant and similar measurements.

Equal volumes (1 litre) Vaal River water were treated with different Fe^{3+} concentrations at various pH conditions in a Jar Test apparatus. Water samples were taken at Balkfontein near Bothaville. The turbidity, chlorophyll-*a* concentrations and spectral absorption coefficient at 254 nm (SAC 254) were measured before and after each treatment.

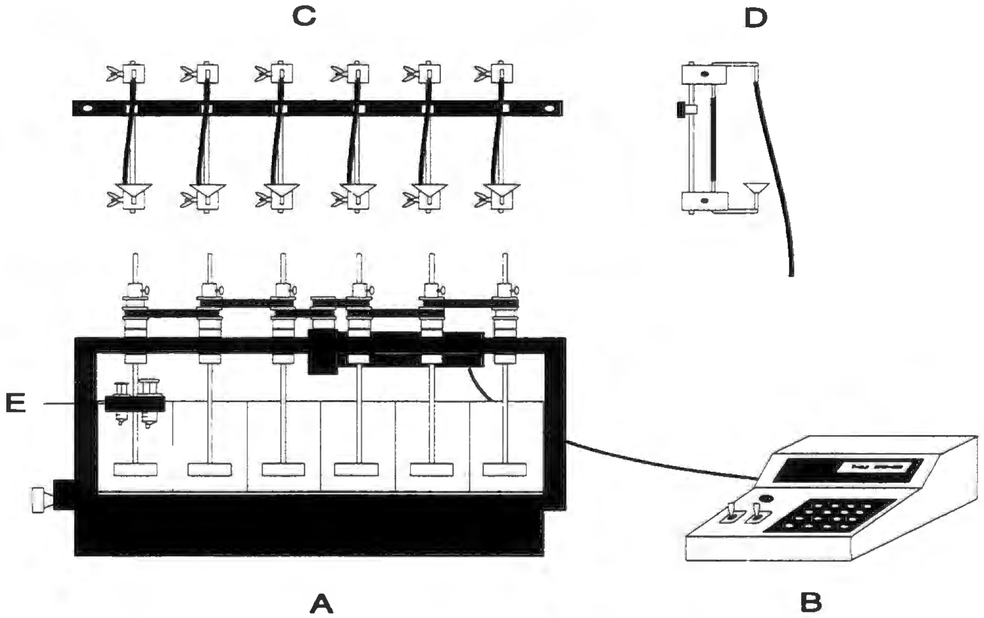


Figure 2.5 The Jar Test Apparatus used in this investigation with (A) the Jar Test Apparatus with six standard jars, (B) the control unit, (C) multiple funnels (front view), (D) multiple funnels (side view) and (E) syringes in a syringe holder.

The jar test apparatus used for the experiments is illustrated in Fig. 2.5. This apparatus consists of several standard jars (19,9 X 9,5 X 9,5 cm) and a mixing device with standard mixing paddles (stirrer flaps 2,5 X 7,5 cm) as seen in Fig. 2.5(A). The jars are made of clear plastic, sometimes with a sampling tap 10 cm below the water level to withdraw water. In addition, water can be withdrawn from the jars with multiple funnels (Fig. 2.5, C and D).

The sampling taps or funnels enable the sampling of supernatant water without sampling sedimented material. The jars and all glassware were cleaned before each experiment.

Iron(III) salts, i.e. ferric chloride ($FeCl_3$) and ferric sulphate ($Fe_2(SO_4)_3$), were used as flocculants. The $FeCl_3$ stock solution contained 4,8376 g $FeCl_3.6H_2O$ (1 g l^{-1} Fe^{3+}) and the $Fe_2(SO_4)_3$ stock solution 3.578 g $Fe_2(SO_4)_3.XH_2O$ (1 g l^{-1} Fe^{3+}) to which 2,5 ml l^{-1} 15% HCl was added to stabilise the solution.

The pH range for flocculation with Fe^{3+} was adjusted to between 5 and 11. The pH adjustment chemicals used were 0.1 M HCl, 0.1 M and 0.4M NaOH and lime. In one treatment, water from the Vaal River was put through the jar test while following exactly the same experimental procedures as for the different treatments, but without the addition of any flocculant. Only pH adjustment was done. For the control treatment, raw water was analysed without being put through the jar test. Neither flocculant nor pH adjustment chemicals were added.

During experimentation the following procedures were followed. Samples were thoroughly mixed prior to the experiment. The volume of pH adjustment chemicals was determined prior to the experiment by a standard titration method. To achieve different Fe^{3+} concentrations, the $FeCl_3$ solution was added to 250 ml of Vaal River water. Due to the acidity of the $FeCl_3$ stock solution, the pH of the water for each Fe^{3+} concentration had to be adjusted. This adjustment was done with either HCl, NaOH or lime.

Multiple syringes were used to add the flocculant and pH adjustment chemicals to the 12 jars (of two Jar test apparatii). A syringe holder (Fig. 2.5E) was used for this purpose.

The following procedures were followed to fill the syringes with the appropriate volumes. The flocculant ($FeCl_3$ or $Fe_2(SO_4)_3$) or pH adjustment chemicals were drawn into the syringe and ejected repeatedly until the syringe tip was filled without any bubbles. The plunger with rubber bulb was removed and the exact volume of chemicals were pipetted into the syringe. The plunger was replaced without ejecting the chemicals.

Each jar was filled with 1 litre thoroughly mixed Vaal River water. Tests for the optimisation of the coagulant dosage was conducted in the following way.

1. While rapid mixing (350 rpm) the water for one minute, the pH adjustment chemicals were added near the impeller with multiple syringes as illustrated in Fig. 2.2E into the twelve jars of two jar test apparatii that contain the same raw water.
2. The water was rapidly mixed for another 30 seconds at the maximum mixing intensity (350 rpm) and, while mixing, the coagulant ($FeCl_3$ or $Fe_2(SO_4)_3$) was added simultaneously near the impeller, also with multiple syringes.
3. To allow flocculation to proceed, the suspension was then slowly mixed for 10 minutes at 40 rpm.
4. After flocculation, the flocs were allowed to settle for 30 minutes without stirring.
5. Supernatant water was withdrawn with multiple funnels (Fig. 2.5 C and D).

Chlorophyll-*a* concentrations, turbidity and spectral absorption coefficient (SAC 254) of the settled water were determined.

2.2.2 Chlorophyll-*a* determination

Settled water samples were taken from the jars and chlorophyll-*a* was measured by a modified pigment extraction method described by Sartory (1982). 250 ml of the 1000 ml (I litre Vaal River water) was filtered through a Whatman - GF/C filter.

Chlorophyll-*a* was extracted from the algal cells in suspension in 10 ml of 95% ethanol for a period of 5 minutes at 78°C. After the ethanol samples were allowed to cool to room temperature, the absorption of the dissolved chlorophyll was determined at 750 and 665 nm with a spectrophotometer. 100 µl of 0,3 N HCl was added to the extract and the absorption was again determined at 665 nm. The chlorophyll-*a* concentration (µg l^{-1}) was then determined with the following formula (Sartory, 1982):

$$\text{Chl-}a\ (\mu g\ l^{-1}) = \frac{[(665_o-750_o)-(665_a-750_a)] \times 28{,}66 \times 10\ \text{ml}}{250\ \text{ml}}$$

where 665_o = adsorption before addition of acid
665_a = adsorption after addition of acid
750_o = background value before addition of acid
750_a = background value after addition of acid

2.2.3 Spectral absorption coefficient (SAC) determination

Settled water samples were taken from the jars and SAC was measured in a way slightly modified from the method used by Bernhardt *et al.* (1985). 10 ml of the supernatant was filtered through a Whatman membrane filter (pore size, 0.65 µm). Absorbance, here taken as SAC (SAC m^{-1}), was measured on the membrane-filtered water at 254 nm in a quarts cuvet.

SAC 254 of the membrane filtered water was taken to represent total particulate and dissolved organic matter, and is often used as a simple surrogate method for dissolved organic carbon (DOC; Edzwald *et al.*, 1985). SAC 254 of the membrane filtered water was taken to represent DOC. SAC 254 (m^{-1}) was calculated using the following formula:

$$\text{SAC 254 (SAC } m^{-1}) = \frac{\text{Absorbance (254 nm)} \times 100\ \text{(cm)}}{1\ \text{(cm; cuvet length)}}$$

2.2.4 Turbidity determination

The turbidity (NTU) of the raw and chemically treated water was determined with a Aqualytic Turbidimeter AL 1000.

2.2.5 Addition of algal cells to increase the chlorophyll-*a* concentration

Algal cells and colonies (*Monoraphidium minutum* and *Pandorina morum* respectively) were grown in culture medium, GBG 11, for a period of 10 days. The light intensity was 43 µE m^{-1} s^{-1} and the temperature 18 °C. Due to the fact that the growth rate of *Cyclotella meneghiniana* is lower than that of the other algal species, *C. meneghiniana* was grown for a period of 18 days before the cells were added to Vaal River suspensions.

The algal cells, except *Cyclotella* cells were centrifuged at 5500 rpm for 10 minutes to concentrate the cultures so that 5 ml contained ±60 µg l^{-1} chlorophyll-*a*. Because of low cell numbers it was difficult to concentrate the chlorophyll-*a* of *C. meneghiniana* to 60 µg l^{-1}; therefore the *C. meneghiniana* culture was concentrated so that 7.5 ml contained ±30 µg l^{-1}. The volume of culture medium that was needed to add ±60 µg l^{-1} of chlorophyll-*a* to Vaal River water was calculated (in case of *C. meneghiniana*, ±30 µg l^{-1}). The cells were allowed for 30 minutes to adapt to the conditions in which they were concentrated.

Different treatments were applied to distinguish between the effects of algal cells separate from components excreted by the cells. After the cells were centrifuged, the supernatant of the culture medium was kept for control experiments to determine the effect of the cations (not adsorbed by algal cells) as well as excreted organic sustances present in the culture medium, on the flocculation process. Autoclaved culture medium was also added as a control to determine what effect the dissolved inorganic matter, present in the culture medium in which cells did not grow, had on the flocculation process.

Experiments were also conducted where the algal cells were removed from the culture medium (by centrifugation) and resuspended in distilled water. The algal cells were then centrifuged and resuspended three times to make sure that the original culture medium and all dissolved substances were effectively removed. These algal cells were then suspended in distilled water and added to Vaal River water in the volume and concentrations given above. The aim was to determine what effect algal cells only had on the flocculation processes.

In addition, experiments were conducted where only distilled water, the same volume as given above (5 ml for *Monoraphidium minutum* and *Pandorina morum*, and 7.5 ml for *Cyclotella meneghiniana*), was added to Vaal River water. This was to determine the (possibly small) effect of dilution when algal cells suspended in distilled water, were added to Vaal River water.

2.2.6 Composition of GBG 11

The composition of the growth medium, GBG 11 (Krüger and Eloff, 1978), a modified BG 11 (Stanier *et al.*, 1971), used for all three algal species are given in Tables 1 and 2. The addition of culture medium changed the ion composition of the Vaal River water and the added ions may act as point charges, which could influence the coagulation process.

Table 1 Composition of GBG 11, major elements

Constituents	Stock-solution	Volume of stock
$NaNO_3$	15.00 g l^{-1}	10 ml l^{-1}
$K_2HPO_4.3H_2O$	6.93 g l^{-1}	10 ml l^{-1}
$MgSO_4.7H_2O$	7.50 g l^{-1}	10 ml l^{-1}
$CaCl_2.2H_2O$	3.60 g l^{-1}	10 ml l^{-1}
$NaSiO_3$	10.00 g l^{-1}	10 ml l^{-1}
Na_2CO_3	2.00 g l^{-1}	10 ml l^{-1}
EDTA	0.10 g l^{-1}	10 ml l^{-1}
Citric acid	1.20 g l^{-1}	10 ml l^{-1}
$FeSO_4.7H_2O$	1.10 g l^{-1}	10 ml l^{-1}
Minor elements	*	1 ml l^{-1}

* see Table 2

However, when culture medium in which algal cells grew, were added to Vaal River water, organic substances excreted by the algal cells were also added. To determine the possible effect of excreted organic material on coagulation and sedimentation, results of added medium in which cells grew were compared with results of added medium in which cells did not grow.

Table 2 Composition of GBG 11, minor elements

Constituents	Stock-solution
H_3BO_3	2.86 g l^{-1}
$MnCl_2.4H_2O$	1.13 g l^{-1}
$ZnSO_4.7H_2O$	0.22 g l^{-1}
$NaMoO_4.5H_2O$	0.39 g l^{-1}
$Co(NO_3)_2.6H_2O$	0.049 g l^{-1}
$CuSO_4.5H_2O$	0.079 g l^{-1}

2.2.7 Analysis of extracellular organic substances

As described earlier, algal cells were removed from the culture medium by centrifugation. The supernatant of each culture was freeze-dried and 80 mg of the weighed, dried substrate was dissolved in 1.0 ml distilled water. Organic substances were extracted in ether and analysed, by the Department of Biochemistry (PU vir CHO), using the methods described in Van Rooyen *et al.* (1994). The concentration of the different excreted organic substances was determined and normalised to the chlorophyll concentration of the original respective culture. In the present study all individual excreted organic components were summed into, and listed in major categories, i.e. monocarboxylic acids, dicarboxylic acids and aromatic acids. In addition, glycerol and phosphoric acid, present respectively in *Monoraphidium minutum* and *Pandorina morum* cultures, were listed separately.

It is possible that the supernatant of the cultures have contained intact algal and bacterial cells from which organic substances could also have been extracted. It can, however, be assumed that these cells made a minor contribution, if at all, to the organic substances actually excreted by the live cells that grew in the culture.

2.2.7.1 Identification and quantification of organic acids

The following reagents were used: Distilled ethylacetate, distilled di-ethylether, 26.25 mg / 50 ml 3-Phenylbutyrate (internal standard), 5 N HCl.

Freeze-dried material (20 mg) was dissolved in 1 ml double distilled water. Identification and quantification of organic acids were achieved by utilising a modified procedure described in Van Rooyen *et al.* (1994). 3-Phenylbutyrate (100 µl;5:1 / 2.5 M per mg creatinine) was added as internal standard to each sample. The solution was subsequently acidified to a pH value of one with 5 N HCl. The prepared sample was extracted under continuous shaking with 6 ml cold distilled ethyl acetate for 30 minutes and then centrifuged for 3 min at 40 x *g*. The resulting supernatant phase, containing the ethyl acetate, was finally extracted by adding 3 ml distilled diethyl ether and shaking it for 10 min, after which the sample was centrifuged as before. After removing the diethyl ether phase, 6 ml ethyl acetate and 0.6 g Na_2SO_4 was added. Once the solution was centrifuged (3 min at 40 x *g*) the supernatant phase was dried under a stream of dry nitrogen for 1 hour.

The dried residue was derivatised for GC analysis with N.O-bis(trimethysilyl) trifluoroacetamide (2:1 per mg creatinine) and tri-methylchlorosilane (0.4 per mg creatinine). Analysis was carried out on a Hewlett Packard 5880 A series gas chromatograph and a SE (25 cm x 0.32 mm i.d.) capillary column. The derivatised sample was heated at 60 °C for 1 hour and 0.5 µl of the sample was injected for analysis A helium flow rate of 1 ml min^{-1} was used and during the analysis the temperature was programmed from 60 °C to 120 °C at 4° min^{-1} and then to 280 °C at 10 ° min^{-1}. An ionised energy of 70 eV was used.

2.2.7.2 Identification and quantification of amino acids and amines

The freeze-dried material (20 mg) was dissolved in 2,5 ml double distilled deionised water and the pH value of each samples were determined. During this study the pH value of each sample was higher than 8. The samples solution (2.5 ml) was subsequently purified with cation exchange resin by mixing 1.75 ml Dowex with the sample solution and allowing it to stand for 20 minutes. The sample-resin preparation was then transferred to a glass column and equilibrated with 20 ml of double distilled water. Compounds were eluted from the resin using 10 ml 2N NH_4OH. The ammonium fraction was concentrated by cooling it for 30 minutes at -70° C followed by freeze drying of the sample. The dried sample was redissolved in 0.5 ml double distilled water and acid-hydrolysed by adding 1 ml Bu HCl to the sample and heating it for 1 minute at 100 % power and 1 minute at 60 % power in a 750 watt microwave oven. After the samples has cooled down to room temperature the hydrolysate was dried under a stream of nitrogen gas at room temperature. The dried sample was resuspended in 10 µl CH_2Cl_2 and dried again as described. Following resuspention in 200 µl CH_2Cl_2 and 600 µl TFAA, the samples were boiled at 120° C for 5 minutes in an oil bath. After the sample has cooled, it was dried under a stream of nitrogen gas at room temperature for 5-10 minutes, washed in 100 µl CH_2Cl_2 and dried again for approximately 5 minutes. The dried sample was redissolved in 75 µl and 25 µl Piridine. Separation of the amino acids were achieved using a GS and MS spectrophotometer.

2.2.7.3 Protein concentration determination

The freeze-dried material (20 mg) was dissolved in 2,5 ml double distilled deionised water and the pH value of each sample was determined. Bicinchoninic acid protein assay kit, SIGMA procedure no. TPRO-562 (for kit no. BCA-1 and product no. B-9643), was used for the determination of the protein concentration. The following reagents were supplied with kit no. BCA-1: Bicinchoninic acid solution (B-9643) Reagent A: For determination of total protein per SIGMA procedure TPRO-562. A 1000 ml solution containing bicinchoninic acid, sodium carbonate, sodium tartrate and sodium bicarbonation 0.1 N NaOH (pH 11.25). Copper(II)sulphate pentahydrate 4% solution (C-2284) Reagent B: For determination of total protein per SIGMA procedure TPRO-562. A 25 ml solution containing 4% (w/v) cupric sulphate.5H_2O. Protein standard solution (P-914). A package of 5 flame sealed glass ampoules containing 1.0 ml of 1.0 mg ml^{-1} bovine serum albumin in 0.15 M NaCl with 0.05% sodium azide as a preservative.

The following assay procedure was used:

A standard curve as described below was prepared in order to determine the protein concentration of unknown samples. Any number of protein samples can be determined as long as the net absorbance 562 nm falls within the range of the standard curve. Some samples may have to be diluted to meet the requirements. The dilution factor should be recorded in the assay data table.

1. The required amount of protein determination reagent was prepared by adding 1 part copper(II)sulphate pentahydrate 4% solution (C-2284) to 50 parts bicinchoninic acid solution (B-9643).
2. Test tubes were labelled as indicated below in the assay set-up table.
3. The indicated amounts of water, protein standards and unknown protein samples were quantitatively added to the appropriate tubes.
4. 2.0 ml of the protein determination reagent was added to each test tube and vortexed.
5. The tubes were incubated at 37° C for 30 minutes.
6. The tubes were cooled to room temperature and the absorbance at 562 nm were determined in a spectrophotometer, using water to zero the instrument
7. The absorbance of the blank (tube 1) was subtracted from the absorbance of the remaining assay tubes to obtain the net absorbance due to protein.
8. A standard curve was prepared by plotting the net absorbance at 562 nm vs. the known added µg protein standard. The standard curve was used to determine the amount of protein in the unknown samples.

9. The protein concentration (mg ml^{-1}) of the unknown samples were calculated as follows:

$$\text{Protein (mg ml}^{-1}\text{)} = \frac{\text{(mg unknown protein per assay)(dilution factor)}}{\text{(ml unknown used for assay)}}$$

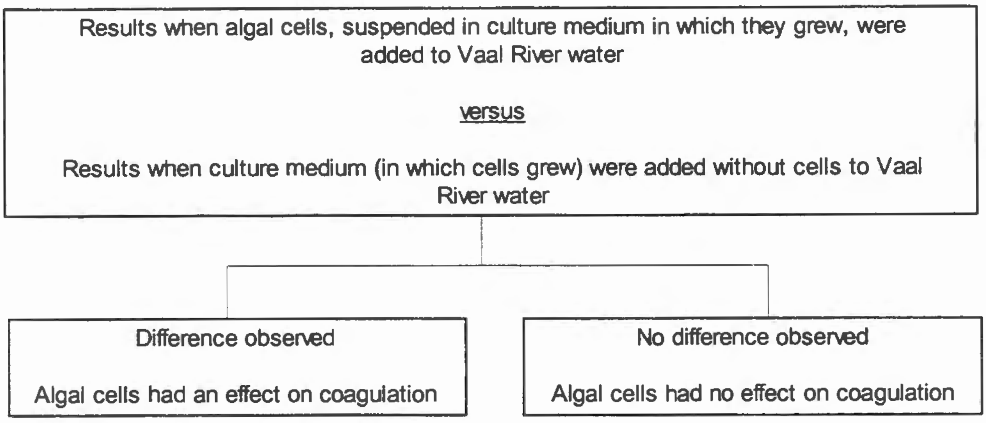
2.2.7.4 Oligosaccharide determination

The freeze-dried material (20 mg) was dissolved in 1 ml double distilled deionised water and used for oligosaccharide determination. Oligosaccharides were separated on a 5553 Thin layer chromatography plate using standard sugar solution (10 g l^{-1} in 10 percent v/v isopropanol). Ten microlitres of each of the prepared samples were applied to the plate after which it was developed for 90 minutes in an oli-bial system (150 ml bythanol, 75 ml acetic acid and 75 ml water). The plate was left to dry for at least 30 minutes between each change (2X developed). Thirty minutes after the last development, the plates were submersed in a fresh solution of ornicol sufuric acid (40 mg ornicol, 80 ml acetone and 4 ml H_2SO_4). After consideration of the results obtained it was decided to analise sugars utilising the gas chromatograph and / or ß-manno system consisting of 252 ml propanol, 3 ml acetic acid and 45 ml water.

2.2.8 Interpretation of results

The interpretation of the results where algal cells together with culture medium were added to Vaal River water was done very carefully. The experiments were conducted to determine the effect of increased algal concentration and excreted dissolved organic carbon (DOC) on the coagulation conditions of Vaal River water. The addition of excreted DOC to Vaal River water was done by adding culture medium in which the cells grew to Vaal River water. This gave rise to an experimental problem, because removal was then not only affected by DOC excreted by algal cells, but also by inorganic substances present in the culture medium. For this reason, medium in which algal cells did not grow, was also added to Vaal River water. The following illustrations explain the way in which the results from the different treatments were interpreted.

2.2.8.1 The effect of algal cells only



Results when algal cells, suspended in culture medium in which they grew, were added to Vaal River water

versus

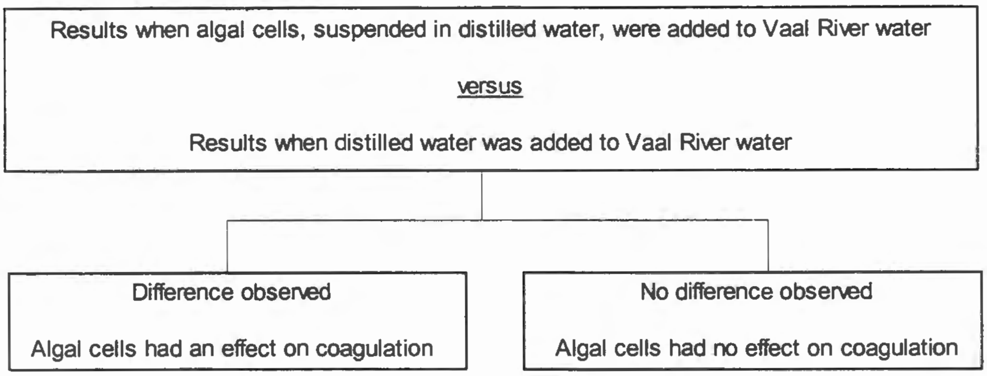
Results when culture medium (in which cells grew) were added without cells to Vaal River water

Difference observed

Algal cells had an effect on coagulation

No difference observed

Algal cells had no effect on coagulation



Results when algal cells, suspended in distilled water, were added to Vaal River water

versus

Results when distilled water was added to Vaal River water

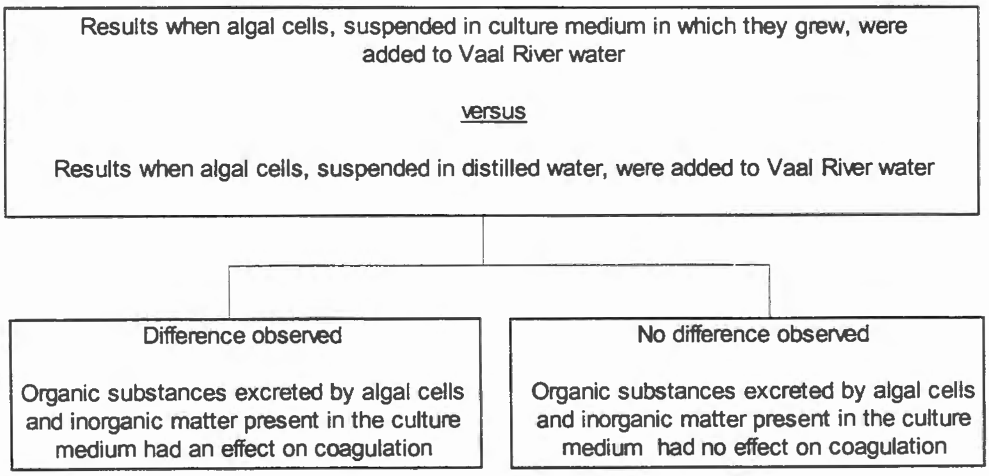
Difference observed

Algal cells had an effect on coagulation

No difference observed

Algal cells had no effect on coagulation

2.2.8.2 The effect of organic and inorganic substances



Results when algal cells, suspended in culture medium in which they grew, were added to Vaal River water

versus

Results when algal cells, suspended in distilled water, were added to Vaal River water

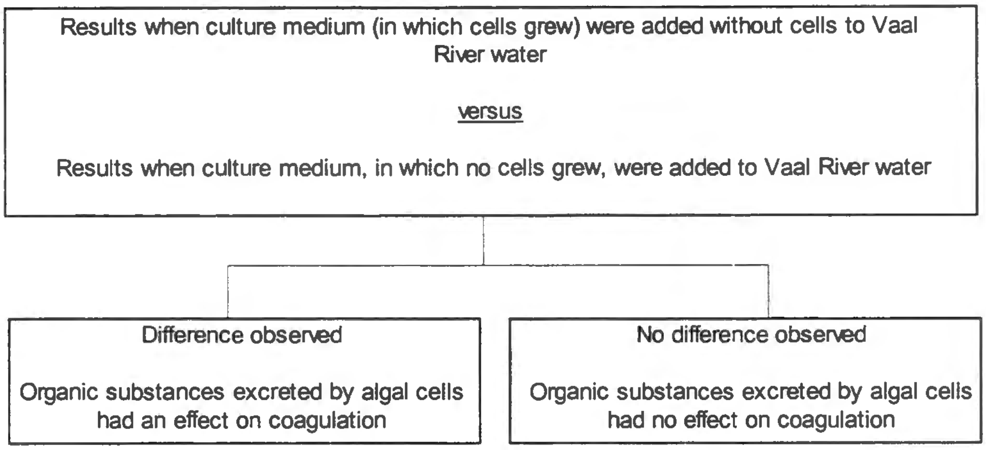
Difference observed

Organic substances excreted by algal cells and inorganic matter present in the culture medium had an effect on coagulation

No difference observed

Organic substances excreted by algal cells and inorganic matter present in the culture medium had no effect on coagulation

2.2.8.3 The effect of organic substances only



Results when culture medium (in which cells grew) were added without cells to Vaal River water

versus

Results when culture medium, in which no cells grew, were added to Vaal River water

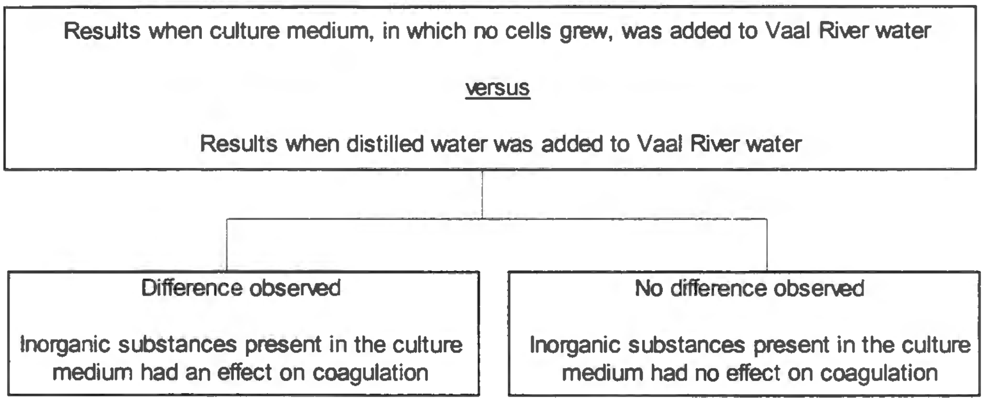
Difference observed

Organic substances excreted by algal cells had an effect on coagulation

No difference observed

Organic substances excreted by algal cells had no effect on coagulation

2.2.8.4 The effect of inorganic substances only



Results when culture medium, in which no cells grew, was added to Vaal River water

versus

Results when distilled water was added to Vaal River water

Difference observed

Inorganic substances present in the culture medium had an effect on coagulation

No difference observed

Inorganic substances present in the culture medium had no effect on coagulation

CHAPTER 3: EFFECT OF DIFFERENT FLOCCULANTS AND pH ADJUSTMENT CHEMICALS ON COAGULATION AND SEDIMENTATION PROCESSES

3.1 The effect of increased iron concentration on the removal of total suspended solids and dissolved organic carbon

The aim of the experiment was to determine the efficiency of $FeCl_3$ as flocculant by using the jar test apparatus, and to determine the optimum pH for coagulation at various Fe^{3+} concentrations.

The pH was adjusted to 5 and 7 with 0.1 M HCl, to 9 with 0.1 M NaOH, and to 11 with 0.4 M NaOH. The various Fe^{3+} concentrations used in this experiment was between 0 and 22 mg l^{-1}. Chlorophyll-*a*, turbidity and SAC 254 was determined, and the results are illustrated in Figs 3.1-3.4. The formation of flocs, floc size and amount of flocs were visually observed.

No flocks formed with the addition of 2 mg l^{-1} Fe^{3+} when the pH was adjusted to 5 with 0.1 M HCl. After the flocculation period, the treated water seemed turbid. With the addition of higher flocculant concentrations, increase in the floc size were observed together with increased Fe^{3+} concentration. The amount of small flocs formed on the surface of the treated water also increased with an increase in Fe^{3+} concentration, possibly an indication of flocculant overdose.

As illustrated in Fig. 3.1, a decrease in chlorophyll-*a* and turbidity levels occurred with the addition of 4 mg l^{-1} Fe^{3+}. At higher concentrations, an effective removal of suspended solids and chlorophyll-*a* were observed. There was also a total removal (total removal represent removals past the detection limit) of chlorophyll-*a* at 18 mg l^{-1} Fe^{3+}, leading to the conclusion that phytoplankton was effectively removed with the addition of 18 mg l^{-1} Fe^{3+}.

Flocs were formed with the addition of 4 mg l^{-1}, when the pH was adjusted to 7 with 0.1 M HCl, but the floc size was probably insufficient for effective sedimentation.

An increase in the amount of flocs formed on the surface of the treated water was observed together with an increase in Fe^{3+} concentration, an indication that flocculant overdosing possibly occurred.

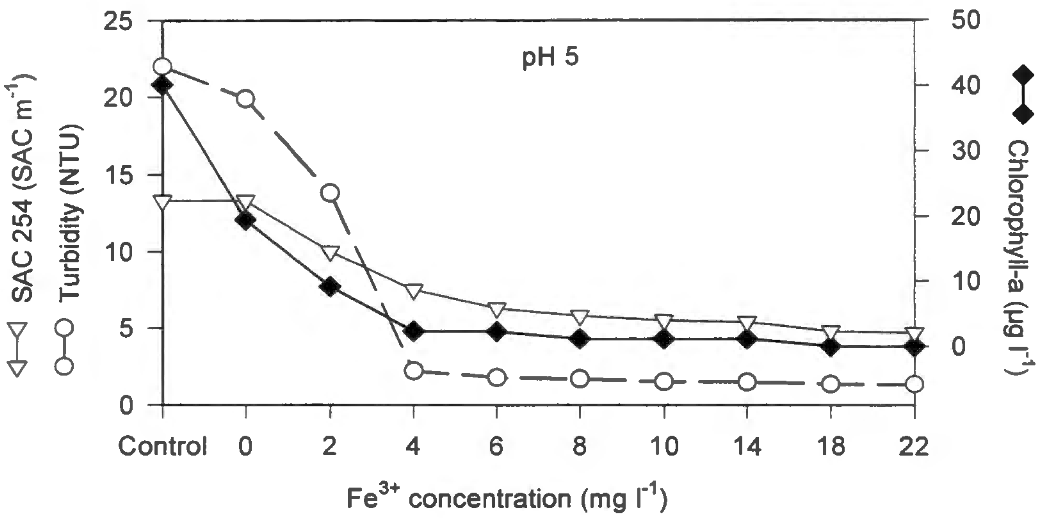


Figure 3.1 Variation in turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 5 with HCl. The turbidity of the raw water was 22 NTU, chlorophyll-*a* was 40.124 µg l^{-1}, and SAC was 13.3 SAC m^{-1}.

A decrease in chlorophyll-*a* levels with the addition of flocculant at pH 7 and at Fe^{3+} concentrations above 2 mg l^{-1} was observed as illustrated in Fig. 3.2.

The removal of total suspended solids, as indicated by turbidity, was more gradual in contrast to chlorophyll-*a*, but more effective than the removal of DOC, as indicated by SAC 254.

With the adjustment of the pH to 9 (Fig. 3.3) with 0.1 M NaOH, only small flocs formed when 2 mg l^{-1} flocculant was added. The size of the flocs was apparently insufficient for sedimentation, but after a 30 min sedimentation period, the turbidity of the treated water was lower than the Vaal River water (see control).

As illustrated in Fig. 3.3, high Fe^{3+} concentrations displayed effective removal of total suspended solids in the water, but not the removal of DOC. With the addition of 2 mg l^{-1} Fe^{3+} at pH 9, a decrease in chlorophyll-*a* and turbidity levels occurred. At high concentrations a slight additional decrease with the addition of Fe^{3+} was observed. Chlorophyll-*a* (i.e. algal cells) was not as effectively removed at pH 9 compared with pH 7 (see Fig. 3.2). At Sedibeng Water experience showed that it is not necessary to remove total suspended solids to values below 5 NTUs, because the rapid gravity sand filters are able to remove the remaining suspended matter. However, it is important to remove chlorophyll-*a* to values below 1 µg l^{-1}, because some algae have the ability to penetrate sand filters. This causes undesirably high chlorophyll-*a* concentrations in the final effluent.

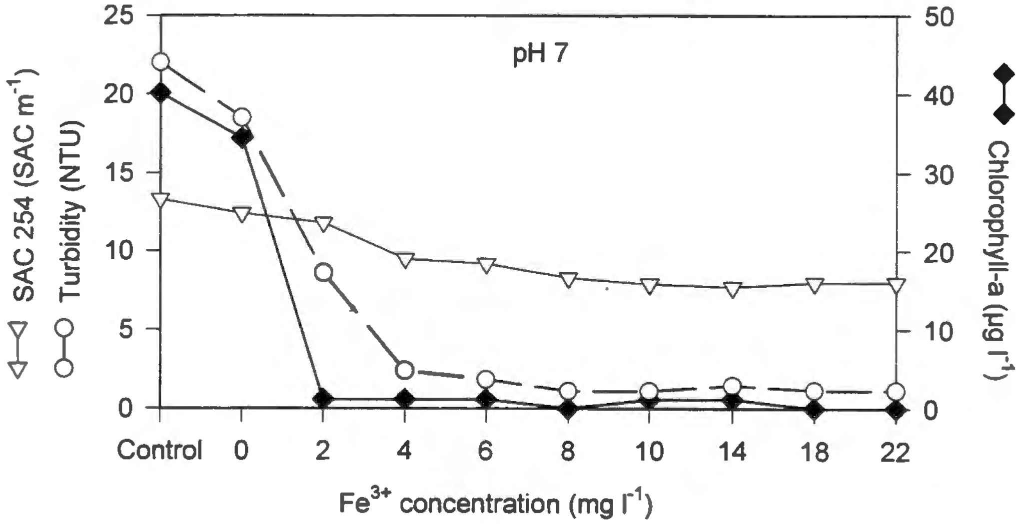


Figure 3.2 Variation in turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 7 with HCl. The turbidity of the raw water was 22 NTU, chlorophyll-*a* was 40.124 µg l^{-1}, and SAC was 13.3 SAC m^{-1}.

In the jar test at pH 11, flocs formed without the addition of $FeCl_3$ (Fig. 3.4). The pH was adjusted with 0.4 M NaOH and the ions produced by NaOH in solution may have acted as point charges, which may have been responsible for floc formation.

An increase in floc size and number, occurring together with an increase in Fe^{3+} concentration, was visually observed.

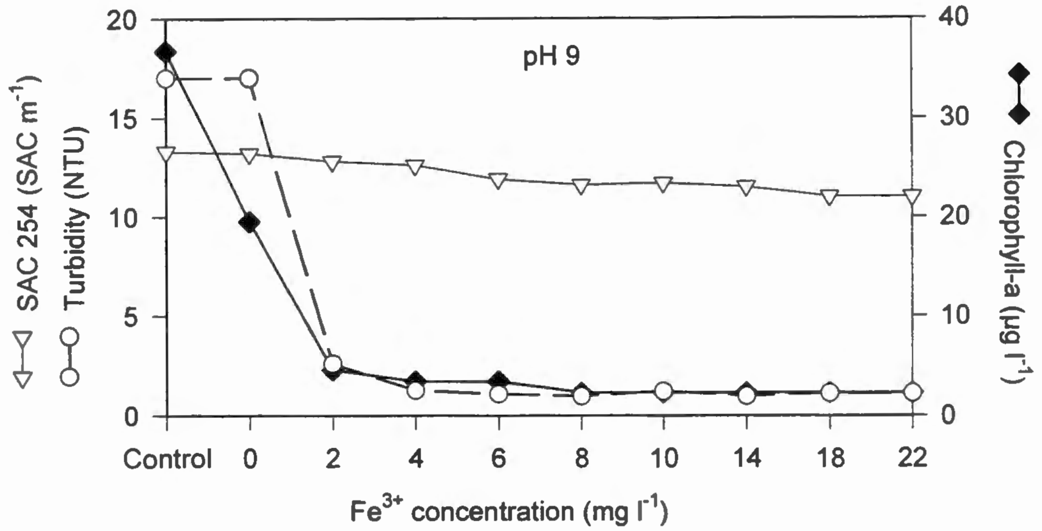


Figure 3.3 Variation in turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 9 with NaOH. The turbidity of the raw water was 17 NTU, chlorophyll-*a* was 36.68 µg l^{-1}, and SAC was 13.3 SAC m^{-1}.

As illustrated in Fig. 3.4, the removal of total suspended solids was ineffective with the addition of lower Fe^{3+} (up to 6 mg l^{-1}) concentrations. A decrease in chlorophyll-*a* values with the addition of 2 mg l^{-1} Fe^{3+} occurred at pH 11. The removal of DOC occurred, but was ineffective in contrast with total suspended solids.

The effect of pH on coagulation-flocculation and sedimentation on Vaal River water can be summarised as follows: Results from Figs 3.1 – 3.4 showed that changes in pH did not affect the removal of suspended solids significantly, the same turbidity values were obtained with after sedimentation with different pH conditions and and various Fe^{3+} concentrations . This indicates that, when low turbidity levels occur in the Vaal River at Balkfontein, it is not necessary to change the pH for turbidity removal. Increasing the pH may increase chemical cost without increasing the reduction in turbidity levels.

By increasing the pH, removal of algal biomass will definitely increase (see Figs 3.1-3.4). Low pH levels, i.e. pH of 5, showed the worst biomass removal. Figs 3.1 – 3.4 also showed that it is not necessary to increase the pH to high pH levels (i.e. pH levels of 9) for algal biomass removal, but that at higher pH levels (i.e. pH levels of 11), higher removals occur.

It is clear from Figs 3.1 – 3.4 that increased pH levels, decrease dissolved organic carbon removal. The best removal of DOC occurred at pH 5 (compare Fig. 3.1 with Figs 3.2 – 3.4). A pH of 5 will be impractical to use at Balkfontein, because water with this acidity may possibly be too aggressive to concrete structures of the Balkfontein plant.

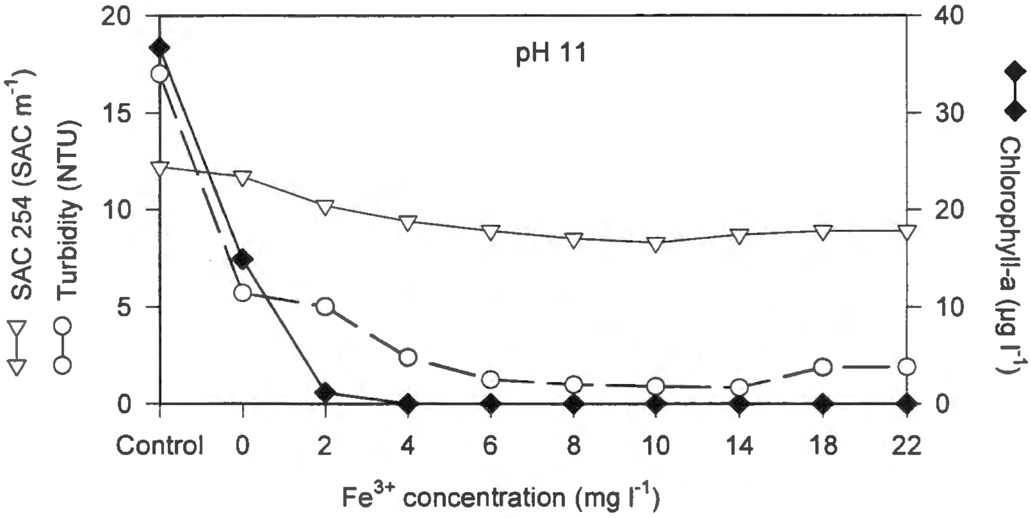


Figure 3.4 Variation in turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 11 with NaOH. The turbidity of the raw water was 17 NTU, chlorophyll-*a* was 36.68 µg l^{-1}, and SAC was 12.2 SAC m^{-1}.

3.2 The effect of NaOH and lime as pH-adjustment chemicals

The aim of the next group of experiments was to compare the use of NaOH and Lime as pH adjustment chemicals.

The pH for flocculation with Fe^{3+} was tested at 9 and 11. The pH adjustment chemicals used was 0.1 M NaOH, 0.4 M NaOH, lime supernatant solution (lime slaked by Midvaal Water Company was left undisturbed so that the lime particles could settle out and the clear supernatant was used) and a supersaturated lime suspension (lime slaked by Midvaal Water Company was well mixed). Supersaturated lime suspension was acquired from the Midvaal Water Company. The lime supernatant used in this experiment was withdrawn with a pipette from the supersaturated lime suspension. The lime suspension was diluted 4:1 with distilled water. The same Fe^{3+} concentrations as in the previous experiments were used, except that the highest concentration used here was 18 mg l^{-1}. Chlorophyll-*a*, turbidity and SAC 254 were determined as described in the materials and methods section, and the results are illustrated in Figs 3.5-3.10. The formation of flocs, floc size and amount of flocs were visually observed.

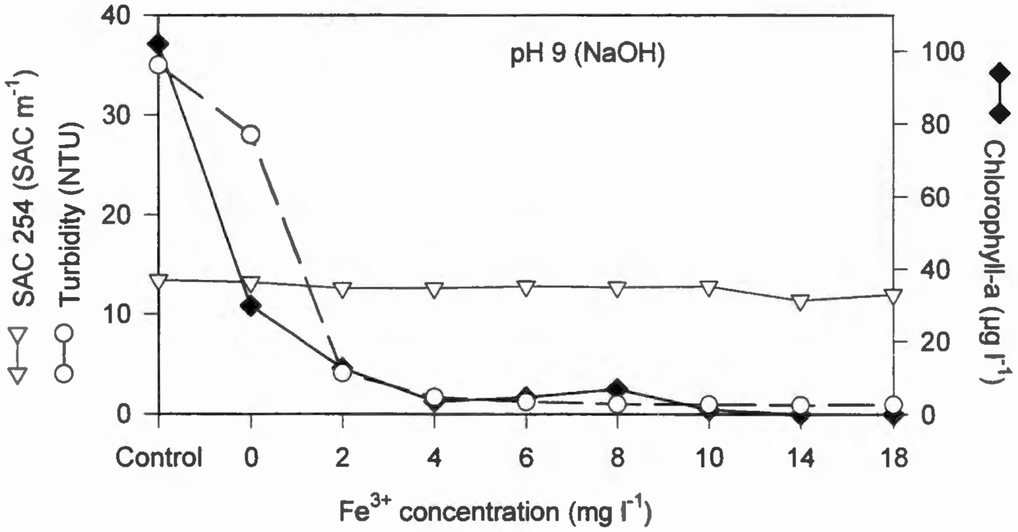


Figure 3.5 Variation in turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 9 with NaOH. The turbidity of the raw water was 35 NTU, chlorophyll-*a* was 102.03 µg l^{-1}, and SAC was 13.4 SAC m^{-1}.

When the pH was adjusted to 9 with 0.1 M NaOH, the size of the flocs was insufficient for sedimentation when small amounts of flocculant was added. A decrease in chlorophyll-*a*, as illustrated in Fig. 3.5, together with the addition of increased Fe^{3+} concentrations was observed, but high Fe^{3+} concentrations (> 14 mg l^{-1}) was necessary for the removal of total suspended solids. Dissolved organic matter was not removed from the treated water with the addition of $FeCl_3$ at pH 9.

At pH 11 (Fig. 3.6), the NaOH solution used caused the formation of flocs without the addition of $FeCl_3$. With the addition of flocculant, large flocs formed. After a sedimentation period of 30 min, there were still flocs in suspension and attached to the sides of the jars.

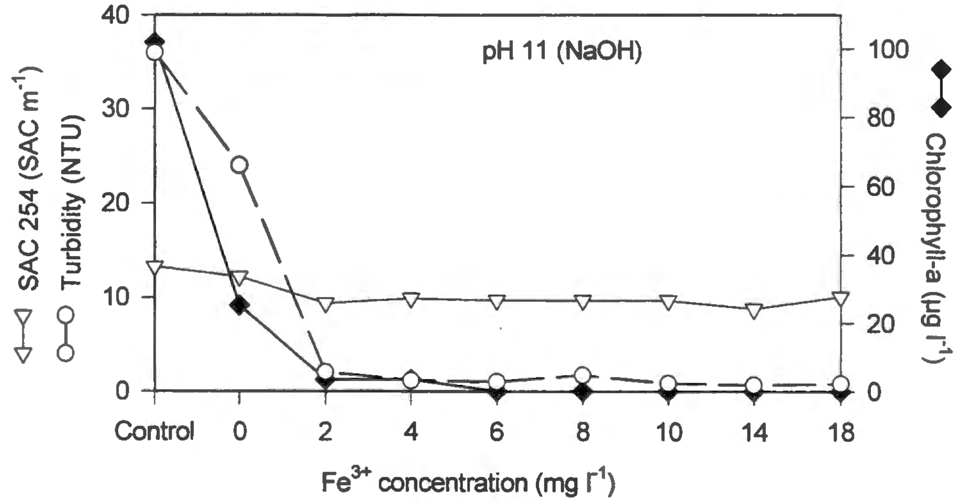


Figure 3.6 Variation in turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 11 with NaOH. The turbidity of the raw water was 35 NTU, chlorophyll-*a* was 102.03 µg l^{-1}, and SAC was 13.4 SAC m^{-1}.

Fig. 3.6 shows a decrease in chlorophyll-*a* and turbidity with the addition of 2 mg l^{-1} Fe^{3+} and higher. The removal of chlorophyll-*a* was most effective with the addition of high Fe^{3+} concentration ($\geq$ 6 mg l^{-1}). Almost no removal of dissolved organic matter was observed, as indicated by SAC 254 values.

Results from the experiment where pH was adjusted with NaOH and lime supernatant were in general similar (compare Figs 3.5 and 3.7; 3.6 and 3.8). After the lime supernatant was added as pH adjustment chemical at pH 9, the time period in which the flocs were formed, observed visually, was longer than with the addition of NaOH.

A removal of chlorophyll-*a* and suspended solids in the treated water together with the addition of 2 mg l^{-1} Fe^{3+} was observed (Fig. 3.7). No removal of dissolved organic matter at pH 9 was observed.

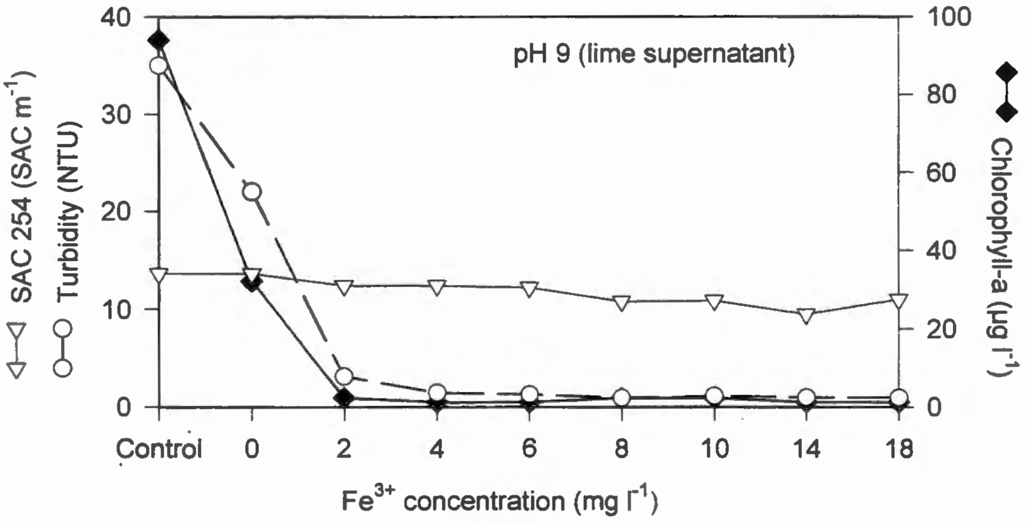


Figure 3.7 Variation in turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 9 with lime supernatant. The turbidity of the raw water was 35 NTU, chlorophyll-*a* was 94.01 µg l^{-1}, and SAC was 13.6 SAC m^{-1}.

When the pH was adjusted to 11 with lime supernatant (Fig. 3.8), a noticeable removal of chlorophyll-*a*, without the addition of $FeCl_3$, occurred. The removal of chlorophyll-*a* and other suspended particles was effective with the addition of increased Fe^{3+} concentration (above 4 mg l^{-1}). The flocculation and removal of dissolved organic matter was inefficient at pH 11, as indicated by SAC 254.

The addition of the supersaturated lime suspension caused an increase in the formation of flocs, observed visually, in contrast to the previous treatments. The size of the flocs also increased visually with the addition of lime as pH adjustment chemical.

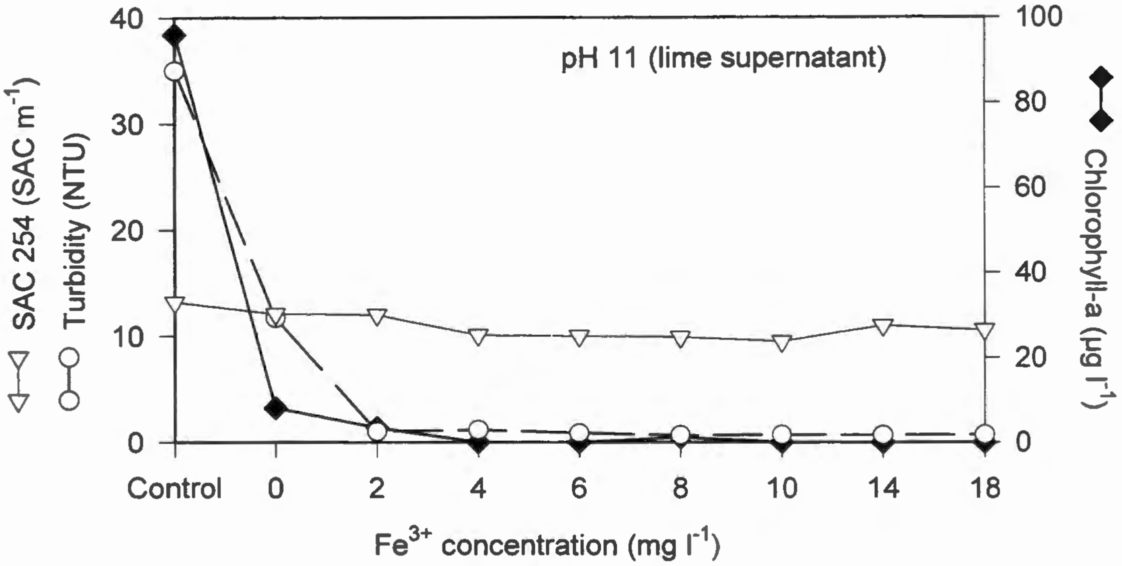


Figure 3.8 Variation in turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 11 with lime supernatant. The turbidity of the raw water was 35 NTU, chlorophyll-*a* was 96.29 µg l^{-1}, and SAC was 13.2 SAC m^{-1}.

As illustrated in Fig. 3.9, a decrease in total suspended solids in the treated water with the addition of 2 mg l^{-1} Fe^{3+} was observed at pH 9. A total removal of chlorophyll-*a* with the addition of 10 mg l^{-1} Fe^{3+} and higher concentrations was observed. The removal of total suspended solids was decreased to 0.79 NTUs with the addition of 14 mg l^{-1} Fe^{3+}. As mentioned earlier, the high iron concentration may possibly represent overdosing, because a turbidity of 5 NTU would be sufficient for water after sedimentation just before filtration. The high iron concentrations for the removal of chlorophyll-*a* makes it easier for the filters to remove the unwanted algae that are present in the water. This is essential because high chlorophyll-*a* values may possibly increase the chlorine demand of the final effluent and cause bacteriological problems in the distribution system.

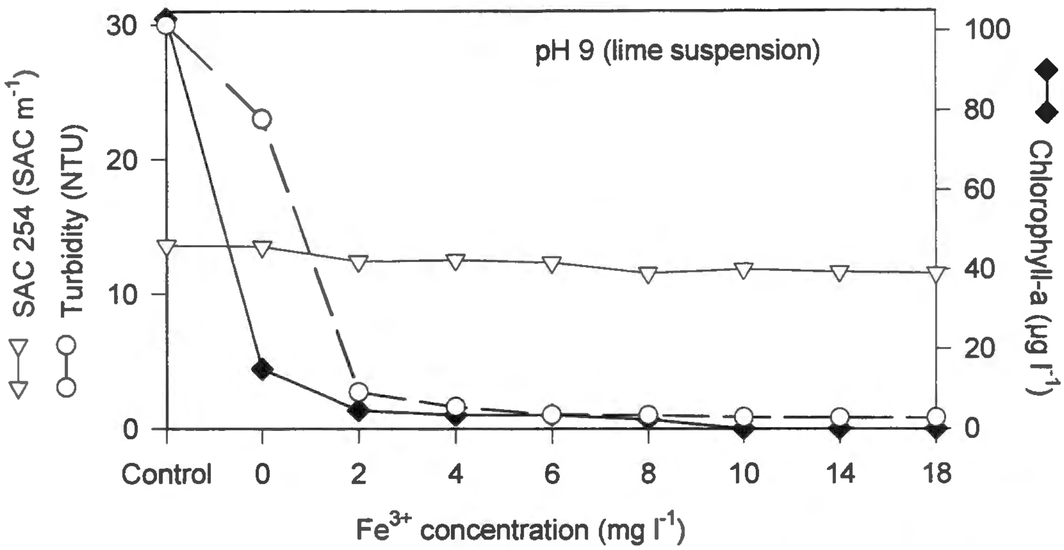


Figure 3.9 Variation in turbidity (NTU), chlorophyll-a concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 9 with Lime suspension. The turbidity of the raw water was 30 NTU, chlorophyll-a was 103.18 µg l^{-1}, and SAC was 13.6 SAC m^{-1}.

No removal of dissolved organic matter at pH 11 was observed (Fig. 3.9).

It was extremely difficult to keep particles of the lime suspension in suspension before it was added to the untreated water. After the sedimentation period, the treated water was turbid (due to suspended particles from the lime suspension) when no flocculant was added.

An increase in turbidity with the addition of lime to the untreated water was observed (Fig. 3.10), but with the addition of increased Fe^{3+} concentration, the turbidity decreased to a minimum of 0.59 NTUs.

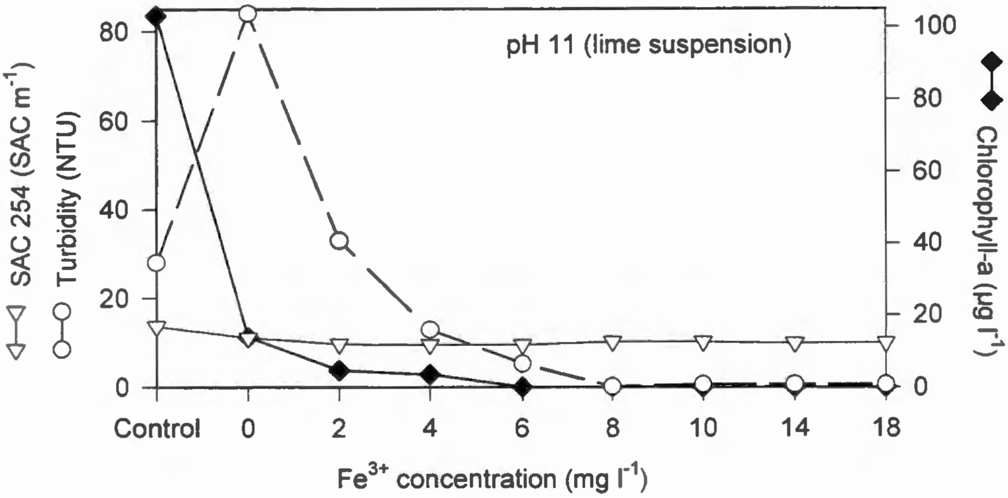


Figure 3.10 Variation in turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 11 with Lime suspension. The turbidity of the raw water was 28 NTU, chlorophyll-*a* was 103.18 µg l^{-1}, and SAC was 13.6 SAC m^{-1}.

Chlorophyll-*a* was efficiently removed at higher Fe^{3+} concentrations ($\geq$ 6 mg l^{-1}). As illustrated in Fig. 3.10, in accordance with the previous experiments, dissolved organic matter was unsuccessfully removed at pH 11.

The effect of different pH adjustment chemicals may be summarised as follows: At pH 9 (see Figs 3.5, 3.7 and 3.9), the best removal of suspended material occurred when lime was used as pH adjustment chemical. Effective removal also occurred when NaOH was used, but taking into account the cost of the pH adjustment chemicals, the use of lime is recommended.

When a supersaturated lime suspension was used as pH adjustment chemical at pH 11, the turbidity of the water increased when no flocculant was added (see Fig. 3.10). If this is compared to what happens on the Balkfontein plant, the same results were observed. When high pH levels (higher than pH 11) is used at Balkfontein, 5 mg l^{-1} $FeCl_3$ (as 48% $FeCl_3$ solution) is added to decrease turbidity levels to below 5 NTU's. Therefore it is recommended to use lime instead of NaOH, in accordance with practice, because lime is a cheaper alternative and more effective than NaOH.

3.3 The effect of $FeCl_3$ and $Fe_2(SO_4)_3$

The aim of the experiment was to compare the removal of algal biomass, other suspended particles and organic substances when $Fe_2(SO_4)_3$ (ferric-sulphate) and $FeCl_3$ (ferric-chloride) are used as coagulants. The experiment was conducted to compare the stabilisation ability of the two coagulants at various pH levels when lime was used as pH adjustment chemical. The concentrations used in this experiment was the same as those used in the previous experiment. The $Fe_2(SO_4)_3$ stock solution contained 3.578 g $Fe_2(SO_4)_3.H_2O$ (1 g.l^{-1} Fe^{3+}) to which 2.5 ml l^{-1} 15% HCl was added to stabilise the solution. The pH for flocculation with Fe^{3+} as $FeCl_3$ and $Fe_2(SO_4)_3$ was tested at 9 and 11. The pH adjustment chemical used was lime suspension.

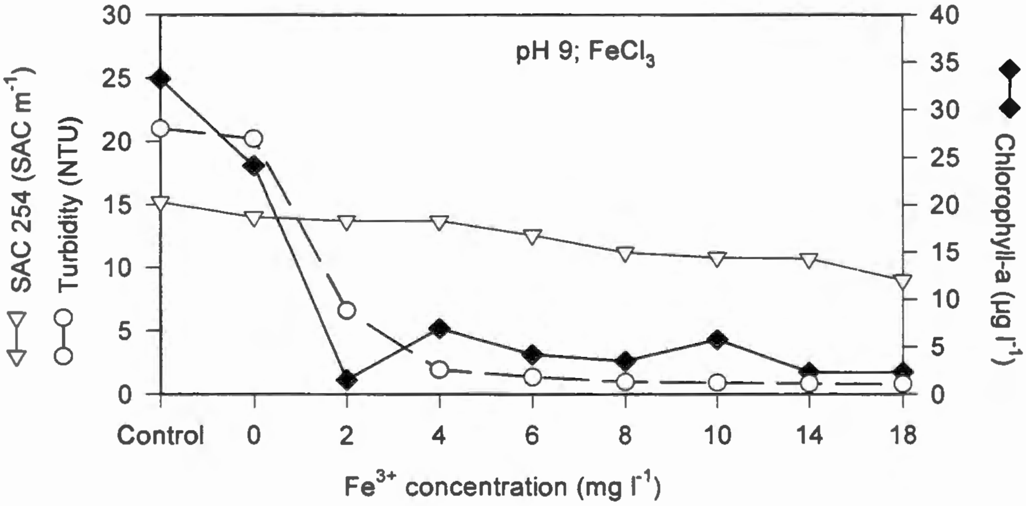


Figure 3.11 Variation in turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 9 with Lime suspension. The turbidity of the raw water was 22.1 NTU, chlorophyll-*a* was 33.25 µg l^{-1}, and SAC was 15.2 SAC m^{-1}.

When $FeCl_3$ was used as flocculant at pH 9, as illustrated in Fig. 3.11, a decrease in total suspended solids was observed as the Fe^{3+} concentrations increased.

The actual removal of chlorophyll-*a* and suspended inorganic matter in the treated water occurred under higher Fe^{3+} concentrations ($\geq$ 6-18 mg l^{-1}). Slight removal of dissolved organic matter occurred (Fig. 3.11) at 6 mg l^{-1} Fe^{3+} and higher concentrations.

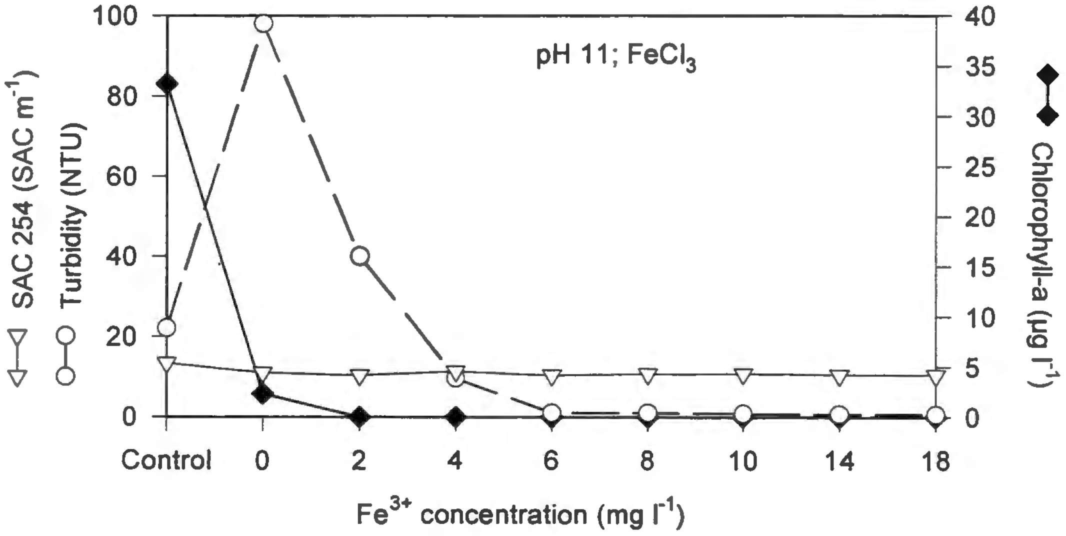


Figure 3.12 Variation in turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 11 with Lime suspension. The turbidity of the raw water was 22.1 NTU, chlorophyll-*a* was 33.25 µg l^{-1}, and SAC was 13.4 SAC m^{-1}.

With the addition of increased Fe^{3+} concentrations ($\geq$ 4 mg l^{-1}), the turbidity was efficiently decreased to NTUs lower than 1.0. More than 80 % of the chlorophyll-*a* of the Vaal River water was removed without the addition of flocculant. The removal may have been due to the aid of lime as flocculant.

As illustrated in Fig. 3.12, dissolved organic matter was not removed from the treated water at pH 11.

When $Fe_2(SO_4)_3$ was used as flocculant (Fig. 3.13), the results obtained was similar to the results obtained from the addition of Fe^{3+} as $FeCl_3$ at pH 9 adjusted with lime.

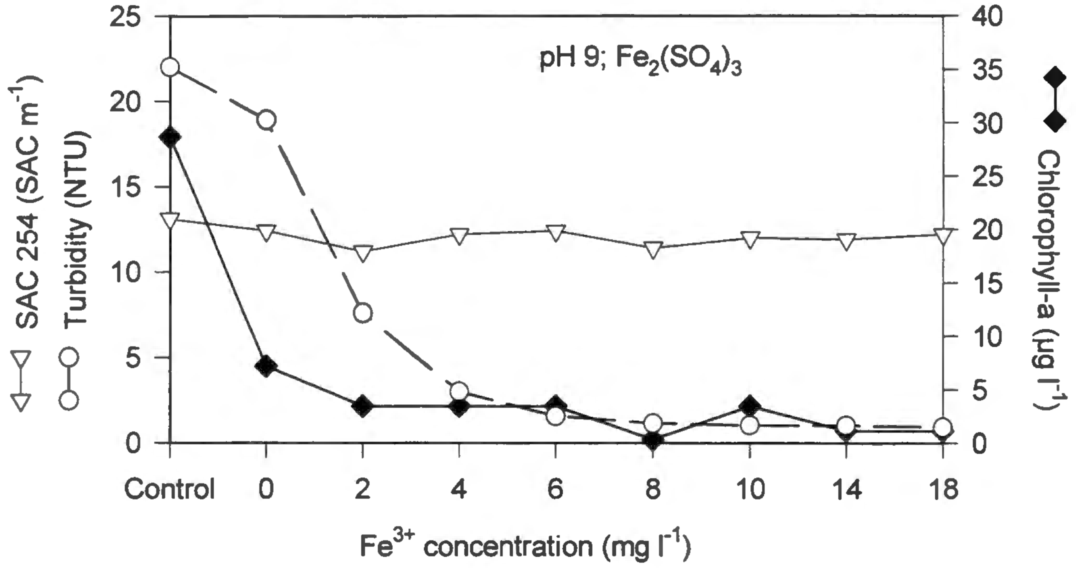


Figure 3.13 Variation in turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 9 with Lime suspension. The turbidity of the raw water was 22 NTU, chlorophyll-*a* was 28.66 µg l^{-1}, and SAC was 13.1 SAC m^{-1}.

Differences between the $FeCl_3$ versus $Fe_2(SO_4)_3$ treatments were observed in the removal of chlorophyll-*a*. With the addition of 14 mg l^{-1} Fe^{2+} as $Fe_2(SO_4)_3$, chlorophyll-*a* was decreased to 4% of that of the raw water Figs 3.13 and 3.14. Therefore, the addition of a high concentration of $Fe_2(SO_4)_3$ was effective in the removal of chlorophyll-*a* in the untreated water. This is an indication of effective removal of phytoplankton.

When lime suspension was added at pH 11, as illustrated in Fig. 3.14, as in the case of the previous experiment where lime was added to pH 11 (Fig. 3.10), turbidity increased initially. With an increase in $Fe_2(SO_4)_3$ concentration, turbidity decreased. Chlorophyll-*a* was effectively removed with the addition of increased $Fe_2(SO_4)_3$ concentrations (above 14 mg l^{-1}).

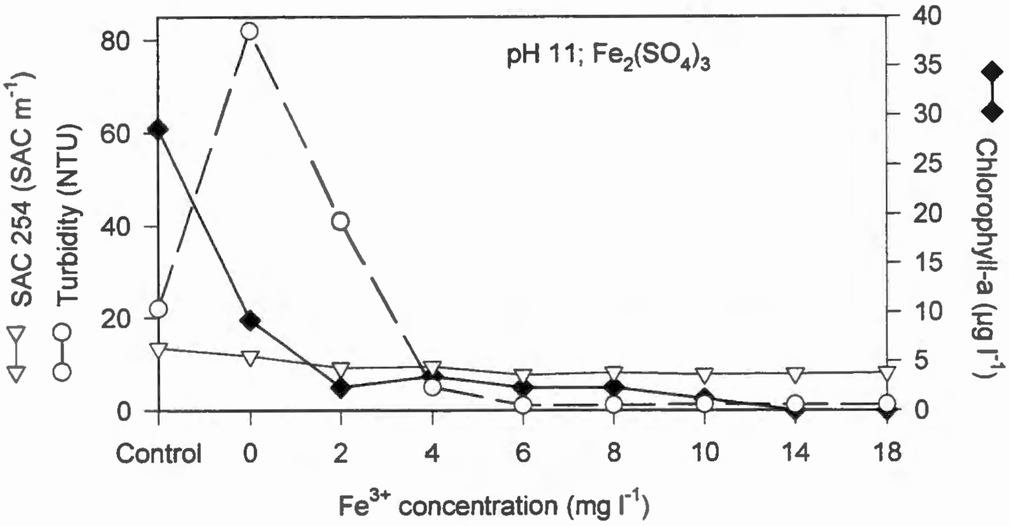


Figure 3.14 Variation in turbidity (NTU), chlorophyll-a concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased iron concentrations (mg l^{-1}) for pH adjusted to 11 with Lime suspension. The turbidity of the raw water was 22 NTU, chlorophyll-a was 28.66 µg l^{-1}, and SAC was 13.1 SAC m^{-1}.

A slight removal of dissolved organic matter was observed at pH 11 with the addition of increased flocculant concentrations. This can be due to the effect of lime in the flocculation and removal of dissolved organic matter. This aspect need to be investigated further.

The effect of different flocculants could be summarised as follows:

With the removal of turbidity, no difference occurred when $FeCl_3$ and $Fe_2(SO_4)_3$ were used, even at different pH conditions.

During conditions with a low pH (pH 9), the removal of algal biomass was more effective with $Fe_2(SO_4)_3$. During conditions with a higher pH (pH 11), the removal of algal biomass was more effective with $FeCl_3$.

For the removal of dissolved organic carbons, with a lower pH $FeCl_3$ would be the most effective flocculant to be used. During higher pH conditions no difference was observed.

3.4 The effect of pH > 11 on the removal of suspended material

The aim of this experiment was to determine the effect of the removal of chlorophyll-*a*, suspended solids and dissolved organic matter when the pH was increased to above 11. At the Balkfontein purification plant, experience showed that DOC was effectively removed when the pH was raised to 11.4.

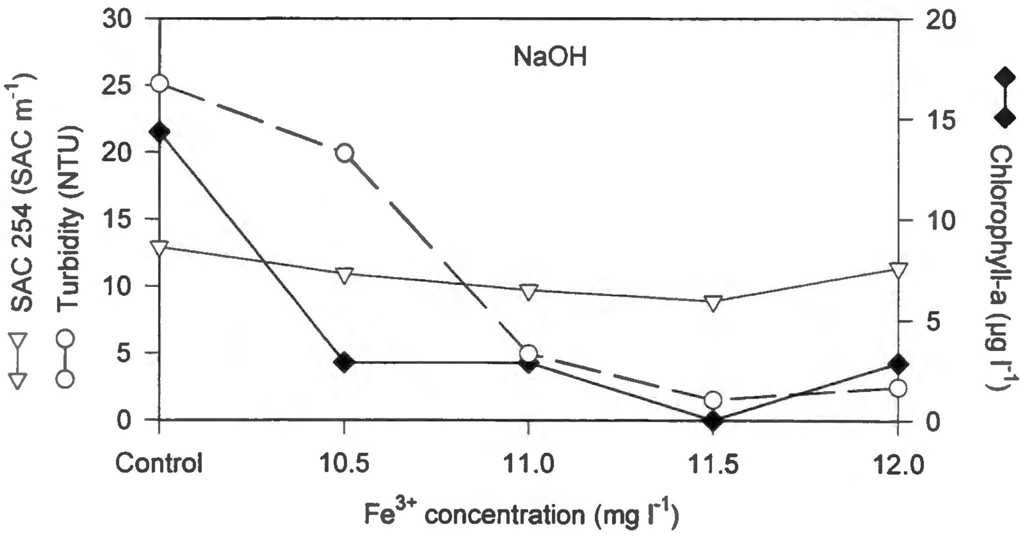


Figure 3.15 Variation in turbidity (NTU), chlorophyll-a concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased pH. pH adjusted with NaOH.

When the pH was adjusted with NaOH and no flocculant was added, removal of chlorophyll-*a* increased with an increase in pH (Fig. 3.15). Total removal of chlorophyll-*a* was observed at pH 11.5. When the pH was raised to 12, the removal decreased slightly. Suspended solids were also effectively removed at pH 11.5, with almost no removal at pH 10.5. DOC removal was not effective, which can be due to the absence of added Fe^{3+}, but the best removal was observed at pH 11.5

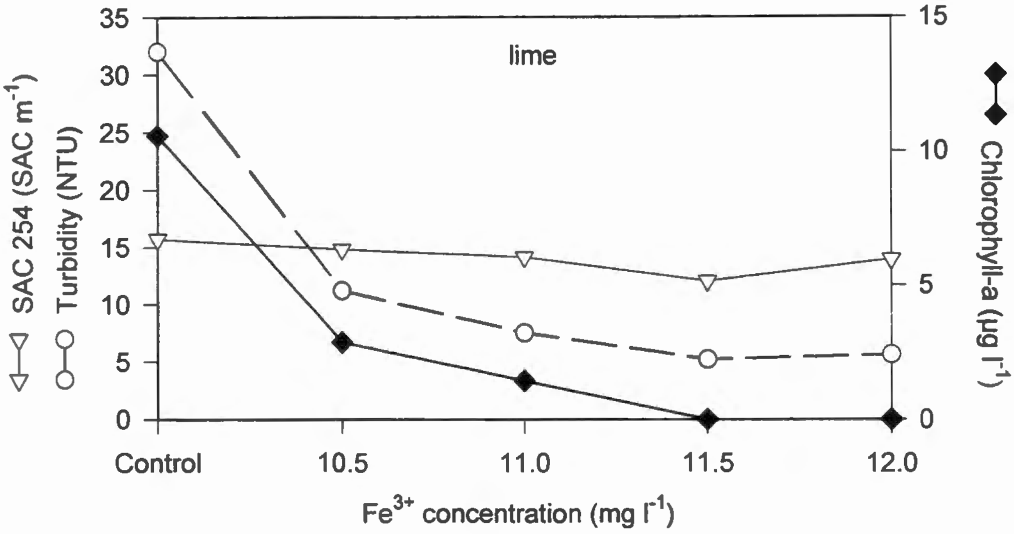


Figure 3.16 Variation in turbidity (NTU), chlorophyll-a concentration (μg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased pH. pH adjusted with lime.

When the pH was adjusted with lime, the removal of chlorophyll-a was ineffective at pH 10.5, but when the pH was increased to 11.5 and higher, total removal of chlorophyll-*a* occurred (Fig. 3.16). The removal of suspended solids was ineffective when the pH was adjusted with lime (compare Figs 3.15 and 3.16) which can be due to the suspended particles in the lime suspension which may have increased the turbidity. Fe^{3+} was not added, and previous experiments (Figs 3.10 and 3.12) showed that added lime increased turbidity when Fe^{3+} was not added as coagulant. The lowest turbidity values were obtained when the pH was adjusted to 11.5. DOC was also ineffectively removed when pH was adjusted with lime, but lower SAC values were obtained at pH 11.5

When 2 mg l^{-1} Fe^{3+} was added together with increased pH, total removal of chlorophyll-a occurred. The removal of suspended solids was effective and almost the same for all pH conditions (Fig. 3.17). DOC was again not effectively removed. The lowest SAC value was observed at pH 11.5 (Fig. 3.17). If high concentrations of DOC occur in the Vaal River, it becomes more difficult to remove these organic substances with the ordinary processes available at the Balkfontein plant of Sedibeng Water. Processes like high lime treatment is the only real efficient method to remove the DOC, and therefore the results obtained during this study showed that this method is possibly the only effective one to remove organic material at the Balkfontein plant.

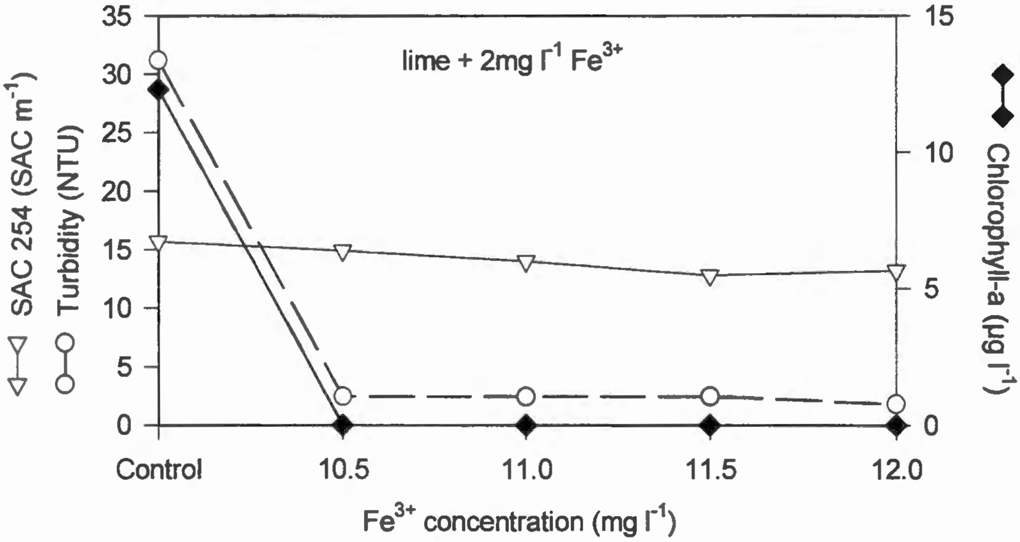


Figure 3.17 Variation of turbidity (NTU), chlorophyll-*a* concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased pH. pH adjusted with lime and 2 mg l^{-1} was added to Vaal River water.

The effect of pH 11 on the removal of suspended materials can be summarised as follows:

Increasing pH levels to pH 11, removal of suspended materials occurred, whilst a further increasing of pH, resolved in more effective removal.

The addition of a flocculant at lower concentrations (2 mg l^{-1} Fe) shows that different pH levels without flocculants are not liable for better removal of suspended materials.

3.5 The effect of the Ca^{2+} ions present in lime on the flocculation processes

In the high pH lime experiment (see Figs 3.4 and 3.6), high concentrations of Ca^{2+} was added to the untreated Vaal River water in the form of $Ca(OH)_2$. The aim of the following experiment was to determine the effect of this added Ca^{2+} ions on the flocculation processes.

The actual calcium concentration of the supersaturated lime suspension ($Ca(OH)_2$ acquired from the Midvaal Water Company), that was used for pH adjustment, was determined by the Soil Laboratory of the PU vir CHO. The Ca^{2+} concentration of the lime which was added as pH adjustment agent in the previous experiments was then calculated. The highest calculated Ca^{2+} concentration (volume of lime added to Vaal River water to raise the pH to 12) was 0.741 mg l^{-1}. The various Ca^{2+} concentrations used in this experiments were 0.55, 0.65, 0.75, 0.85 and 1 mg l^{-1}. Ca^{2+} as $CaCl_2$ was added to Vaal River water, without pH adjustment (Fig. 3.19), and also when the pH was adjusted to 11.5 with NaOH (Fig. 3.18).

When the pH was adjusted to 11.5 (Fig. 3.18), total removal of chlorophyll-*a* was observed in all the treatments. The removal of suspended solids was also effective, with the best removal observed at Ca^{2+} concentrations of 0.75-0.85 mg l^{-1}. DOC was, however, poorly removed when Ca^{2+} ions were added as $CaCl_2$ (Fig 3.18) when pH was adjusted to 11.5. Ca^{2+} had no effect on chlorophyll-*a* removal, because when 0 mg l^{-1} was added, total chlorophyll-*a* removal also occurred. The removal of algal biomass was, therefore, the result of high pH conditions.

When pH was not adjusted, and increased calcium concentrations were added to raw Vaal River water, almost no visually observed flocs formed. Slight removal of chlorophyll-*a* occurred at high Ca^{2+} concentrations (> 0.75 mg l^{-1}). The removal of DOC did not occur, and the turbidity values increased with the addition of high calcium concentrations (Fig. 3.19).

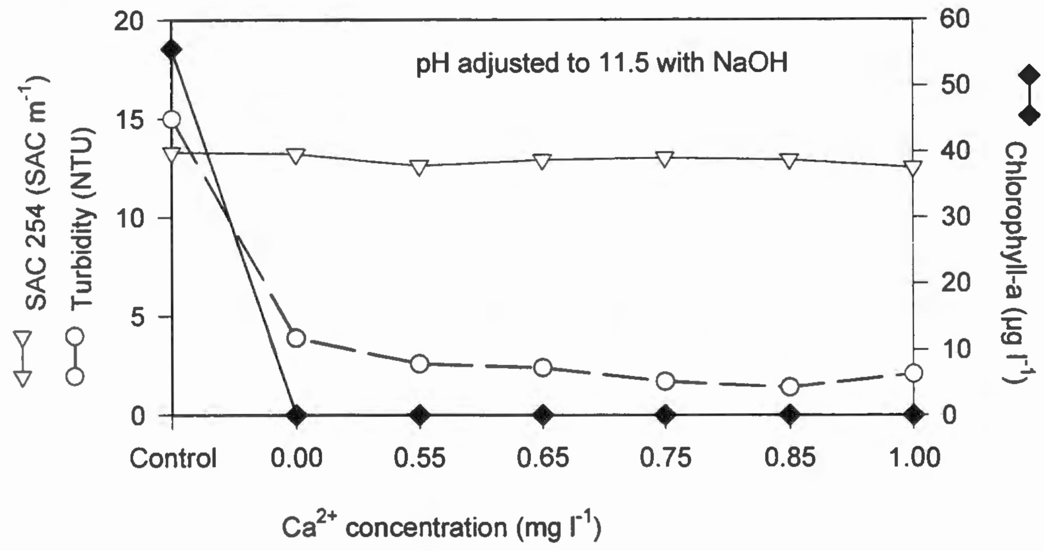


Figure 3.18 Variation in turbidity (NTU), chlorophyll-a concentration (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased Calcium concentrations (mg l^{-1}) for pH adjusted to 11.5 with NaOH.

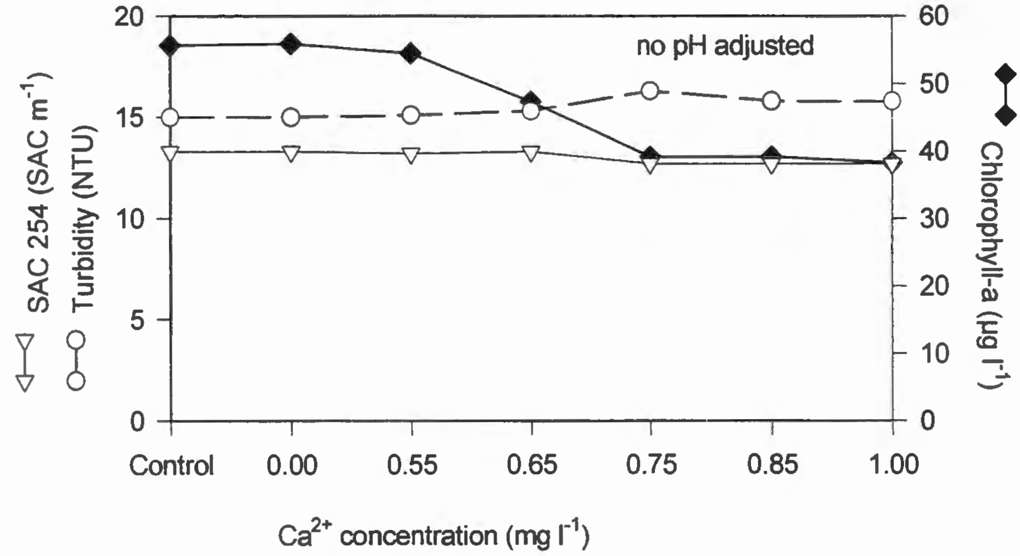


Figure 3.19 Variation in turbidity (NTU), chlorophyll-a concentrations (µg l^{-1}) and spectral absorption coefficient (SAC m^{-1}) with increased Calcium concentrations (mg l^{-1}). pH was not adjusted.

Figs 3.18 and 3.19 showed that Ca^{2+} had no effect on the removal of chlorophyll-*a*, suspended solids and dissolved organic matter. pH played an important part in flocculation or it can be possible that pH 11.5 caused calcium ions (and magnesium ions present in Vaal River water) to form precipitates which assist in flocculation, because suspended particles can be adsorbed by these precipitates (Parker *et al.*, 1975; Dziubek and Kowal, 1984).

The only conclusion drawn from this experiment was that calcium alone cannot be used as flocculant, but that pH adjustment are necessary for removal of suspended materials.

CHAPTER 4: EFFECT OF EXCRETED ORGANIC SUBSTANCES BY THREE ALGAL SPECIES ON COAGULATION PROCESSES

4.1 Introduction

Certain algal species penetrate purification processes in full-scale water treatment plants (Pieterse *et al.*,1993). The present investigation focused on algae which possibly resist coagulation, flocculation and sedimentation. Because algae probably resist coagulation, flocculation and sedimentation, additional flocculants must be added to remove the remaining algal cells. One of the possible reasons for the penetration of algal cells in purification processes is the possible effects of algal-extracellular products on destabilisation of suspended particles.

Algal-extracellular products could influence water purification by affecting the following processes or characteristics of the suspended particles. Because algal cells generally carry negative surface charges (Tebutt, 1977: Ronen, 1981), positively charged ions (e. g. Al^{3+} or Fe^{3+}) are added during purification for charge neutralisation. The purpose of the positive ions is to bind by means of attraction forces, to the negative charged particles in suspension. In this way biotic and abiotic particles agglomerate to form flocs that increase in size so that removal by sedimentation becomes possible. Negative charged substances can be biotic (e.g. algal and bacteria cells) or abiotic (e. g. colloids, and dissolved ions such as SO_4^{2-} and NO_3^-).

The characteristics of the biotic particles are not the same as that of the abiotic partcles, because biotic particles are alive and can excrete different substances (Lüsse *et al.*, 1985). These extracellular organic substances can go into suspension or solution or could attach to the cell wall and influence their surface characteristics, such as the surface charge. If the excreted substances go into suspension, it could remain stable or dissolved in the water or react with other substances, such as with chlorine, to form trihalomethanes (THMs) which are considered to be potentially carcinogenic compounds (Basson and

Pieterse, 1993). The occurrence of THM substances have been demonstrated in the Balkfontein purification plant as well as in raw water from the middle Vaal River (Basson & Pieterse, 1993).

Algal-extracellular organic substances in suspension contribute to the particulate organic carbon (POC) fraction in the water, while algal-extracellular products which dissolve in the water, contribute to the dissolved organic carbon (DOC) fraction. The POC and DOC may compete with the algal cells for the oxidation reagent, e.g. chlorine. One possibility is when algal cells are under stress, as might happen under the influence of the added oxidation chemicals, more algal extracellular excretion could occur, providing organic substances which can react with the oxidation reagent. In certain fragile species the cells may break up to release their constituents into the external medium. The result is that the algal-extracellular products are most probably oxidised in preference to algal cells which may cause some algal cells not to be oxidised.

If algal-extracellular products occur, they would contribute to the total organic carbon (TOC) fraction in the water. The particulate organic carbon fraction in the water together with the dissolved organic carbon fraction, gives the total organic fraction in the water. If the TOC concentration is less than 1-2 mg l^{-1}, flocculation is generally improved, but if the concentration is in excess of 1-2 mg l^{-1}, flocculation is disturbed (Lüsse *et al.*, 1985; Hoyer *et al.,* 1985).

Charge neutralisation is necessary for the formation of flocs. Algal-extracellular products which attach to the cell wall may affect the surface charge characteristics of the algal cells. If the algal-extracellular product concentration is high, the effect on the surface charge characteristics will possibly be more extensive. The net result of the effects of algal-extracellular products is that charge neutralisation possibly does not occur completely and flocs containing algal cells are not readily formed or are destablished.

Some algal-extracellular products could be polysaccharides. Polysaccharidic substances serve as precursors of humin-like compounds containing aliphatic and aromatic structures with phenolic polymers. Portions of aliphatic structures in humic acids can be considerably larger than portions of aromatic structures, particularly when the humic substances originate in aquatic environments (O'Colla, 1962).

Different studies were done on the mechanisms involved in unit processes in a water purification plant (Tebutt, 1977 and Gregory, 1989). Based on these studies, an understanding of the influence of algal-extracellular substances on the water purification process became possible.

In order to develop such an understanding, different characteristics of algae must be investigated, such as the reaction of algal cells to flocculation and sedimentation and the characteristics of cell walls and mucilages (O'Colla, 1962) excreted by algal cells. In terms of the possible reaction of algal cells, it is important to know whether algal cells excrete organic substances in reaction to oxidation and flocculation chemicals.

Based on aspects referred to in the previous paragraphs, the aim of the experiments into organic substances excreted by algal cells was to identify and quantify extracellular products. Special attention was given in other studies to the isolation and characterisation of extracellular organic matter from algae (Hoyer *et al.,* 1985; Lüsse *et al.,* 1985; Fogg, 1962: Hellebust, 1974). The removal of different specific extracellular products was not investigated *per se* in this study, but the influence of organic matter (excreted by algal cells) on the removal of algal cells, suspended solids and natural occurring organic matter was investigated.

The first part of this chapter deals with the different types of organic matter excreted by the three algae used in this study, namely Mono- and Dicarboxylic acids, Fatty acids, Aromatic acids and Amino acids. The second part of this chapter deals with the possible effect these extracellular products may have on the removal of algae and other suspended material.

4.2 Organic substances excreted by algal cells

Tables 4.1 to 4.6 show organic substances excreted by the algae in culture. A diverse amount of organic, fatty and aromatic acids as well as dimers, glycerol and phosphoric acid were found in the culture media.

Table 4.1 Componentes of extracellular organic substances from a uni-algal culture of *Monoraphidium minutum* (10 days old), a unicellular green alga, isolated from the Vaal River

Compound	Formula	Mol. Mass	Concentration (µg 1^{-1})	ng µg^{-1} Chlorophyll-*a*
1. **Monocarboxylic acids**				
Lactic acid	$CH_3CH0HCO_2H$	90.08	34.4	9.7
Hydroxy-Acetic/ Glycolic adic	$HOCH_2CO_2H$	76.05	135.7	38.2
3-Hydroxy-Propanoic acid	$HOCH_2CH_2CO2H$	90.08	47.7	13.4
3,4-dihydroxy-Butyric acid	$HOCH_2CHOHCH_2CO_2H$	104.11	137.0	38.5
2. **Dicarboxylic acids**				
Oxalic acid	HO_2CCO_2H	90.04	251.8	70.9
Succinic acid	$HO_2CCH_2CH_2CO_2H$	118.09	14.2	4.0
3. **Fatty acids**				
Lauric acid	$CH_3(CH_2)_{10}CO_2H$	200.32	131.5	37
Myristic acid	$CH_3(CH_2)_{12}CO_2H$	228.36	79.4	22.3
Palmitic acid	$CH_3(CH_2)_{14}CO_2H$	256.43	58.7	16.5
Stearic acid	$CH_3(CH_2)_{16}CO_2H$	284.49	61.8	17.4
4. **Aromatic acids**				
Methylenediphenol	$C_{13}H_{12}0_2$	344	51.5	14.5
Hydroxyethylenediphenol	$C_{14}H_{14}O_3$	446	76.9	21.6
Hydroxy-propylenediphenol	$C_{15}H_{16}O_3$	460	103.4	29.1
5. **Other**				
Glycerol	$HOCH_2CHOH_2OH$	92.1	34.3	9.7

Monoraphidium minutum excreted the largest number of substances (Table 4.1), followed by *Pandorina morum* (Table 4.5) and *Cyclotella meneghiniana* (Table 4.3). High concentrations of Hydroxy-Acetic/Glycolic acid, 3,4-dihydroxy-Butyric acid, Oxalic acid, Lauric acid, Myristic acid, Stearic acids and Hydroxy-propylenediphenol were excreted by *Monoraphidium minutum* (Table 4.1). If the extracellular products of the two green algae are compared with each other, Table 4.1 and Table 4.3 indicates that only Lactic-, Oxalic- and Succinic acids were excreted by both algae. *Cyclotella meneghiniana* (Table 4.5) excreted also Lactic-, Oxalic-, Succinic-, and Palmitic acids similar to the green algae, but higher concentrations occurred in the culture medium of *Cyclotella meneghiniana* (Tables 4.5 and 4.6). A large number of organic acids were excreted by *Monoraphidium minutum* (Table 4.1), but except for Oxalic acid, only low concentrations were detected when compared with *Cyclotella meneghiniana* (Table 4:5)

Table 4.2 Amino acids from a uni-algal culture of *Monoraphidium minutum* (10 days old), a unicellular green algae, isolated from the Vaal River

Amino acid	Mol. Mass	Concentration µmol l^{-1}	Ng μg^{-1} Chlorophyll-*a*
Glysine	132	50.7	0.690
Alanine	146	10.6	0.159
α-Aminobutyric acid	160	2.4	0.039
Serine	162	131.1	2.192
Threonine	176	20.6	0.374
Proline	172	11.4	0.202
Ornithine	168	13.1	0.227
Valine	174	12.9	0.231
Leucine	131	10.4	0.140
Lycine	203	447.8	9.382
Lycine	186	160.9	3.088
Methionine	206	28.6	0.608
Sithruline	215	58.4	1.295
α-Amino adipic acid	218	15.4	0.346
Aspartic acid	246	12.6	0.319
Glutamic acid	260	49.6	1.331

Table 4.3 Components of extracellular organic substances from a uni-algal culture of *Pandorina morum* (10 days old), a colonial green flagellate, isolated from the Vaal River

Compound	Formula	Mol. Mass	Concentration (µg 1^{-1})	ng µg^{-1} Chlorophyll-a
1. **Monocarboxylic acids**				
Lactic acid	$CH_3CH0HCO_2H$	90.08	34.4	10.8
2. **Dicarboxylic acids**				
Oxalic acid	HO_2CCO_2H	90.04	251.8	79.4
Succinic acid	$HO_2CCH_2CH_2CO_2H$	118.09	14.3	4.5
5. **Aromatic acids**				
Bezoic acid	$C_6H_5CO_2H$	122.12	109.6	34.6
6. **Other**				
Phosphoric acid	$(C_2H_5O)_2POOH$	118.09	6.7	2.1

Pandorina morum excreted, compared with *Monoraphidium minutum* only few organic acids, but high concentrations of Oxalic and Benzoic acids were detected (Table 4.3).

Table 4.4 Amino acids from a uni-algal culture of *Pandorina morum* (10 days old), a colonial green flagellate, isolated from the Vaal River

Amino acid	Mol. Mass	Concentration µmol l^{-1}	ng µg^{-1} Chlorophyll-*a*
α-Aminobutyric acid	160	5.1	0.125
Serine	162	223.2	5.575
Proline	172	4.1	0.108
Valine	174	2.5	0.067
Leucine	131	32.8	0.662
Lycine	203	121.5	3.803
Lycine	186	101.1	2.899
Homo-cysteïne	192	6.5	0.192
Methionine	206	26.3	0.835
Histidine	212	17.1	0.559
1&3-Methylhistidine	226	13.9	0.484
Sithruline	215	58.5	1.939
α-Amino adipic acid	218	40.9	1.374
Aspartic acid	246	271.0	10.280
Glutamic acid	260	22.7	0.910

A diverse number of amino acids were found in all the culture media. *Cyclotella meneghiniana* excreted 15 different amino acids (Table 4.4), and *Pandorina morum* (Table 4.6) and *Monoraphidium minutum* (Table 4.2) excreted 16 different amino acids. High concentrations of Serine and Histidine were observed in the culture medium of *Monoraphidium minutum* and *M. minutum* was the only algal species that excreted Phenylalanine and Thyrosine. (Table 4.2). *Pandorina morum* excreted Glysine. Glycine was not detected in the culture media of the other two species (compare Table 4.4 with 4.2 and 4.6). Higher concentrations of Lycine was detected in the medium of *Pandorina morum* compared with *M. minutum* and *C. meneghiniana* (compare Table 4.4 with 4.2 and 4.6). Cyclotella meneghiniana excreted high concentrations of Leucine, α-Amino adipic acid and Aspartic acids (Table 4.6). Although a relatively large number of amino acids were excreted by the algae, compared to organic acids, only low concentrations of amino acids were detected in the different culture media.

Table 4.5 Componentes of extracellular organic substances from a uni-algal culture of *Cyclotella meneghiniana* (18 days old), a unicellular centric diatom, isolated from the Vaal River

Compound	Formula	Mol. Mass	Concentration ($\mu g\ 1^{-1}$)	ng μg^{-1} Chlorophyll-a
1. **Monocarboxylic acids**				
Lactic acid	$CH_3CH0HCO_2H$	90.08	188.9	219.1
2. **Dicarboxylic acids**				
Oxalic acid	HO_2CCO_2H	90.04	236.4	274.2
Succinic acid	$HO_2CCH_2CH_2CO_2H$	118.09	65.9	76.4
Citric acid	$HO_2C(CH_2CO_2H)CO_2H$	192.12	45.7	53.0
3. **Fatty acids**				
Palmitic acid	$CH_3(CH_2)_{14}CO_2H$	256.43	39.9	46.3

A number of proteins were present in the culture media of the three algae, but difficulty to identify the various proteins allow only the determination of concentrations of the three algal species used in this study, *Monoraphidium minutum* has 12.3 mg l^{-1}, *Cyclotella meneghiniana* 9.6 mg l^{-1} and *Pandorina morum* 6.5 mg l^{-1}. Because no proteins were identified, it is difficult to make conclusions on the effect of proteins on the removal of algal cells, suspended solids and dissolved organic material.

Table 4.6 Amino acids from a uni-algal culture of *Cyclotella meneghiniana* (18 days old), a unicellular centric diatom, isolated from the Vaal River

Amino acid	Mol. Mass	Concentration µmol l^{-1}	ng μg^{-1} Chlorophyll-*a*
Alanine	146	11.8	0.122
α-Aminobutyric acid	160	0.0	0.0
Serine	162	757.2	0.875
Proline	172	38.3	0.469
Ornithine	168	5.3	0.062
Valine	174	60.6	0.752
Leucine	131	15.7	0.146
Lycine	203	107.5	1.556
Lycine	186	35.3	0.464
Histidine	212	69.8	1.055
1&3-Methylhistidine	226	23.8	0.383
Sithruline	215	17.2	0.263
α-Amino adipic acid	218	29.2	0.454
Phenylalanine	222	12.2	0.193
Thyrosine	238	5.1	0.086
Aspartic acid	246	339.5	5.958
Glutamic acid	260	57.3	1.064

No oligosaccharides were detected in the culture media of the three algae used during this study.

4.3 The effect of *Monoraphidium minutum* cells on coagulation and flocculation

The aim of the experiments were to determine what effect added *M. minutum* cells (representing a *M. minutum* dominance under natural conditions in water from the Vaal River), and their organic excretions, had on flocculation processes. Experimental protocol is as applied in the material and methods chapter.

4.3.1 Removal of biomass (i.e. chlorophyll-*a*)

M. minutum cells suspended in culture medium in which the cells grew (concentrated so that 5.0 ml contains 60 µg l^{-1} chlorophyll-*a*, used for biomass), the same concentration suspended in distilled water, culture medium without cells in which the cells grew (cells were removed using a centrifuge), culture medium in which no cells grew (GBG 11), and distilled water only, were added respectively to Vaal River water. The different treatments were transferred to jar test beakers for further processing.

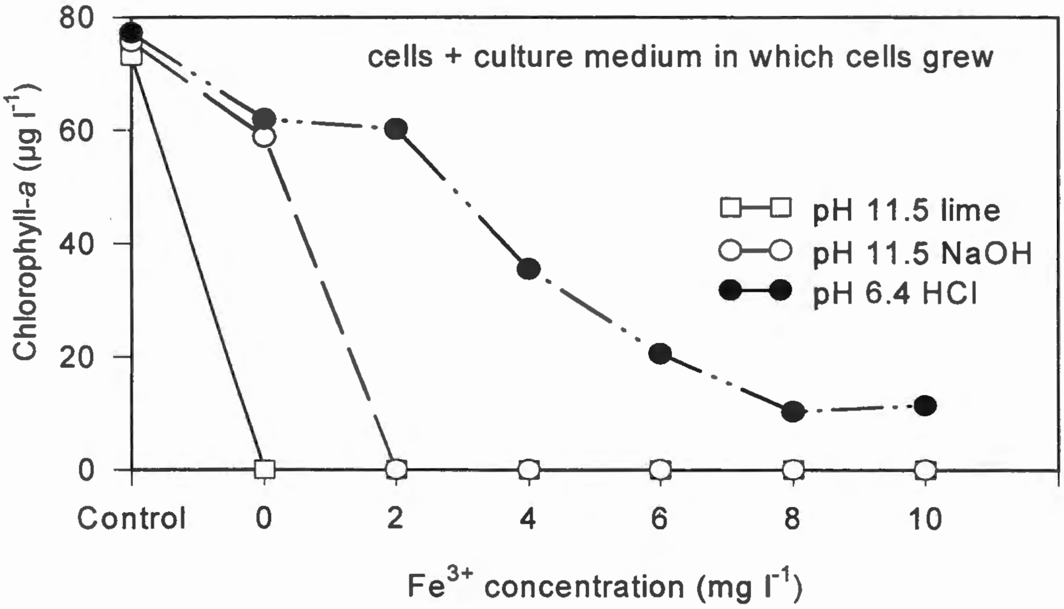


Figure 4.1 Variation in chlorophyll-*a* concentration (µg l^{-1}) with increased Fe^{3+} concentrations. *Monoraphidium minutum* cells, suspended in culture medium in which the cells grew, were added to Vaal River water.

Small flocs formed when HCl was added as pH adjustment chemicals. The treated water was more turbid when lime was added, although larger flocs formed. The treated water, after lime dosing, was visually clearer at the end of the sedimentation period (30 min). When no Fe^{3+} was added, and only pH adjustment chemicals (HCl and NaOH), almost no removal occurred. When lime ($Ca(OH)_2$ was added to adjust pH to 11.5, total removal (total removal represent removal past the detection limit of the chlorophyll-*a* method used) of algal biomass occurred (Fig. 4.1).

The addition of low Fe^{3+} concentrations at pH 6.4 was inefficient in the removal of *M. minutum* biomass when compared to when pH adjustment to 11.5 was made with NaOH and lime. The best removal of algal biomass, at pH 6.4, was obtained when 8 mg l^{-1} Fe^{3+} and higher concentrations were added. Effective removal occurred at pH 11.5 (adjusted with NaOH) when Fe^{3+} was added. The same results were obtained when lime was used (Fig. 4.1). The good removal of algal biomass when lime only was added, represents a possible indication that lime might act as primary coagulant in Vaal River water when chlorophyll-*a*, representing algal biomass, is the most problematic parameter to be removed.

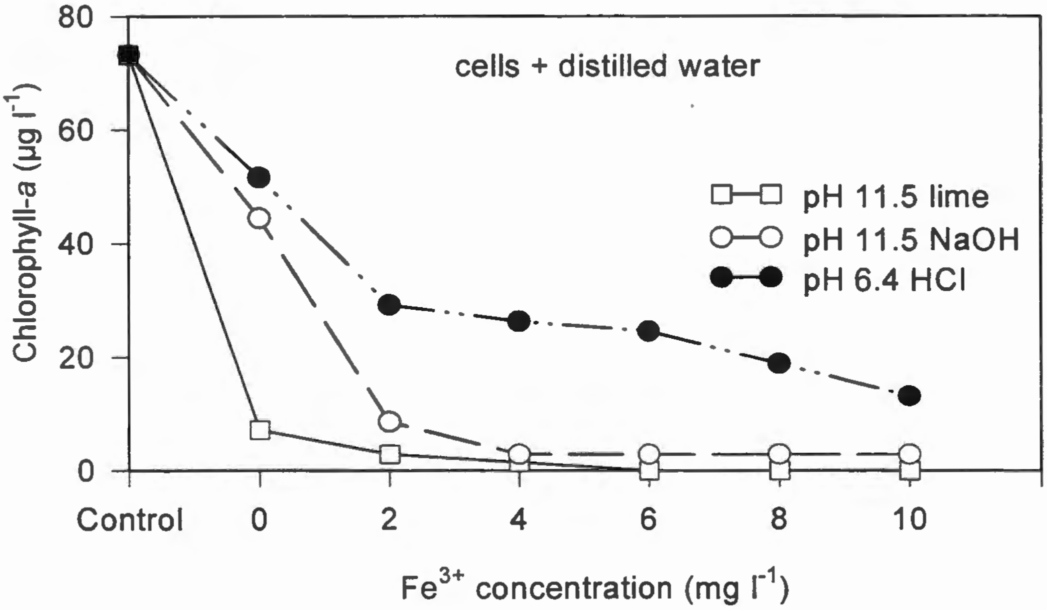


Figure 4.2 Variation in chlorophyll-*a* concentration (µg l^{-1}) with increased Fe^{3+} concentrations. *Monoraphidium minutum* cells, suspended in distilled water, were added to Vaal River water.

When *M. minutum* cells were suspended in distilled water and added to Vaal River water, the floc size of the differently treated water was the same as when cells, suspended in culture medium, were added to Vaal River water. The number of flocs formed on the surface of the treated water was, however, visually less. The inorganic salts in the culture medium may have acted as point charges and can possibly indicate overdosing.

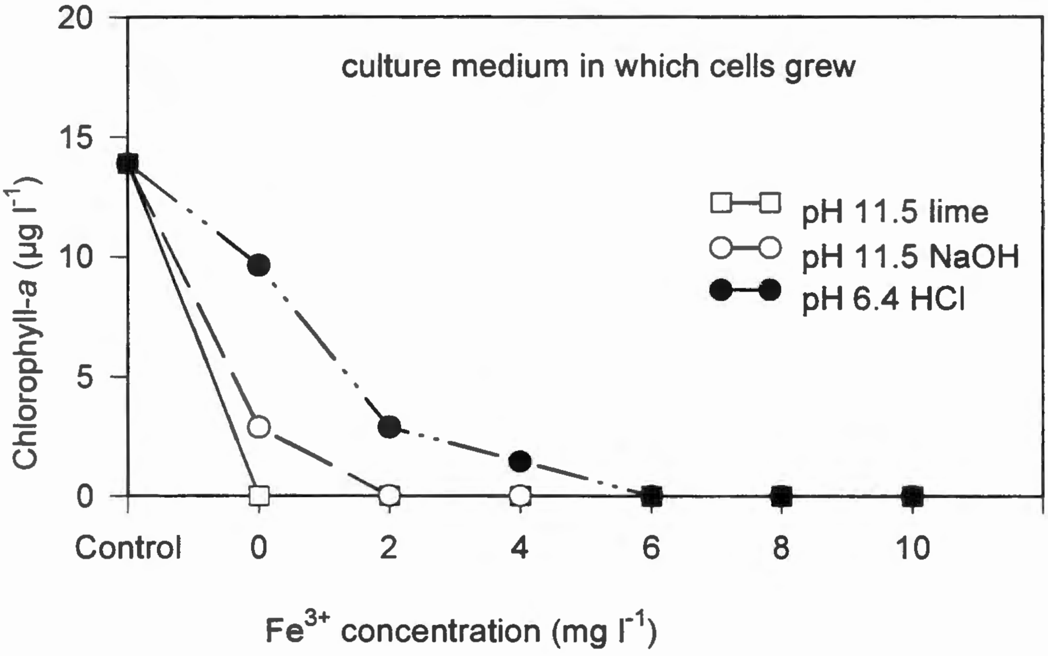


Figure 4.3 Variation in chlorophyll-*a* concentration (µg l^{-1}) with increased Fe^{3+} concentrations. Culture medium, in which *Monoraphidium minutum* cells grew, was added to Vaal River water.

As illustrated in Fig. 4.2, when lime was added as pH adjustment chemical, total removal of algal biomass did not occur at Fe^{3+} < 6 mg l^{-1} (compare with Fig. 4.1). This further indicates that the inorganic salts in the culture medium may possibly enhance coagulation processes. The Fe^{3+} treatment at pH 6.4 was ineffective in the removal of algal biomass. The best removal occurred at the addition of high Fe^{3+} concentrations, with the most effective removal of *M. minutum* biomass when 10 mg l^{-1} Fe^{3+} was added. At pH 11.5, adjusted with NaOH, total removal of algal biomass did not occur, but effective removal was obtained when 4 mg l^{-1} Fe^{3+} and higher iron concentrations were added (Fig. 4.2). Total removal of algal biomass was, however, observed when 6 mg l^{-1} and higher iron concentrations were added to raw water, when the pH was adjusted to 11.5 with lime (Fig 4.2).

When only culture medium in which the cells grew was added to Vaal River water, the chlorophyll-*a* concentration of the Vaal River water was less than 20 µg l^{-1} (Fig. 4.3), compared with Figs 4.1 and 4.2.

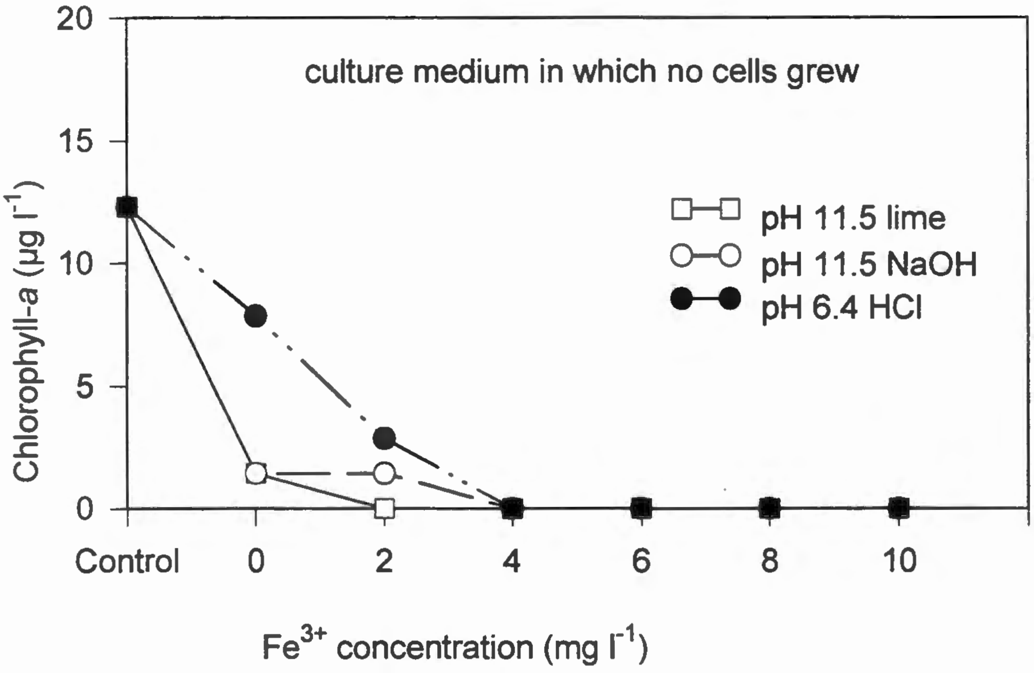


Figure 4.4 Variation in chlorophyll-*a* concentration (µg l^{-1}) with increased Fe^{3+} concentrations. Culture medium, in which no cells grew, was added to Vaal River water.

The removal of natural occurring algal cells (present in raw Vaal River water) was not effective at pH 6.4 when low Fe^{3+} concentrations were used as flocculants, i.e.

< 6 mg l^{-1} Fe^{3+} (Fig. 4.3). At pH 11.5, when the pH was adjusted with NaOH, total removal of algal biomass was obtained when iron was added as flocculant. At pH 11.5, when lime was used as pH adjustment chemical, total removal of algal biomass occurred even when Fe^{3+} was not added (Fig. 4.3). Therefore, lime was apparently responsible for the coagulation of algal cells and removal of algal biomass. Total removal of algal biomass also occurred at pH 6.4 when 6.0 mg l^{-1} and higher iron concentrations were added (Fig. 4.3).

When only culture medium in which cells never grew, containing no algal cells, was added to raw water, the initial chlorophyll-*a* values were also low (Fig. 4.4). At pH 6.4 small flocs formed during slow mixing, but when the pH was adjusted to 11.5, the floc size increased and the treated water visually appeared more turbid. The number of flocs formed on the surface layer also increased from pH 6.4 to pH 11.5.

Slight removal occurred when pH was adjusted to 6.4 when no Fe^{3+} was added, but total removal of algal biomass occurred when 4 mg l^{-1} and higher Fe^{3+} concentrations were added (Fig. 4.4).

Effective removal of algal biomass occurred at pH 11.5 when adjusted with NaOH (Fig. 4.4). The removal was the same when 2 mg l^{-1} Fe^{3+} was added, but total removal of chlorophyll occurred when 4 mg l^{-1} and higher concentrations were added.

Effective removal of algal biomass was observed when lime (pH 11.5) was used as pH adjustment chemical. Total removal occurred when Fe^{3+} (>2 mg l^{-1}) was added to the untreated water (Fig. 4.4).

When only distilled water was added to the Vaal River water (Fig. 4.5), unsuccessful coagulation and removal of natural occurring cells occurred when the pH was adjusted to 6.4. Total removal of algal biomass was observed when 8 mg l^{-1} and higher Fe^{3+} concentrations were added.

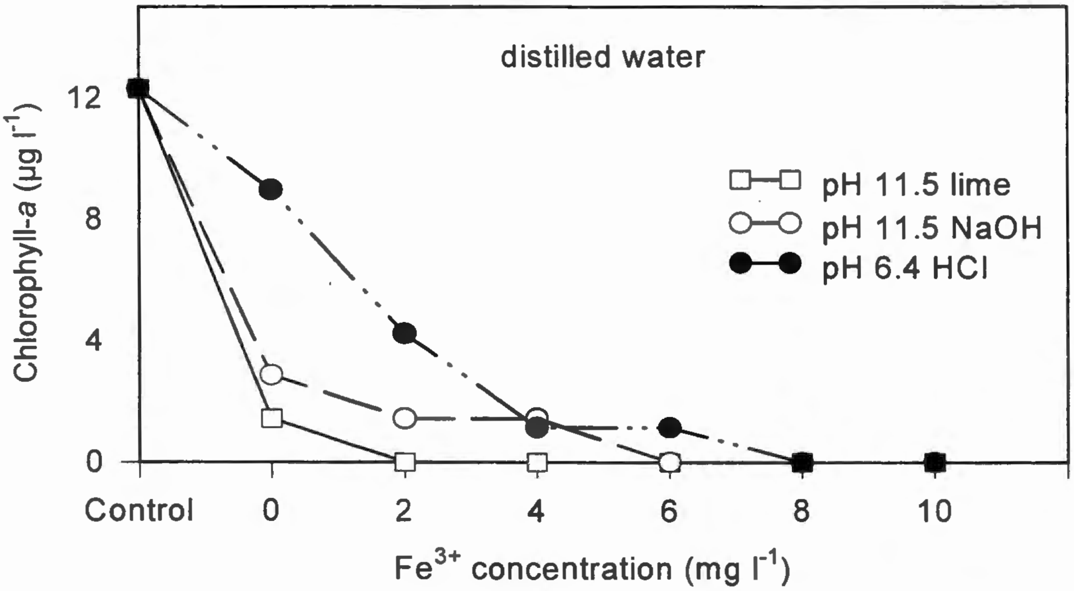


Figure 4.5 Variation in chlorophyll-*a* concentration (µg l^{-1}) with increased Fe^{3+} concentrations. Distilled water only was added to Vaal River water.

Similar results were obtained when the pH was adjusted to 11.5 with NaOH (only distilled water was added to Vaal River water). Lower Fe^{3+} concentrations did not remove biomass. Total removal of algal biomass occurred when 6 mg l^{-1} Fe^{3+} and higher concentrations were added as flocculant (Fig. 4.5).

In the absence of flocculant, lime (at pH 11.5) removed chlorophyll-*a* more effectively than when pH was adjusted with NaOH (pH 11.5) and HCl (pH 6.4). The addition of Fe^{3+} (2 mg l^{-1} and higher), together with lime, gave total removal of algal biomass (Fig. 4.5.).

The following conclusions can be drawn to summarise observations on the removal of algal biomass when *M. minutum* was added to Vaal River water. The addition of

M. minutum cells increased the chlorophyll-*a* concentration with approximately 60 µg l^{-1}. The high concentration of chlorophyll-*a* was removed less efficient at pH 6.4 (see Figs 4.1 and 4.2). When algal cells were added which were suspended in culture medium in which the cells grew (excreted organic substances present), the removal of the cells at pH 11.5 was more effective than when the cells were suspended in distilled water (compare Figs 4.1 and 4.2). Therefore, culture medium in which the cells grew apparently assist in flocculation. The assistance could be due to dissolved inorganic components of the nutrient medium, or excreted organic substances.

When the biomass concentration was increased by adding cells suspended in culture medium, and the pH was adjusted to 6.4, then high Fe^{3+} concentrations, possibly higher than 10 mg l^{-1}, were necessary for effective removal of algal biomass (Figs 4.1). When the chlorophyll-*a* concentrations were low (approximately 12 µg l^{-1} in the untreated water), and culture medium in which the cells grew was added to Vaal River water (Fig. 4.3), effective removal occurred at Fe^{3+} concentrations of 6 mg l^{-1} and higher. Therefore, algal cells apparently obstructed the flocculation process when high chlorophyll-*a* concentration occurred in the water.

When culture medium in which the cells grew (Fig. 4.3) was added to Vaal River water, poorer removal of algal biomass was observed at pH 6.4 than when culture medium in which no cells grew (Fig. 4.4) was added to Vaal River water. Therefore, organic substances possibly excreted by the cells, apparently caused poorer removal of algal biomass.

When culture medium in which no cells grew (Fig. 4.4) was added to Vaal River water, removal of algal biomass was better at pH 6.4 and 11.5 than when distilled water only was added to Vaal River water. Therefore, inorganic components of the nutrient medium assist in removal of algal biomass.

High pH in conjunction with Fe^{3+} removed *M. minutum* cells effectively (Figs 4.1 and 4.2). When cells suspended in culture medium in which they grew, was added to Vaal

River water, pH 11.5 (adjusted with lime) effectively removed biomass without Fe^{3+} addition (Fig. 4.1).

4.3.2 Removal of suspended solids

The effect of *M. minutum* cells on the removal of suspended solids will be illustrated in the following figures (Figs 4.6 to 4.10).

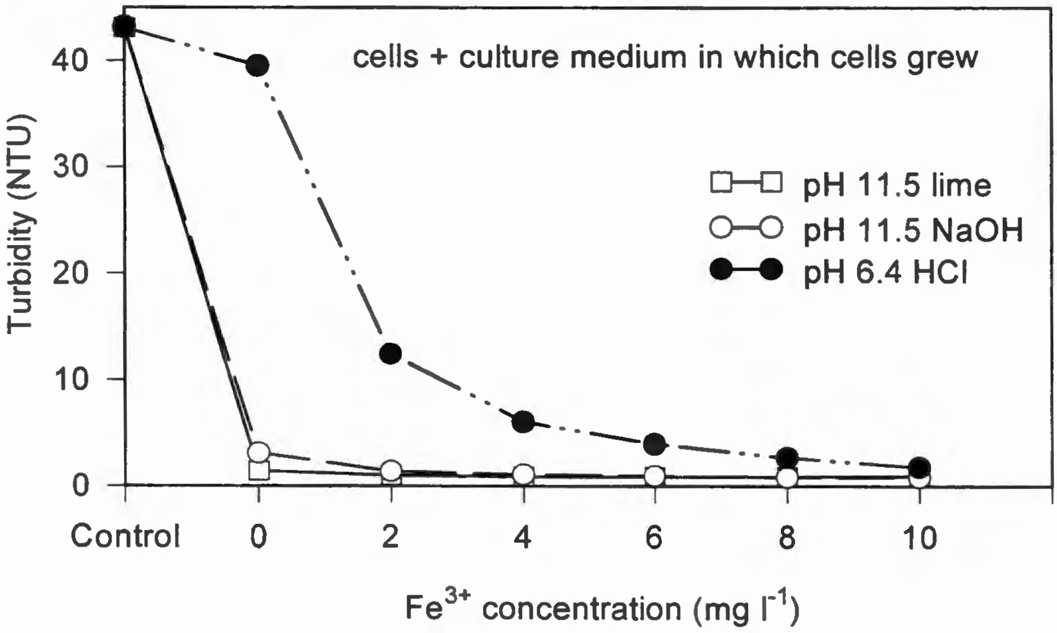


Figure 4.6 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. *Monoraphidium minutum* cells, suspended in culture medium in which cells grew, was added to Vaal River water.

When *M. minutum* cells suspended in culture medium in which they grew, were added to Vaal River water, removal of suspended particles (illustrated as turbidity in Figs 4.6 – 4.10), together with algal cells, were ineffective when the pH was adjusted to 6.4, although effective removal occurred at Fe^{3+} concentrations of 8 mg l^{-1} and higher

(Fig. 4.6). pH 11.5, adjusted with NaOH, did improve the removal of suspended solids, and with the addition of increased Fe^{3+} (>2 mg l^{-1}) concentrations, suspended material was effectively removed. At pH 11.5, when the pH was adjusted with lime, effective removal of suspended material was obtained with increased Fe^{3+} concentrations. Even when no $FeCl_3$ was added, effective removal occurred (Fig. 4.6).

When *M. minutum* cells were suspended in distilled water and then added to Vaal River water, removal of suspended material occurred at pH 6.4, but it was ineffective when compared with results at pH 11.5 (Fig. 4.7). At pH 11.5, when the pH was adjusted with lime, effective removal of suspended material was observed.

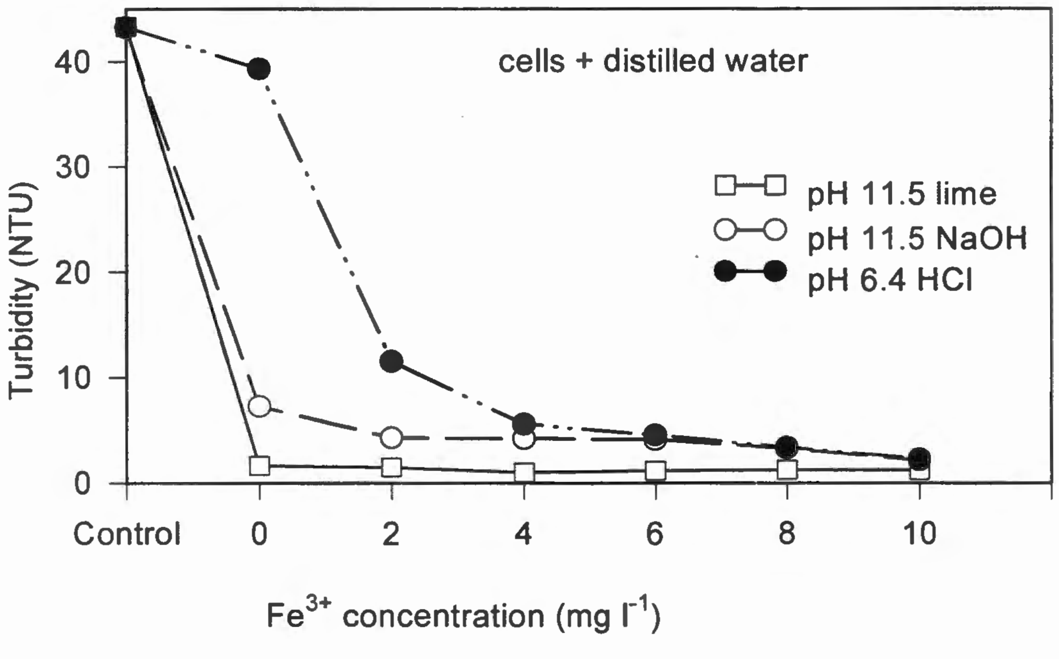


Figure 4.7 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. *Monoraphidium minutum* cells, suspended in distilled water, were added to Vaal River water.

Results illustrated in Fig. 4.8 (when culture medium in which cells grew were added to Vaal River water), in Fig. 4.9 (when culture medium in which no cells grew were added to Vaal River water) and in Fig. 4.10 (when distilled water only was added to Vaal River water) are more or less the same, except for a few small differences.

At pH 6.4, when low iron concentrations were added, the most effective removal of suspended solids occurred when culture medium in which cells did grow (Fig. 4.8) was added to Vaal River water. When 5 ml culture medium in which no cells grew was added (Fig. 4.9), the removal was more effective than when only 5 ml distilled water was added (compare Figs 4.9 and 4.10). This observation confirms a previous one, namely that inorganic components present in the nutrient medium positively affected coagulation and sedimentation.

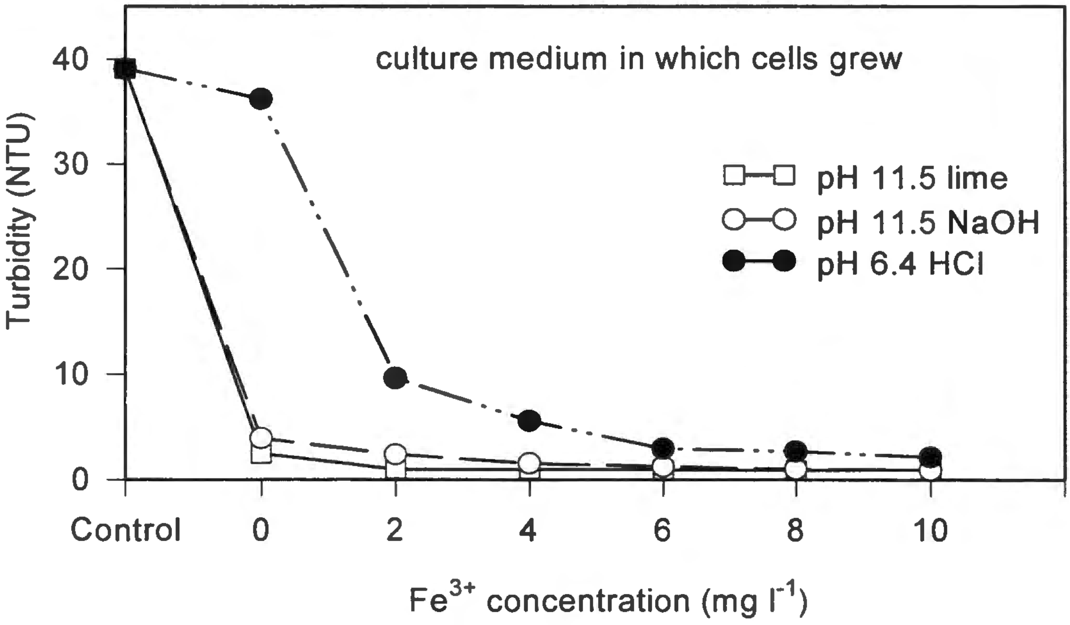


Figure 4.8 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. Culture medium, in which *Monoraphidium minutum* cells grew, was added to Vaal River water.

When culture medium in which cells grew (Fig. 4.8), culture medium in which no cells grew (Fig. 4.9) or distilled water only (Fig. 4.10), was added to Vaal River water, effective removal at pH 6.4 occurred only when the flocculant concentration was higher than 6 mg l^{-1}. At pH 11.5, for both pH adjustment chemicals, the most effective removal was observed at iron concentrations higher or equal to 4 mg l^{-1} Fe^{3+}.

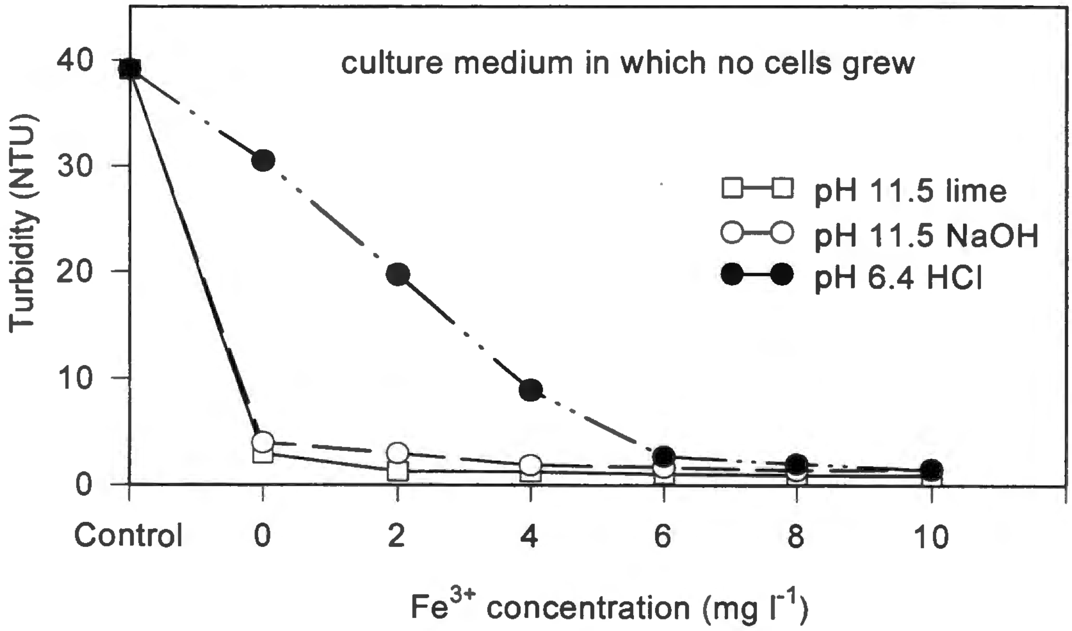


Figure 4.9 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. Culture medium, in which no cells grew, was added to Vaal River water.

The following conclusions can be drawn to summarise observations on the removal of suspended solids when *M. minutum* cells were added to Vaal River water. When *M. minutum* cells were added as 60 µg l^{-1} chlorophyll-*a*, turbidity was 13 NTU's higher than the initial turbidity of the Vaal River water (30.2 NTU; Figs 4.6 and 4.7). When culture medium in which cells grew (Fig. 4.8) or did not grow (Fig. 4.9) was added, turbidity was 8 NTUs higher than the initial Vaal River water. Therefore turbidity was not only increased by algal cells, but also by the culture medium. When distilled water was added to Vaal River water, turbidity was unchanged. Figs 4.6 to 4.10 indicated that a pH of 11.5 was more effective in the removal of suspended solids as was the case with chlorophyll-*a*. At pH 6.4 removal was ineffective, except when Fe^{3+} was dosed in high concentrations (6 mg l^{-1} and higher). At pH 11.5, when *M. minutum* cells together with culture medium in which they grew, was added to Vaal River water, little difference in the removal was observed compared to when NaOH or lime was added (Fig. 4.6).

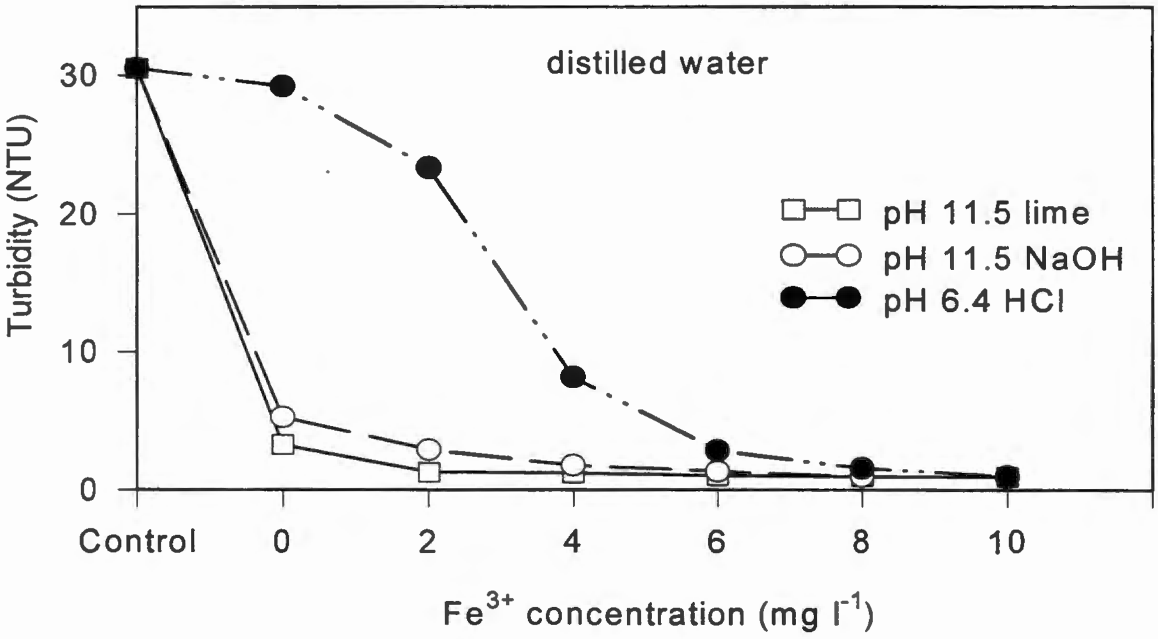


Figure 4.10 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. Distilled water only was added to Vaal River water.

When *M. minutum* cells suspended in culture medium in which they grew was added to Vaal River water (Fig. 4.6), better removal of suspended solids was observed at pH 11.5 (adjusted with NaOH) than when *M. minutum* cells suspended in distilled water was added to Vaal River water. Therefore, dissolved inorganic components of the nutrient medium or the excreted organic substances apparently assisted in flocculation of suspended solids.

When *M. minutum* cells suspended in culture medium in which they grew was added to Vaal River water (Fig. 4.6), removal of suspended solids was similar than when only the culture medium in which cells grew was added to Vaal River water. Therefore, *M. minutum* cells apparently did not affect removal of suspended solids.

When culture medium in which no cells grew was added to Vaal River water (Fig. 4.9), removal of suspended solids was similar to when distilled water only (Fig. 4.10) was added to Vaal River water. Therefore, inorganic components of the nutrient medium did not effect removal of suspended solids.

When culture medium in which cells grew was added to Vaal River water (Fig. 4.8), slightly better removal of suspended solids was observed than when culture medium in which no cells grew (Fig. 4.9) was added to Vaal River water. Therefore, organic substances excreted by the algal cells apparently assisted in removal of suspended matter.

4.3.3 Removal of dissolved organic carbon

The removal of dissolved organic carbon (DOC) when *M. minutum* cells were added to Vaal River water, are illustrated and discussed in the following paragraphs.

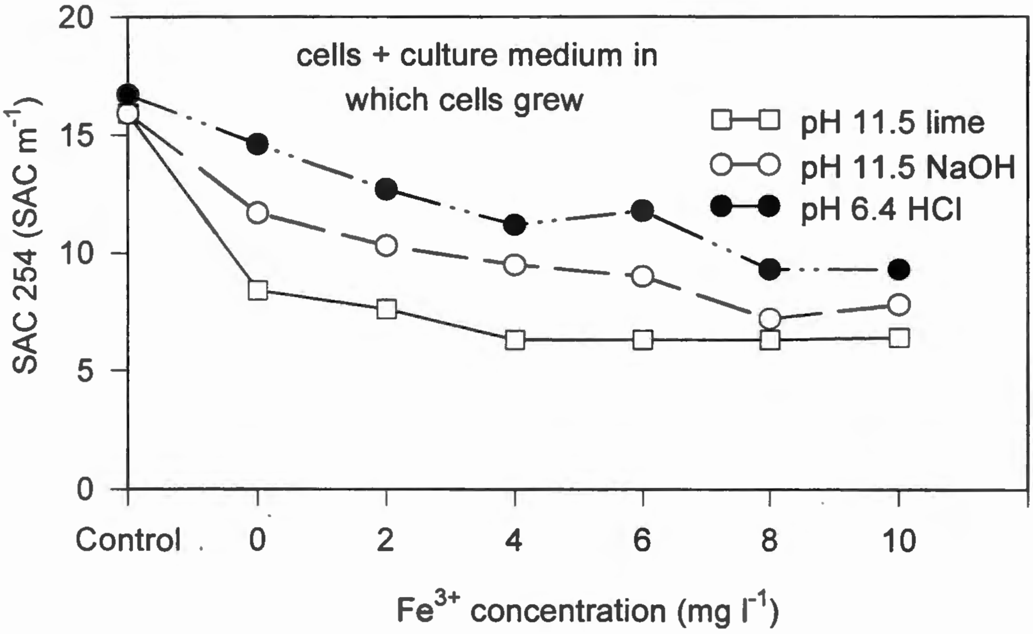


Figure 4.11 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. *Monoraphidium minutum* cells, suspended culture medium in which cells grew, were added to Vaal River water.

When *M. minutum* cells, suspended in culture medium in which cells grew, were added to Vaal River water, removal of DOC increased with increase in Fe^{3+} concentration and pH. At pH 6.4, the same removal as illustrated earlier occurred (Figs 3.1 and 3.2), i.e. at lower pH conditions. When the removal is compared with removal at pH 11.5 (when lime was used), it is clear that a pH of 11.5 gave slightly better removal of DOC (Fig. 4.11). The most effective removal occurred at pH 11.5 when lime was used and 4 mg l^{-1} and higher Fe^{3+} concentrations were added (Fig. 4.11).

When *M. minutum* cells were suspended in distilled water before being added to Vaal River water, the results of DOC removal (Fig. 4.12) appeared to be the same as when cells were suspended in culture medium in which cells grew (Fig. 4.11). The removal of DOC at pH 11.5, when lime was added as pH adjustment chemical, was more effective, probably due to the possibility that DOC was not added together with the cells, or that organic matter was washed off the cells and that the cells adsorbed the natural occurring DOC of the Vaal River.

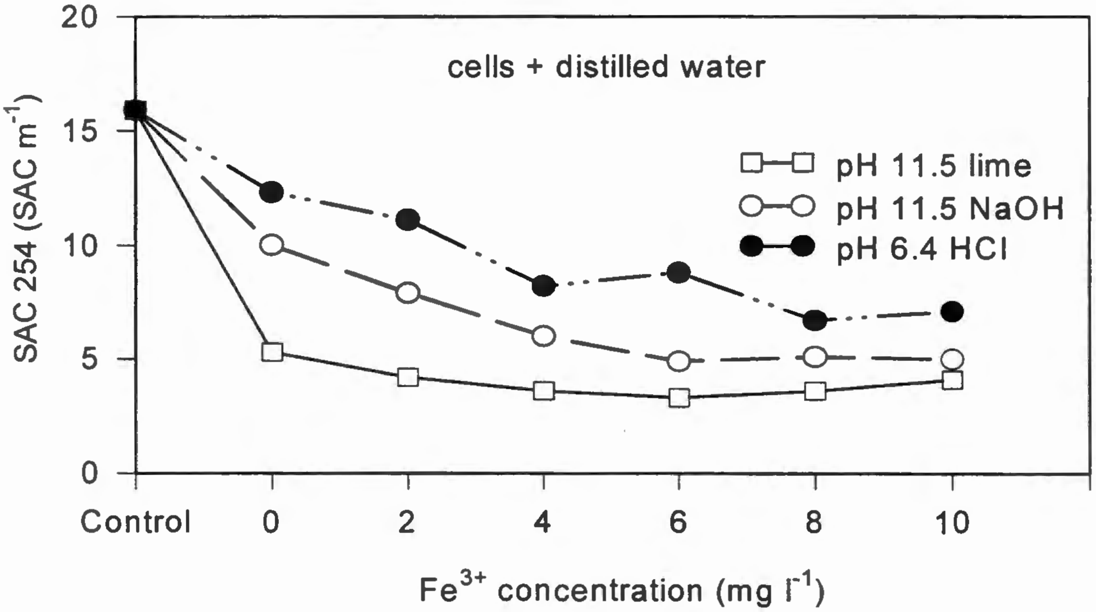


Figure 4.12 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. *Monoraphidium minutum* cells, suspended in distilled water, were added to Vaal River water.

When no *Monoraphidium* cells were added to Vaal River water, but only culture medium (Fig. 4.14) or distilled water (Fig. 4.15), the results differ greatly from when cells suspended in culture medium in which they grew were added to Vaal River water (Fig 4.11) and when cells suspended in distilled water were added to Vaal River water (Fig. 4.12).

The removal of DOC was lower when *M. minutum* cells were not added to Vaal River water (Figs 4.13, 4.14 and 4.15). It appeared that pH had no effect on the removal of DOC. Increased Fe^{3+} concentrations increased the removal of DOC, and effective removal of DOC occurred only when 10 mg l^{-1} and higher Fe^{3+} concentrations were added (Figs 4.13, 4.14 and 4.15).

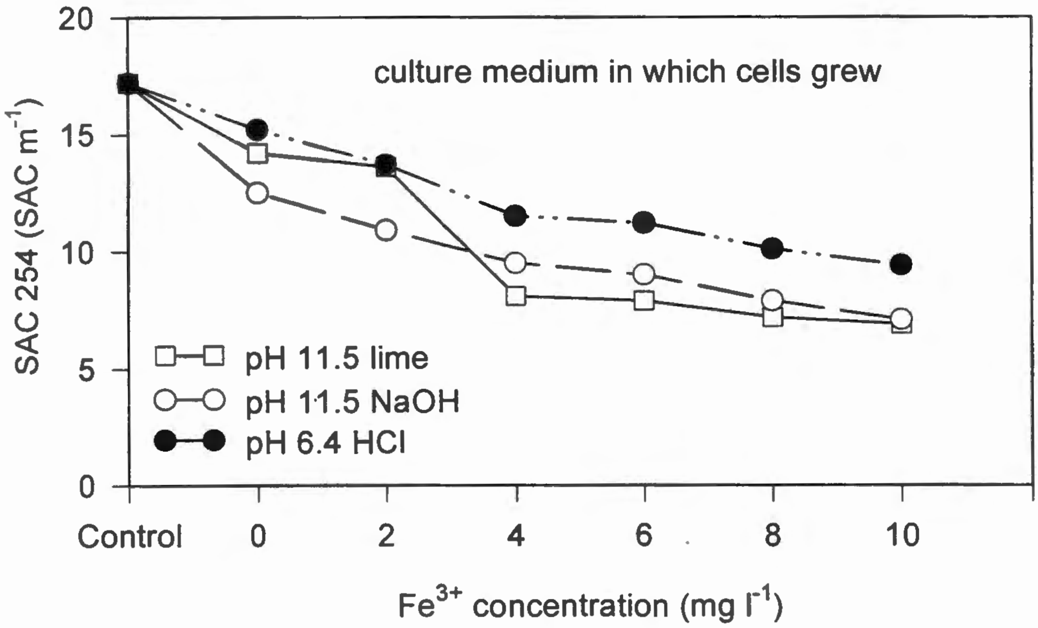


Figure 4.13 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. Culture medium, in which *Monoraphidium minutum* cells grew, was added to Vaal River water.

The following conclusions can be drawn to summarise observation on the removal of dissolved organic matter when *M. minutum* was added to Vaal River water. Figs 4.11 to 4.15 showed that in the initial Vaal River water SAC 254 values were little affected by the addition of *M. minutum* cells and culture medium in which cells grew. It was expected that SAC 254 would increase when algal cells and culture medium in which they grew, were added.

When *M. minutum* cells, suspended in culture medium in which they grew, was added to Vaal River water (Fig. 4.12), removal of DOC was better than when only culture medium in which cells grew was added to Vaal River water (Fig. 4.13). Therefore, *M. minutum* cells apparently assisted in the removal of DOC.

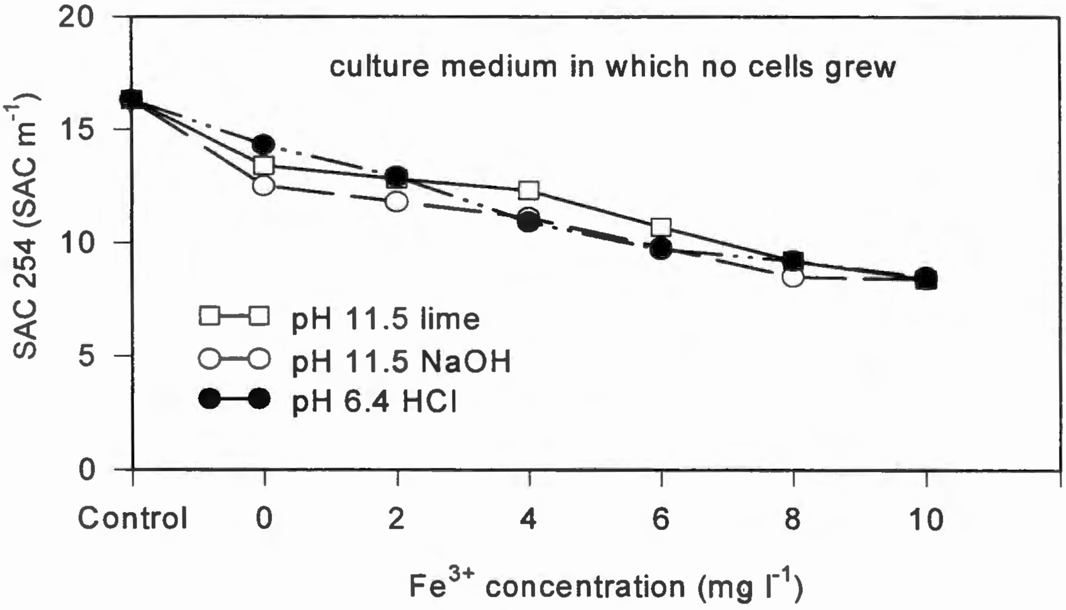


Figure 4.14 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. Culture medium, in which no cells grew, was added to Vaal River water.

When culture medium in which no cells grew was added to Vaal River water (Fig. 4.14), decrease in SAC 254 was similar to when distilled water only (Fig. 4.15) was added to Vaal River water. Therefore, inorganic components of the nutrient medium apparently did not affect removal of DOC.

When culture medium in which cells grew was added to Vaal River water (Fig. 4.13), similar removal of DOC was observed than when culture medium in which no cells grew (Fig. 4.14) was added to Vaal River water. Therefore, organic substances excreted by the algal cells apparently did not affect the removal of DOC.

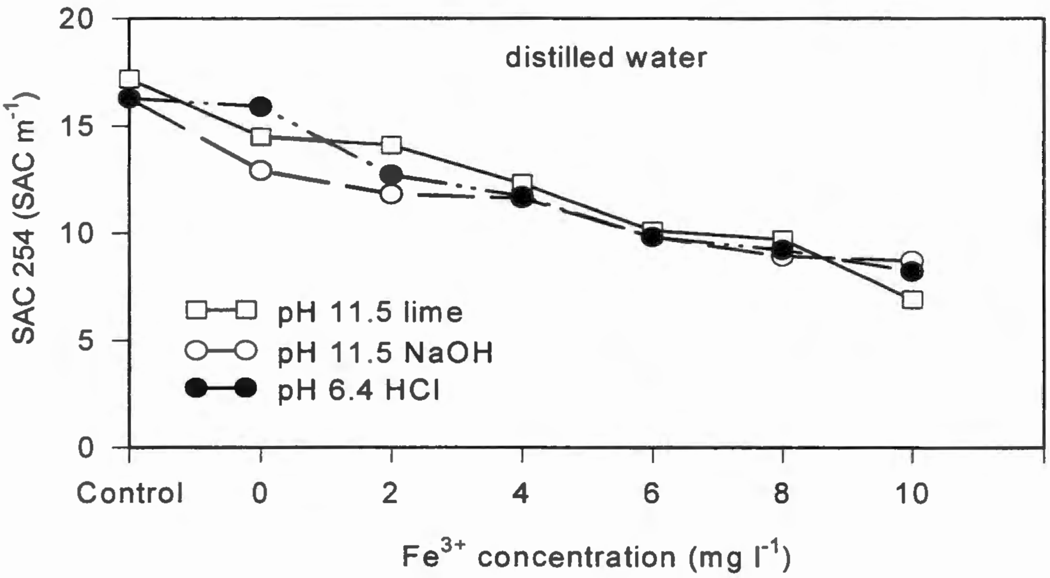


Figure 4.15 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. Distilled water only was added to Vaal River water.

4.4 The effect of *Cyclotella meneghiniana* cells on caogulation and flocculation

The aim of this experiment was to determine what effect added *C. meneghiniana* cells (representing a *C. meneghiniana* dominance under natural conditions), and their organic excretions, had on flocculation processes. Experimental protocol applied are explained in the material and method chapter.

Cyclotella meneghiniana cells grown in culture (concentrated so that 7.5 ml contains approximately 30 µg l^{-1} chlorophyll-*a*) were added to Vaal River water. The chlorophyll-*a* concentration of the raw water was fairly low, and the turbidity values were high, caused by fine silt particles.

4.4.1 Removal of biomass (i.e. chlorophyll-*a*)

When the pH was adjusted to 6.4 with HCl, small flocs formed initially, but the floc size increased with time during the flocculation or slow mixing (40 rpm) period. When pH was adjusted with either NaOH or lime to 11.5, larger flocs formed immediately.

When *C. meneghiniana* and culture medium in which cells grew were added to Vaal River water (Fig. 4.16) and the pH was adjusted to 6.4 with HCl, almost no removal of algal biomass occurred. When flocculant (Fe^{3+}) was added, algal biomass concentration decreased with increased Fe^{3+} concentrations. The removal of algal biomass was, however, effective only when high (>8 mg l^{-1}) Fe^{3+} concentrations were added (Fig. 4.16). The removal of *C. meneghiniana* was, however, better than the removal of *Monoraphidium minutum* at similar conditions (see Fig. 4.1). With the adjustment of pH to 11.5 with either lime or NaOH, total removal of algal biomass occurred.

When *C. meneghiniana* cells were suspended in distilled water and then added to Vaal River water (Fig. 4.17), the results appeared to be the same as when cells were suspended in culture medium in which cells grew before being added to Vaal River water (Fig. 4.16). At pH 6.4 removal increased together with increase in Fe^{3+} concentration; total removal of algal biomass occurred when 8 mg l^{-1} and higher Fe^{3+} was dosed. When the pH was adjusted to 11.5 with NaOH, chlorophyll-*a* was not removed completely, but when flocculant was added, total removal occurred. At pH 11.5, when lime was used as pH adjustment chemical, total removal, even without flocculant, was observed (Fig. 4.17).

When culture medium in which the cells grew was added to Vaal River water (Fig. 4.18), the removal was more or less the same as when cells were added (Fig. 4.16), but the initial chlorophyll-*a* values were less due to the fact that no algal cells were added to the Vaal River water. The algal cells that were present in the raw water was, however, removed inefficiently at pH 6.4 when low Fe^{3+} concentrations were added (2 mg l^{-1}). Total removal of biomass occurred when the pH was adjusted to 11.5.

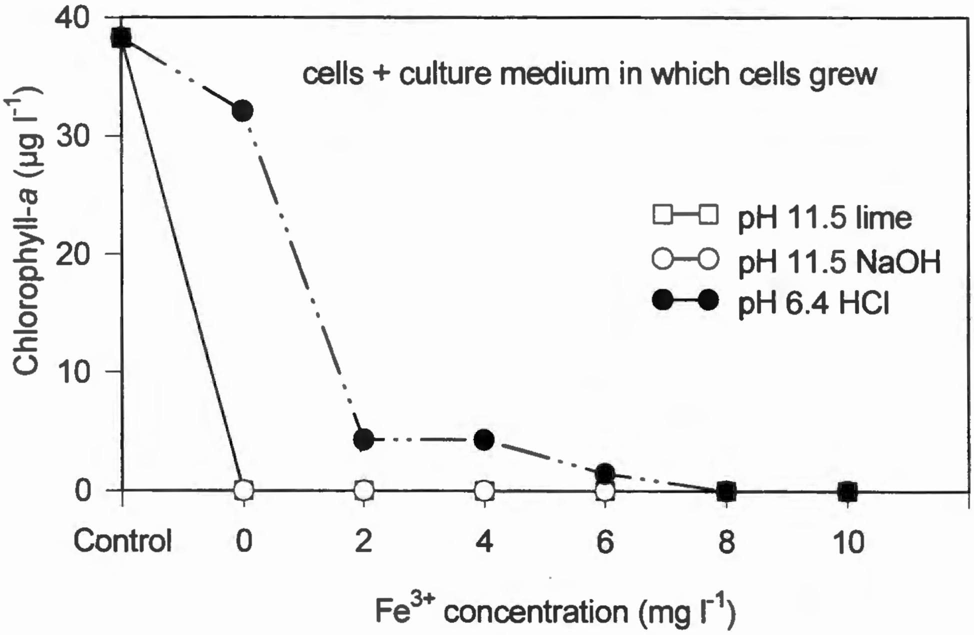


Figure 4.16 Variation in chlorophyll-a concentration (µg l^{-1}) with increased Fe^{3+} concentrations. *Cyclotella meneghiniana* cells, suspended in culture medium in which cells grew, were added to Vaal River water.

Because of the fact that there were only small amounts of biomass present in the raw water, and because the biomass was effectively removed with the addition of chemicals, the effect of the different treatments could not be illustrated clearly. Fig. 4.19 represent results obtained for the treatments where culture medium in which cells did not grow, as well as when distilled water were added. The biomass that was present, was effectively removed.

The following conclusions can be drawn to summarise observations on the removal of algal biomass when *Cyclotella meneghiniana* cells were added to Vaal River water. The addition of *C. meneghiniana* cells increased the biomass concentration with approximately 30 µg l^{-1}. The increased biomass was less efficiently removed at pH 6.4 when lower iron concentrations (< 6 mg l^{-1}) were added (Figs 4.16 and 4.17).

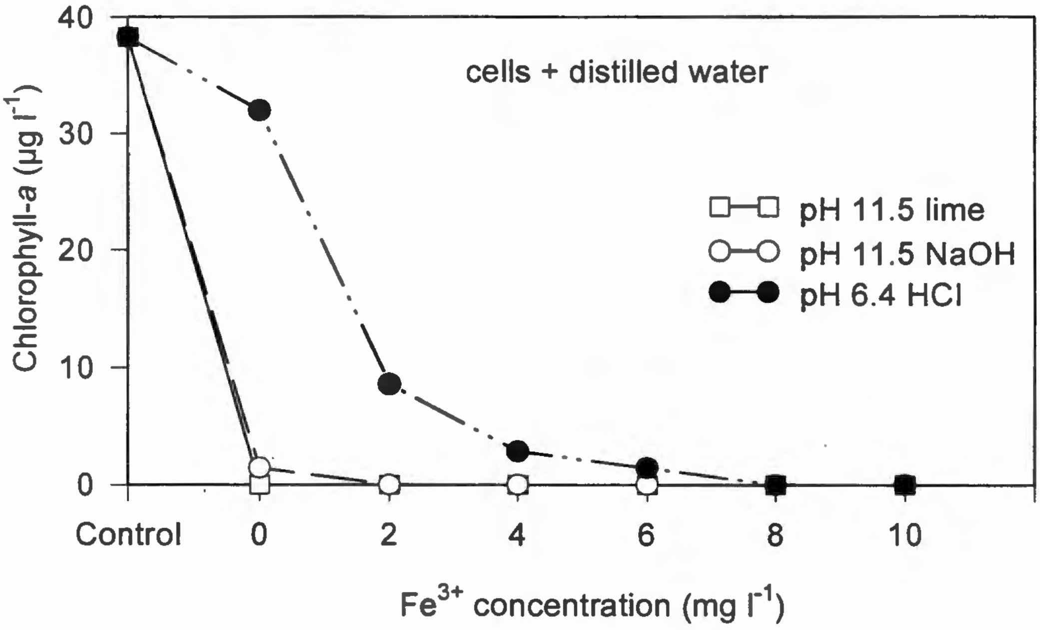


Figure 4.17 Variation in chlorophyll-a concentration (µg l^{-1}) with increased Fe^{3+} concentrations. *Cyclotella meneghiniana* cells, suspended in distilled water, were added to Vaal River water.

When *C. meneghiniana* cells were resuspended in culture medium in which cells grew or in distilled water, and added to Vaal River water, biomass was removed when the pH was adjusted to 11.5 (Figs 4.16 and 4.17). This removal at pH 11.5 indicates that *C. meneghiniana* can easily be removed at this pH.

When *C. meneghiniana* cells suspended in culture medium in which they grew was added to Vaal River water (Fig. 4.16), similar removal of algal biomass was observed than when *C. meneghiniana* cells suspended in distilled water (Fig.4.17) was added to Vaal River water. Therefore, dissolved inorganic components of the nutrient medium or the excreted organic substances possibly did not affect flocculation.

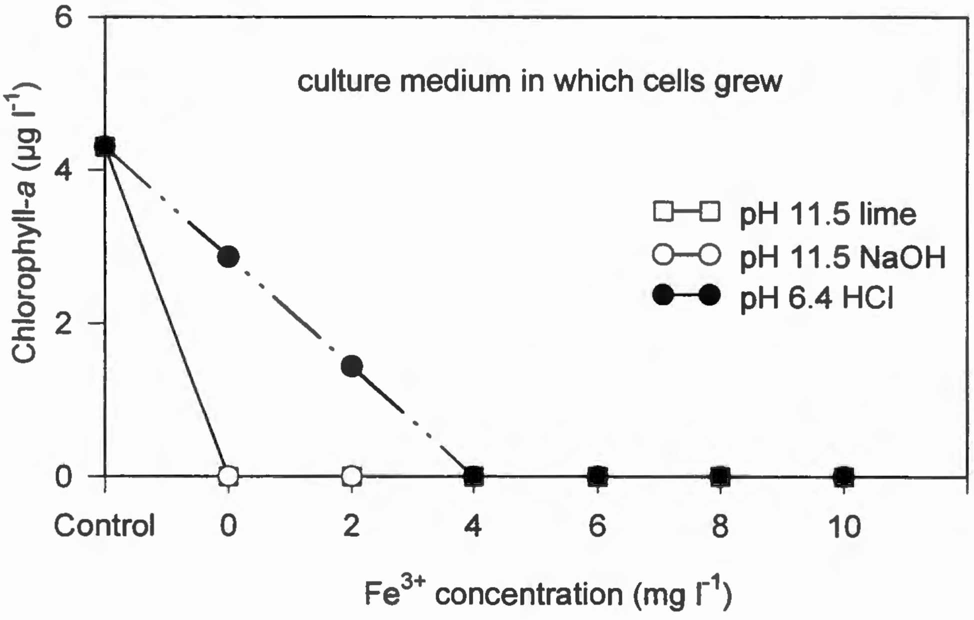


Figure 4.18 Variation in chlorophyll-a concentration (µg l^{-1}) with increased Fe^{3+} concentrations. Culture medium, in which *Cyclotella meneghiniana* cells grew, was added to Vaal River water.

When *C. meneghiniana* cells suspended in culture medium in which they grew was added to Vaal River water (Fig. 4.16), removal of algal biomass was similar at pH 11.5 and slightly poorer at pH 6.4 than when culture medium in which cells grew (Fig. 4.18) was added to Vaal River water. Therefore, *C. meneghiniana* cells did not affect removal of algal biomass at pH 11.5. At pH 6.4, *C. meneghiniana* cells were inefficiently removed.

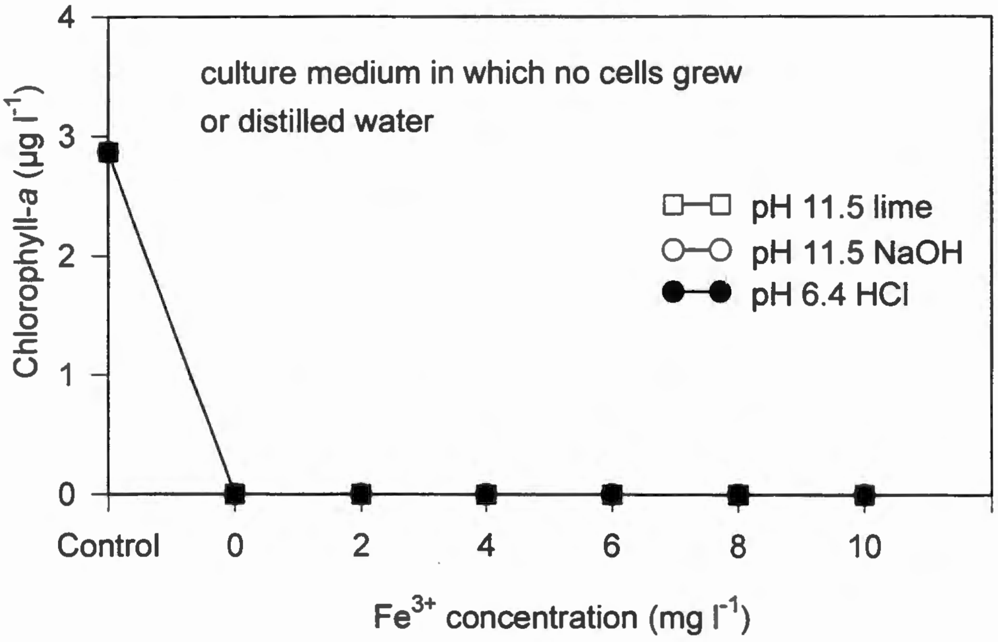


Figure 4.19 Variation in chlorophyll-a concentration (µg l^{-1}) with increased Fe^{3+} concentrations. Either culture medium, in which no cells grew, or distilled water was added to Vaal River water.

When culture medium in which no cells grew was added to Vaal River water (Fig. 4.19), removal of algal biomass was similar to when distilled water only (Fig. 4.19) was added to Vaal River water. Therefore, inorganic components of the nutrient medium did not affect removal of algal biomass.

When culture medium in which cells grew was added to Vaal River water (Fig. 4.18), slightly poorer removal of algal biomass was observed at pH 6.4 than when culture medium in which no cells grew (Fig. 4.19) was added to Vaal River water. Therefore, organic substances excreted by the algal cells inhibited the removal of algal biomass at pH 6.4. At pH 11.5, when culture medium in which cells grew were added to Vaal

River water (Fig. 4.18), removal of algal biomass was similar than when culture medium in which no cells grew (Fig. 4.19) were added to Vaal River water. Therefore, organic substances excreted by the algal cells did not affect the removal of algal biomass at pH 11.5.

When the chlorophyll-*a* concentrations was low (approximately 4 µg l^{-1}), biomass was effectively removed with the addition of Fe^{3+} (2 mg l^{-1} and higher concentrations) at pH 6.4 and pH 11.5 (Fig. 4.19).

4.4.2 Removal of suspended solids

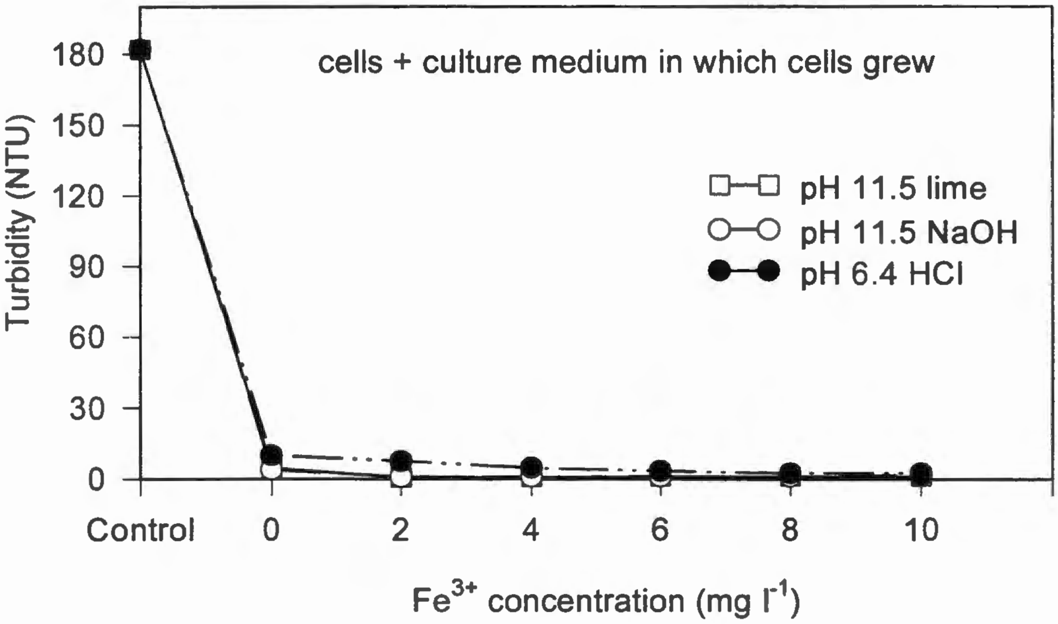


Figure 4.20 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. *Cyclotella meneghiniana* cells, suspended in culture medium in which cells grew, were added to Vaal River water.

Although the initial turbidity was high, the removal of suspended solids were generally effective (Figs. 4.20 - 4.24). Removal was generally less effective at pH 6.4.

When culture medium in which cells grew, culture medium in which no cells grew or distilled water only was added to Vaal River water and the pH was adjusted to 6.4, only high Fe^{3+} concentrations (> 6 mg l^{-1}) removed suspended particles (Figs 4.22, 4.23 and 4.24). During the jar tests, almost no flocs were formed when low iron concentrations were dosed (i.e. 2 - 4 mg l^{-1}). This poor formation of flocs were usually observed at pH 6.4 when the turbidity was high.

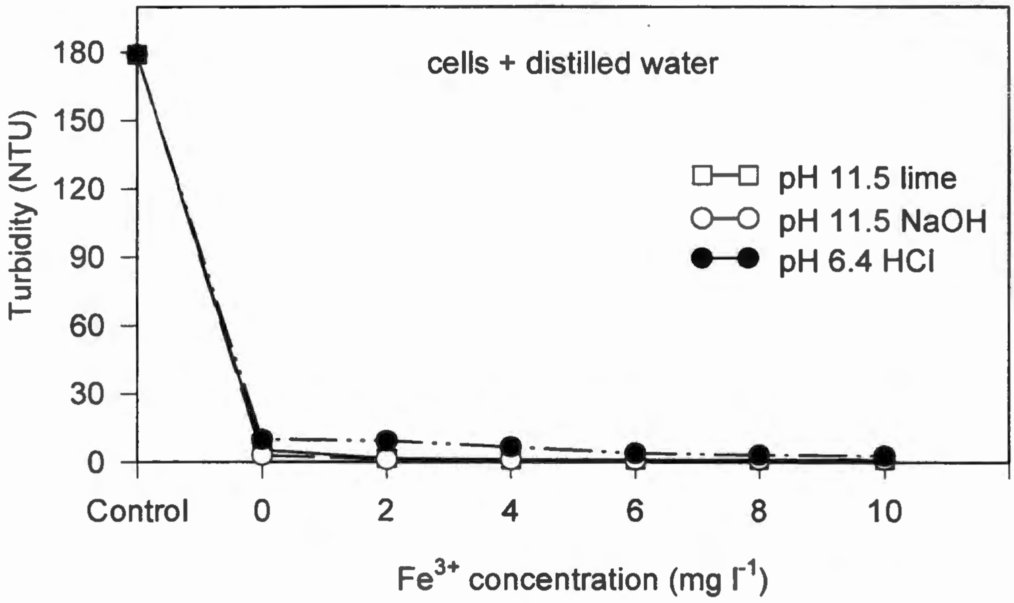


Figure 4.21 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. *Cyclotella meneghiniana* cells, suspended in distilled water, were added to Vaal River water.

When the pH was adjusted to 11.5 with either lime or NaOH, effective removal of suspended matter occurred, even without the addition of $FeCl_3$ (Figs 4.23 to 4.24).

The following conclusions can be drawn to summarise observations on the removal of suspended solids when *C. meneghiniana* cells were added to Vaal River water. When *C. meneghiniana* cells were added as 30 µg l^{-1} chlorophyll-*a*, or when culture medium was added to Vaal River water, the initial Vaal River water turbidity remained unchanged. It is possible that the high concentration of suspended solids in the Vaal River water was the reason why the turbidity remained unchanged or that *C. meneghiniana* cells did not significantly influence turbidity. Figs 4.20 to 4.24 indicated that pH 11.5 played an important part in the removal of suspended solids as was the case when *M. minutum* cells were added (Figs 4.6 to 4.10). pH 6.4 was ineffective in the removal of suspended matter except when high Fe^{3+} (> 6 mg l^{-1}) was added. Effective removal occurred at pH 11.5 at increased Fe^{3+} concentrations, and little difference was observed when NaOH or lime was added.

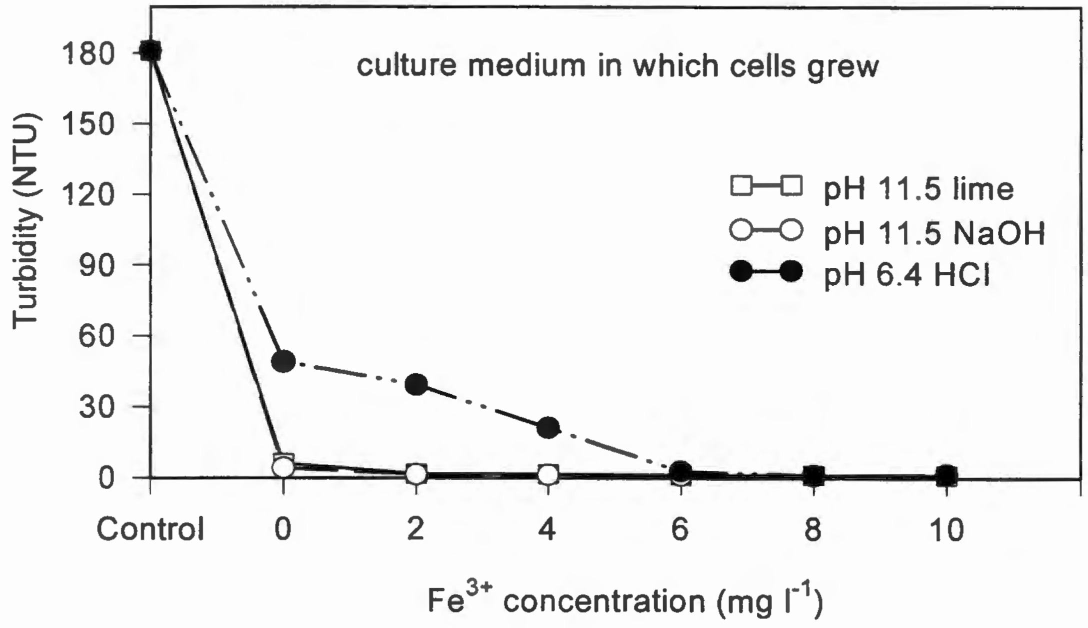


Figure 4.22 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. Culture medium, in which *C. meneghiniana* cells grew, was added to Vaal River water.

When algal cells suspended in culture medium in which they grew (Fig. 4.20) or distilled water (Fig. 4.21) were added to Vaal River water, suspended solids was more effectively removed at pH 6.4 than when culture medium without cells (Fig. 4.22) or distilled water only (Fig. 4.24) was added to Vaal River water. This indicates that *C. meneghiniana* cells possibly assist in the removal of suspended solids at pH 6.4.

When culture medium in which no cells grew was added to Vaal River water (Fig. 4.23), removal of suspended solids was similar to when distilled water only (Fig. 4.24) was added to Vaal River water. Therefore, inorganic components of the nutrient medium apparently did not affect removal of suspended solids.

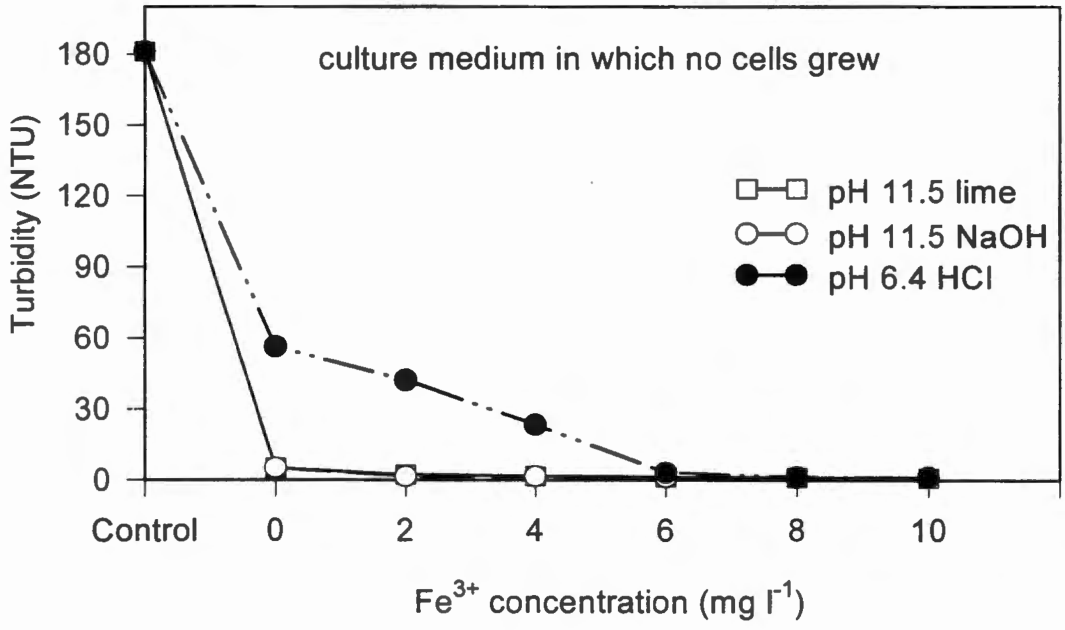


Figure 4.23 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. Culture medium, in which no cells grew, was added to Vaal River water.

When culture medium in which cells grew was added to Vaal River water (Fig. 4.22), similar removal of suspended solids was observed than when culture medium in which no cells grew (Fig. 4.23) was added to Vaal River water. Therefore, organic substances excreted by the *C. meneghiniana* cells did not affect removal of suspended matter.

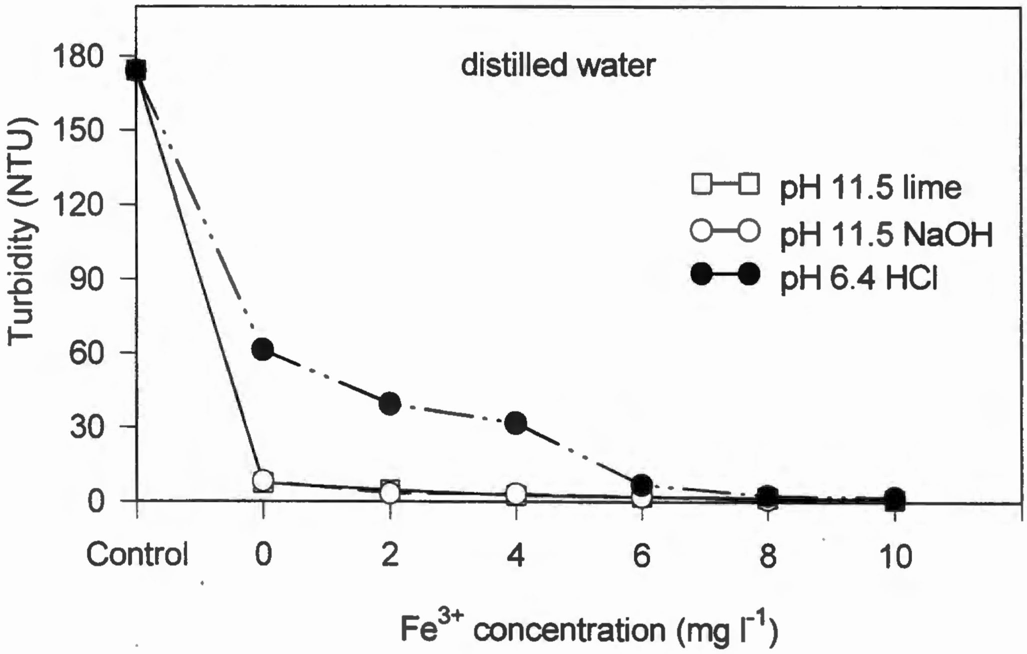


Figure 4.24 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. Distilled water only was added to Vaal River water.

4.4.3 Removal of dissolved organic carbon

The removal of DOC was effective when *C. meneghiniana* cells were added to Vaal River water. SAC values of less than 3 SAC m^{-1} were obtained after sedimentation, when the pH was adjusted to 11.5 with lime.

When *C. meneghiniana* cells and culture medium in which cells grew were added to Vaal River water, and the pH adjusted to 6.4, the removal of DOC was ineffective compared to the removal at a pH of 11.5 (Fig. 4.25). When the pH was adjusted to 11.5, very low SAC values were obtained. When *C. meneghiniana* cells were suspended in distilled water and added to Vaal River water, effective removal was observed, except when pH was adjusted to 6.4 (Fig. 4.26).

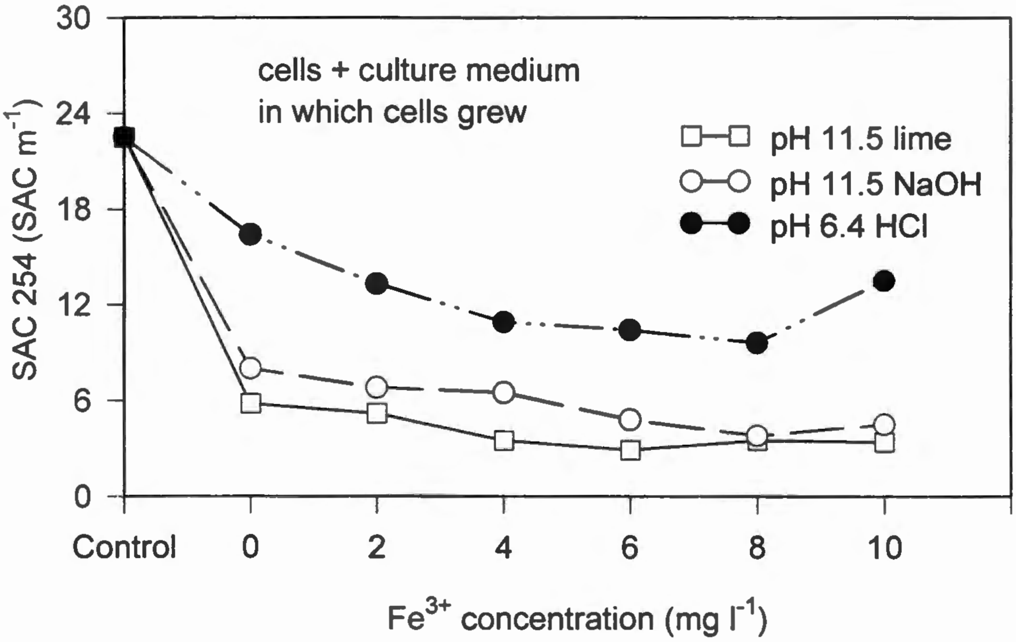


Figure 4.25 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. *Cyclotella meneghiniana* cells, suspended in culture medium in which cells grew, were added to Vaal River water.

When culture medium in which the cells grew (Fig. 4.27), culture medium in which no cells grew (Fig. 4.28) or distilled water (Fig. 4.29) was added to Vaal River water, the removal of DOC increased at pH 6.4 when higher iron concentrations (> 6 mg l^{-1}) were added. When culture medium in which cells grew was added to Vaal River water (Fig. 4.27), removal was still effective at pH 11.5 when lime was used as pH adjustment chemical and high Fe^{3+} concentrations (> 6 mg l^{-1}) were added.

When culture medium in which no cells grew (Fig. 4.28) or distilled water (Fig. 4.29) was added to Vaal River water the removal of DOC was ineffective at pH 11.5 when compared with the situation when algal cells were added to Vaal River water (see Figs 4.25 and 4.26). When the pH was adjusted to 6.4 and Fe^{3+} concentration was higher than 4 mg l^{-1} (Figs 4.27, 4.28 and 4.29), effective removal of DOC was observed.

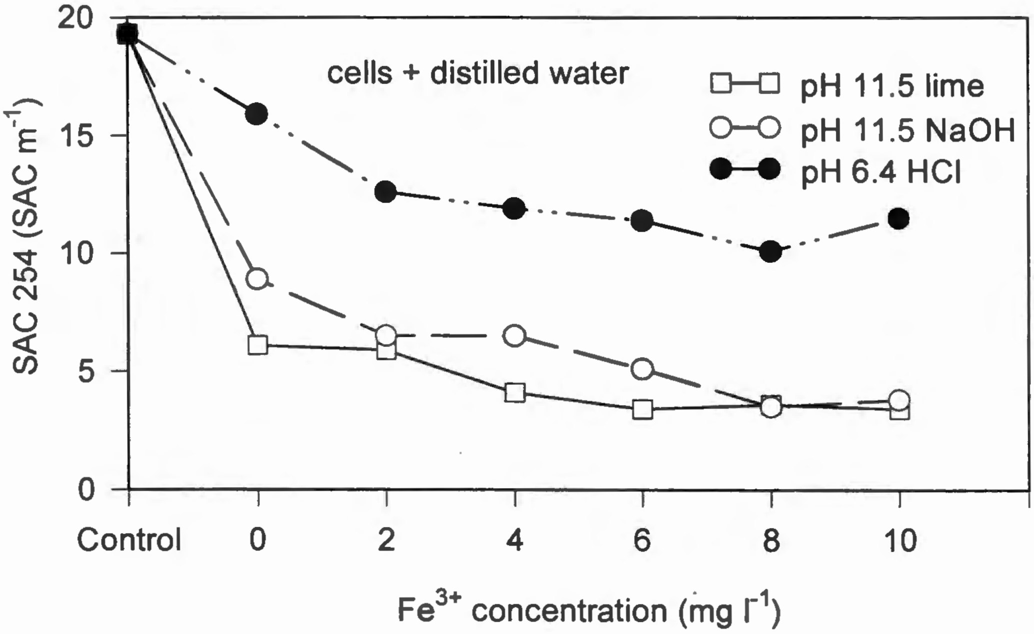


Figure 4.26 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. *Cyclotella meneghiniana* cells, suspended in distilled water, were added to Vaal River water.

The following conclusions can be drawn to summarise observations on the removal of DOC when *C. meneghiniana* cells were added to Vaal River water. Figs 4.25 and 4.27 showed that the initial Vaal River SAC 245 values were increased by approximately 5 units when culture medium in which cells grew were added to Vaal River water. When *C. meneghiniana* cells suspended in culture medium were added to Vaal River water (Fig. 4.25), DOC was similarly removed at pH 11.5 than when culture medium in which cells grew (Fig. 4.27) was added. At pH 6.4 better removal was observed when no cells were added This ineffective removal at pH 6.4 when cells were present, was possibly caused by specific characteristics of *C. meneghiniana* cells.

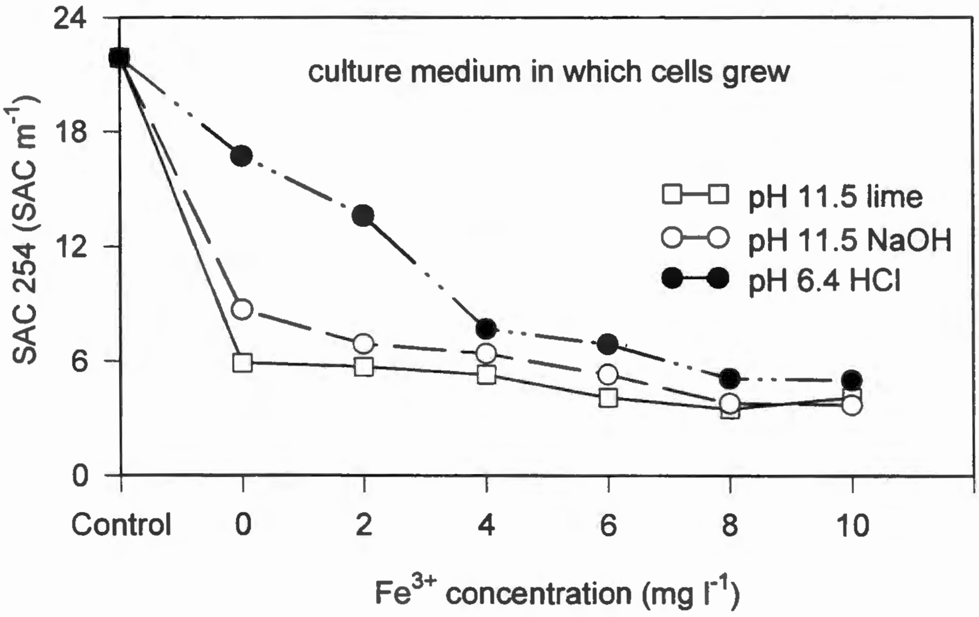


Figure 4.27 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. Culture medium, in which *C. meneghiniana* cells grew, was added to Vaal River water.

When *C. meneghiniana* cells suspended in distilled water were added to Vaal River water (Fig. 4.26), DOC was better removed at pH 11.5 than when distilled water only (Fig. 4.29) was added. Therefore, *C. meneghiniana* possibly assisted in the removal of DOC at pH 11.5. At pH 6.4 better removal was observed when no cells were added. Therefore, removal of DOC was inhibited at pH 6.4 when *C. meneghiniana* cells were present.

When culture medium in which no cells grew was added to Vaal River water (Fig. 4.28), removal of DOC was similar to when distilled water only (Fig. 4.29) was added to Vaal River water. Therefore, inorganic components of the nutrient medium apparently did not affect the removal of DOC.

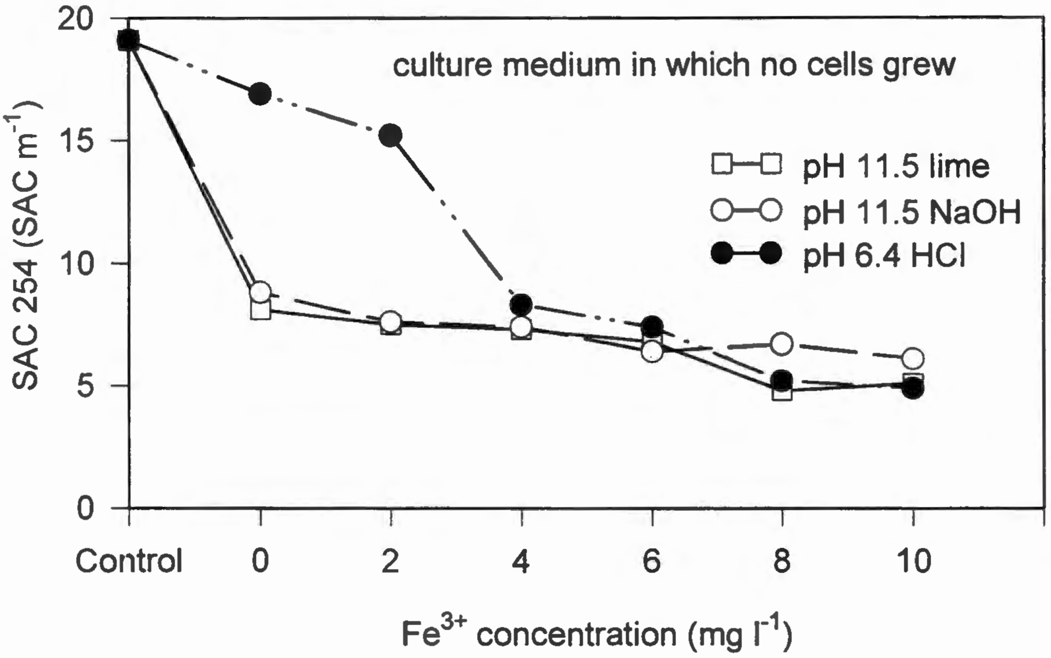


Figure 4.28 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. Culture medium, in which no cells grew, was added to Vaal River water.

When culture medium in which cells grew was added to Vaal River water (Fig. 4.27), better removal of DOC was observed than when culture medium in which no cells grew (Fig. 4.28) was added to Vaal River water. Therefore, organic substances excreted by the algal cells possibly assisted in removal of natural occurring DOC in Vaal River water.

At pH 6.4 ineffective removal of DOC occurred when cells (suspended in culture medium or distilled water) were added to Vaal River water. At pH 11.5 effective removal of DOC was observed. Thus pH 11.5 had an positve effect on the removal of DOC, while pH 6.4 had no effect on the removal..

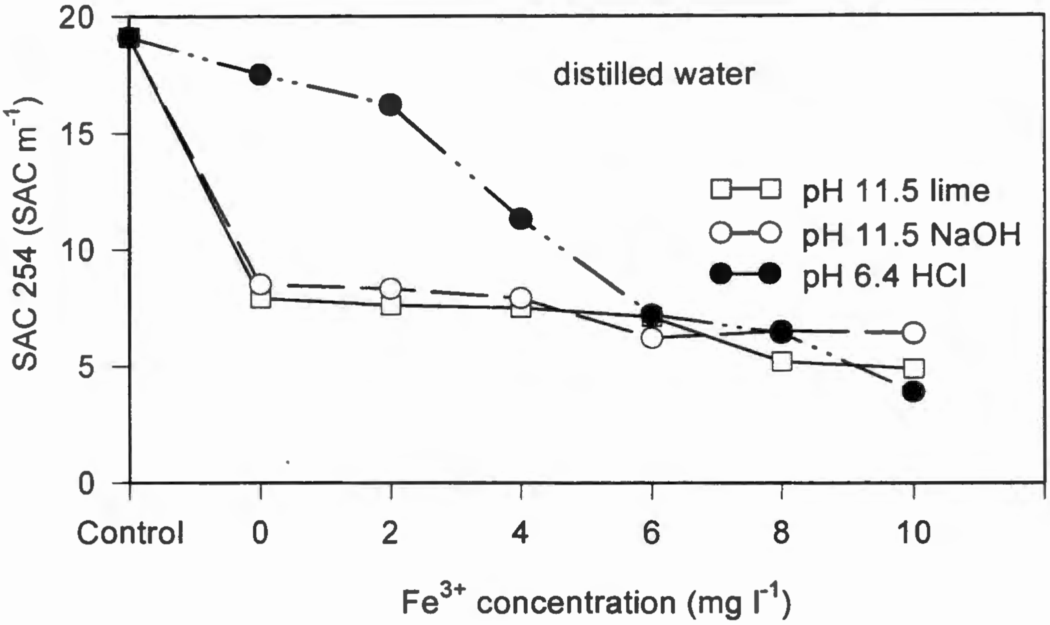


Figure 4.29 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. Distilled water only was added to Vaal River water.

When culture medium in which cells grew (Fig. 4.27) or no cells grew (Fig. 4.28) or distilled water only (Fig. 4.29), were added to Vaal River, the removal of DOC was ineffective at pH 6.4 when low Fe^{3+} concentrations (2 mg l^{-1}) were added. At pH 11.5 effective removal of DOC occurred.

4.5 The effect of *Pandorina morum* colonies on coagulation and flocculation

The aim of this experiment was to determine what effect added *P. morum* colonies (representing a *P. morum* dominance under natural conditions), and their organic excretions, had on flocculation processes. Experimental protocol applied are explained in the material and method chapter.

P. morum colonies suspended in culture medium in which they grew (concentrated so that 5.0 ml culture medium contains approximately 60 µg l^{-1} chlorophyll-*a*), the same concentration suspended in distilled water, culture medium without colonies in which the colonies grew, culture medium in which no colonies grew and distilled water only, were added to Vaal River water. The chlorophyll-*a* concentrations of the raw water was increased from 7.5 µg l^{-1} to ± 67 µg l^{-1}.

4.5.1 Removal of biomass (i.e. chlorophyll-*a*)

When *P. morum* colonies suspended in culture medium in which they grew were added to Vaal River water (Fig 4.30), and the pH was adjusted to 11.5 with lime, total removal of algal biomass was observed. When the pH was adjusted with NaOH, low biomass concentration was observed when 2 mg l^{-1} iron was added, but total removal occurred at higher iron concentrations. At pH 6.4, biomass was effectively removed with the addition of high iron (> 6 mg l^{-1}) concentrations (Fig. 4.30). The removal was, however, better than the removal of *Monoraphidium minutum* under similar conditions (see Fig. 4.1).

When *P. morum* colonies were suspended in distilled water and added to Vaal River water (Fig. 4.31), the same removal occurred as when colonies suspended in culture medium in which they grew were added to Vaal River water (Fig. 4.30), except that biomass was completely removed when the pH was adjusted to 11.5 when NaOH and iron was added.

When culture medium in which colonies grew was added to Vaal River water (Fig. 4.32) of which the biomass concentration was low, effective removal of algal biomass occurred when the pH was adjusted to 11.5 with lime. At pH 11.5, when

NaOH was added, biomass was effectively removed when iron was added at concentrations higher than 2 mg l^{-1}. At pH 6.4 effective removal was observed when Fe^{3+} concentrations were higher than 4 mg l^{-1}.

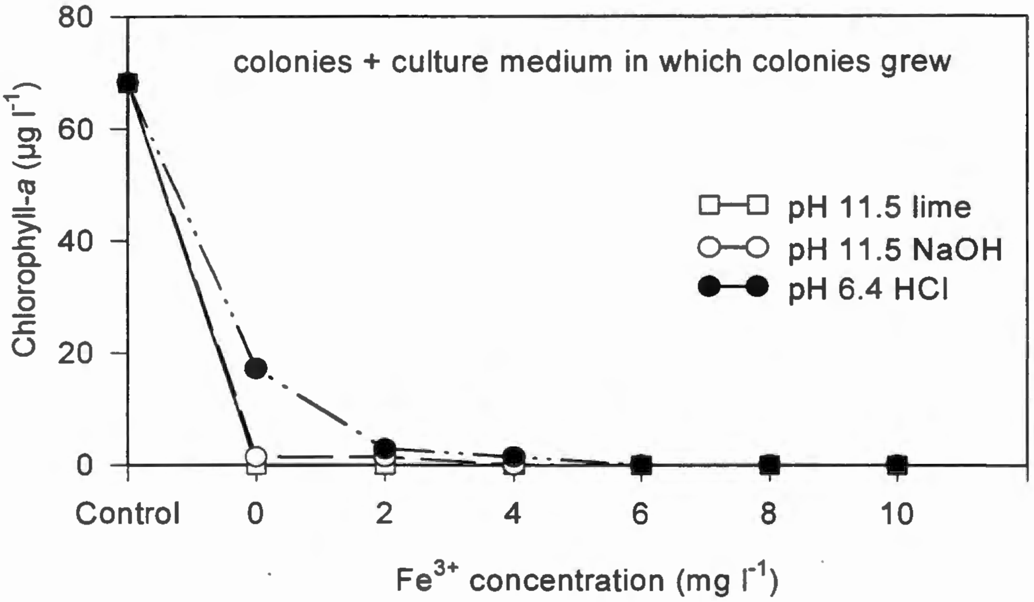


Figure 4.30 Variation in chlorophyll-a concentration (µg l^{-1}) with increased Fe^{3+} concentrations. *Pandorina morum* colonies, suspended in culture medium in which colonies grew, were added to Vaal River water.

Due to the fact that there were only small amounts (approximately 7 µg l^{-1}) of biomass originally present in the raw Vaal River water, and that the chlorophyll-*a* was effectively removed by the addition of different chemicals, the effect of the different treatments designed to investigate excreted organic material, could not be illustrated. Fig. 4.33 represents the results obtained for the treatments where culture medium in which no colonies grew or when distilled water were added. The chlorophyll-*a* that was present, was effectively removed.

The following conclusions can be drawn to summarise observations on the removal of algal biomass when *Pandorina morum* colonies were added to Vaal River water. The addition of *P. morum* colonies increased the chlorophyll-*a* concentration (biomass) with approximately 60 µg l^{-1}. The higher biomass concentration was less efficiently removed at pH 6.4 when lower iron concentrations (< 4 mg l^{-1}) were added (Figs 4.30 and 4.31).

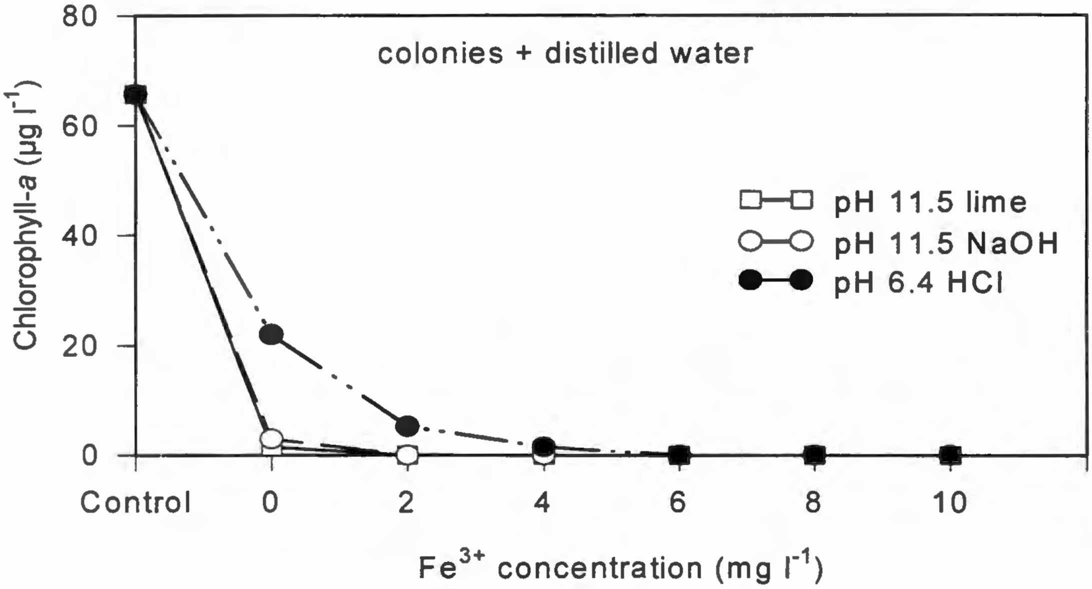


Figure 4.31 Variation in chlorophyll-a concentration (µg l^{-1}) with increased Fe^{3+} concentrations. *Pandorina morum* colonies, suspended in distilled water, were added to Vaal River water.

When *P. morum* colonies suspended in culture medium in which colonies grew were added to Vaal River water, biomass was effectively removed when the pH was adjusted to 11.5 (Fig. 4.30) and increased Fe^{3+} concentrations (> 2 mg l^{-1}) were added. When culture medium in which colonies grew (Fig. 4.32) was added to Vaal River water, removal was better when *P. morum* colonies suspended in culture medium in which the colonies grew, were added to Vaal River water (Fig. 4.30). Therefore, *P. morum* colonies apparently prevent (escape floc formation) the removal of algal biomass, or were removed less efficiently, probably because of the ability of the colonies to move.

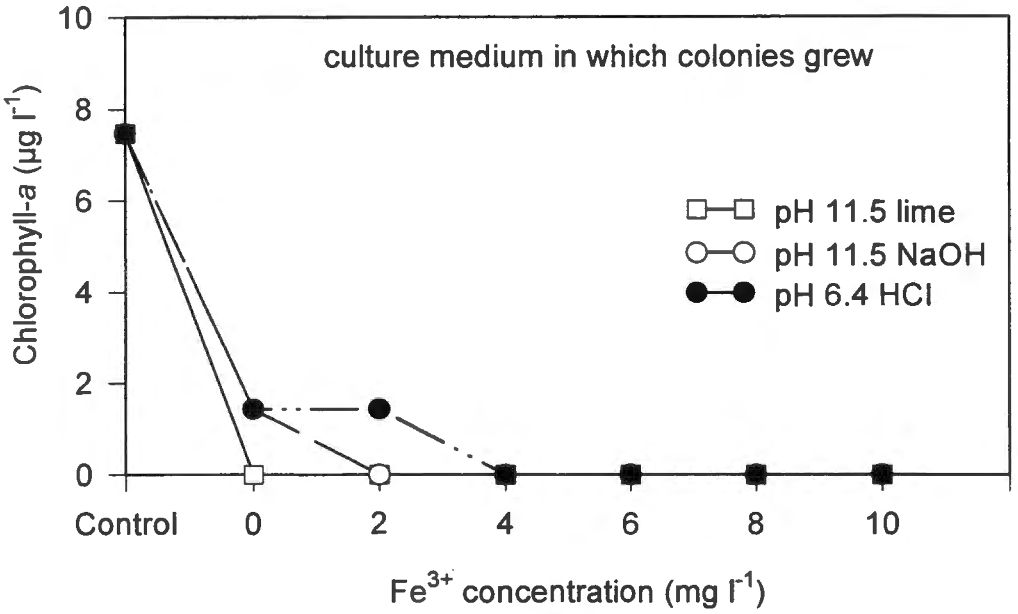


Figure 4.32 Variation in chlorophyll-a concentration (µg l^{-1}) with increased Fe^{3+} concentrations. Culture medium, in which *Pandorin morum* colonies grew, was added to Vaal River water.

When *P. morum* colonies suspended in distilled water (Figs 4.31) were added to Vaal River water, biomass was removed less efficiently than when distilled water only (Fig. 4.33) was added. Therefore, as already shown, *P.morum* colonies apparently inhibited the removal of algal biomass.

When culture medium in which no colonies grew was added to Vaal River water (Fig. 4.33), removal of algal biomass was similar than when distilled water only (Fig. 4.33) was added to Vaal River water. Therefore, inorganic components of the nutrient medium did not affect removal of algal biomass.

When culture medium in which colonies grew was added to Vaal River water (Fig. 4.32), slightly poorer removal of algal biomass was observed than when culture medium in which no colonies grew (Fig. 4.33) was added to Vaal River water. Therefore, organic substances excreted by the algal colonies possibly inhibited the removal of algal biomass.

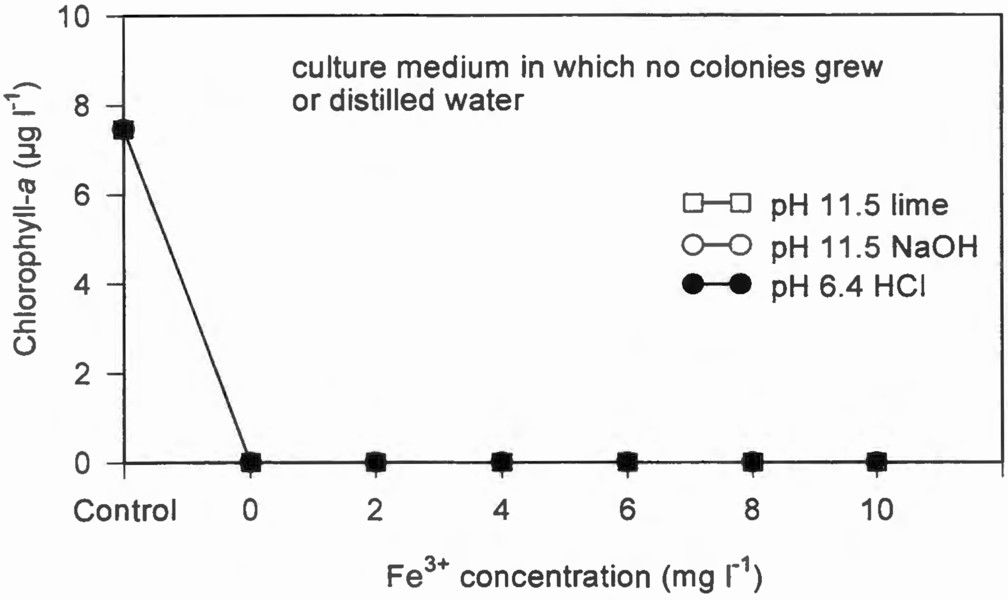


Figure 4.33 Variation in chlorophyll-a concentration (µg l^{-1}) with increased Fe^{3+} concentrations. Either culture medium, in which no colonies grew, or distilled water was added to Vaal River water.

4.5.2 Removal of suspended solids

As illustrated in Figs 4.34 - 4.38, suspended particles in the Vaal River water were removed for most of the treatments when *P. morum* colonies were added to Vaal River water.

When *P. morum* colonies suspended in culture medium in which they grew (Fig. 4.34) were added to Vaal River water and the pH was adjusted to 6.4, higher iron (> 6 mg l^{-1}) concentrations were necessary for the effective removal of suspended particles in the raw water. At pH 11.5, adjusted with NaOH or lime, removal was effective with the addition of increased iron concentrations (> 2 mg l^{-1}).

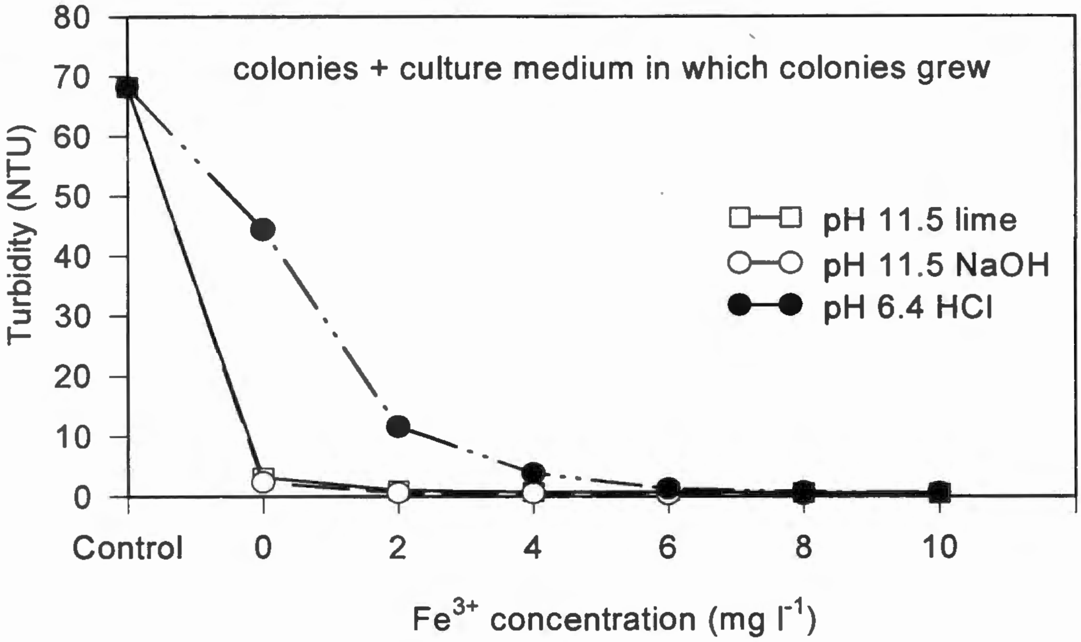


Figure 4.34 Variation in turbidity (NTU) with increased Fe^{3+} concentration. *Pandorina morum* colonies, suspended in culture medium in which colonies grew, were added to Vaal River water.

When *P. morum* colonies suspended in distilled water (Fig. 4.35) was added to Vaal River water and the pH adjusted to 6.4, higher iron (> 6 mg l^{-1}) concentrations were necessary for the effective removal of suspended particles in the raw water. At pH 11.5, adjusted with NaOH or lime, removal was effective with the addition of increased iron concentrations.

When culture medium in which colonies grew (Fig. 4.36) was added to Vaal River water and the pH was adjusted to 6.4, higher iron (> 6 mg l^{-1}) concentrations were necessary for the effective removal of suspended particles in the raw water. At pH 11.5, adjusted with NaOH or lime, removal was effective with the addition of increased iron concentrations.

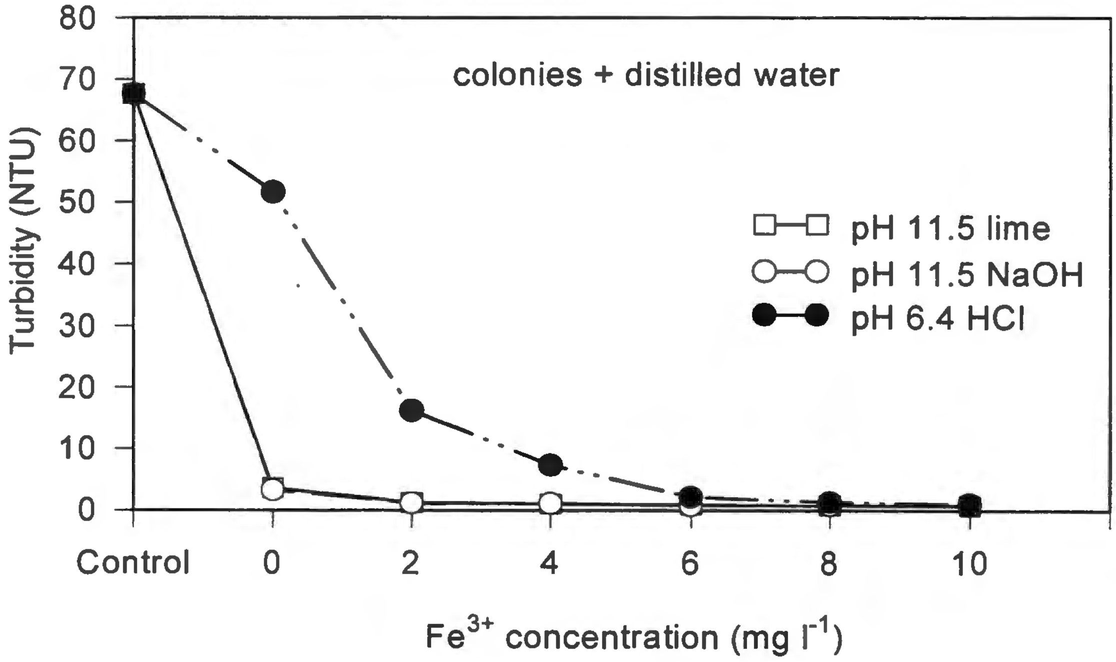


Figure 4.35 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. *Pandorina morum* colonies, suspended in distilled water, were added to Vaal River water.

When culture medium in which no colonies grew (Fig. 4.37) was added to Vaal River water and the pH was adjusted to 6.4, higher iron (> 6 mg l^{-1}) concentrations were necessary for the effective removal of suspended particles in the raw water. At pH 11.5, adjusted with NaOH or lime, removal was effective with the addition of increased iron concentrations.

did not effect turbidity. Figs 4.34 to 4.38 indicated that pH 11.5 played an important role in the removal of suspended solids. pH 6.4 was ineffective in the removal of suspended matter except when high Fe^{3+} (> 6 mg l^{-1}) concentrations were added. Effective removal occurred at pH 11.5 with increased Fe^{3+} concentrations, and little difference was observed when NaOH or lime was added.

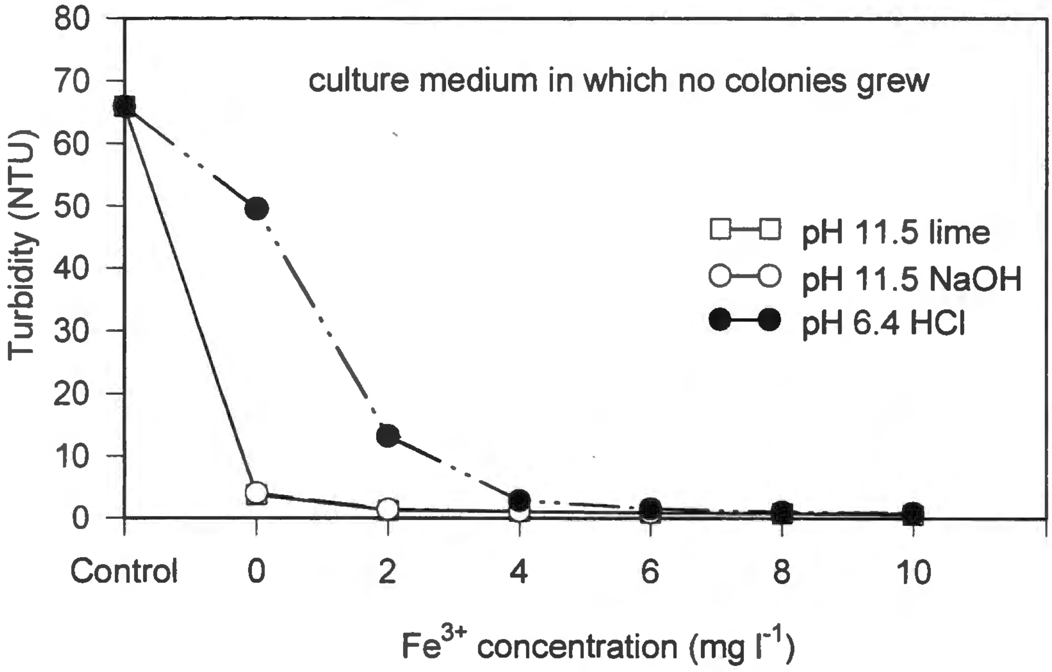


Figure 4.37 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. Culture medium, in which no colonies grew, was added to Vaal River water.

Figs 4.34 to 4.38 showed that the removal of suspended solids was similar for all the treatments designed to investigate excreted organic material. Therefore, removal of suspended solids was apparently not affected by excreted organic substances or *P. morum* colonies.

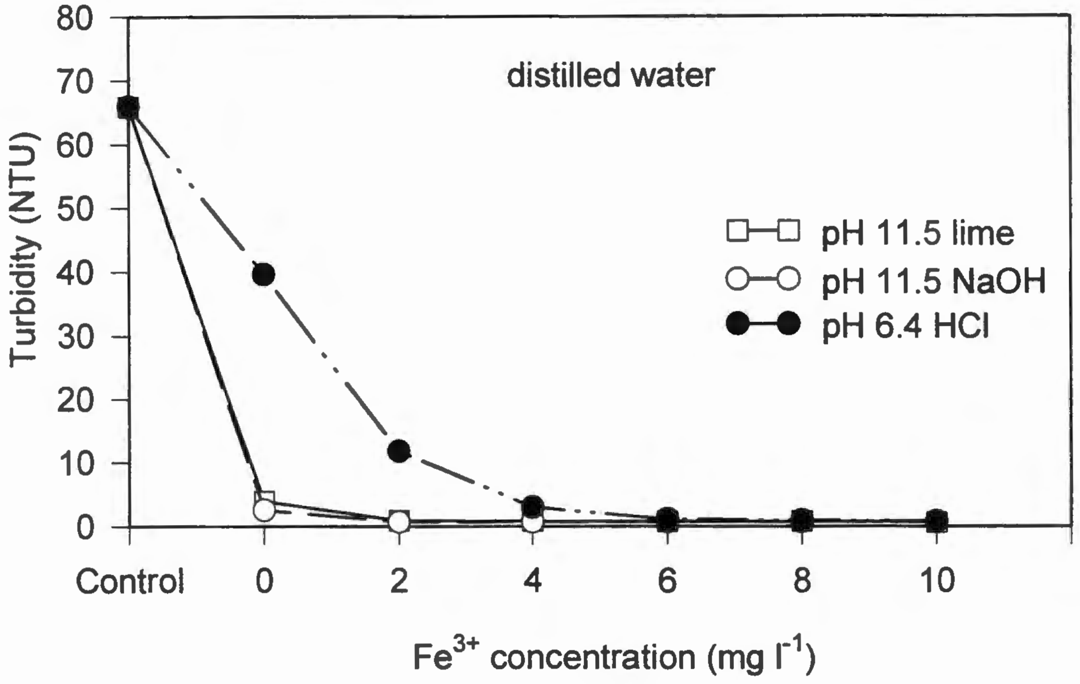


Figure 4.38 Variation in turbidity (NTU) with increased Fe^{3+} concentrations. Distilled water only was added to Vaal River water.

4.5.3 Removal of dissolved organic carbon

When *P. morum* colonies were added to Vaal River water, the DOC concentration did not increase more than 1 SAC unit (Figs 4.39 and 4.40). When *P. morum* colonies suspended in culture medium in which they grew, were added to Vaal River water (Fig. 4.39), effective removal of DOC concentrations occurred only when high iron concentrations were added (10 mg l^{-1} and higher). The removal was not as effective at pH 6.4. The best removal occurred when the pH was adjusted to 11.5 with NaOH, but when lime was used with 10 mg l^{-1} Fe^{3+}, the most effective removal was observed.

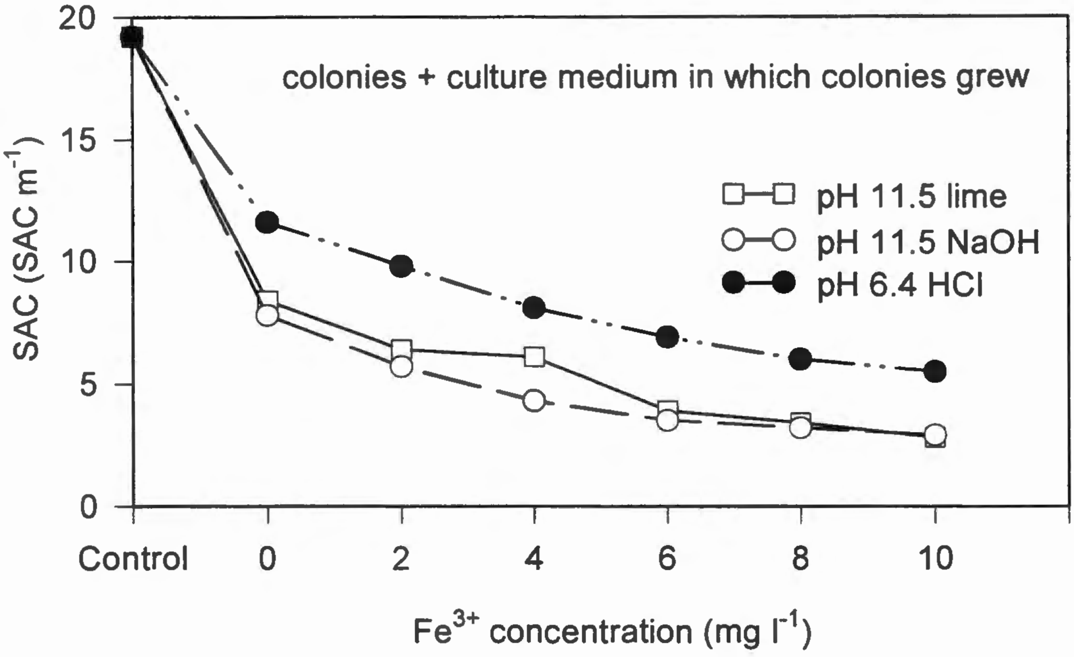


Figure 4.39 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. *Pandorina morum* colonies, suspended in culture medium in which colonies grew, were added to Vaal River water.

When *P. morum* colonies were suspended in distilled water and added to Vaal River water, the removal of DOC seemed to be the same as when algal colonies were added resuspended in culture medium (compare Figs 4.39 and 4.40). When the pH was adjusted to 11.5 with lime, the removal of DOC was slightly less than when *P. morum* colonies resuspended in culture medium were added (Fig. 4.40).

When culture medium in which the colonies grew, or culture medium in which no colonies grew, was added to Vaal River water, the results illustrated in Figs 4.41 and 4.42 were comparable with results obtained when only colonies were added (Figs 4.39 and 4.40). The removal of DOC was, however, poorer when the pH was adjusted to 6.4 and low Fe^{3+} concentrations (< 4 mg l^{-1}) were dosed.

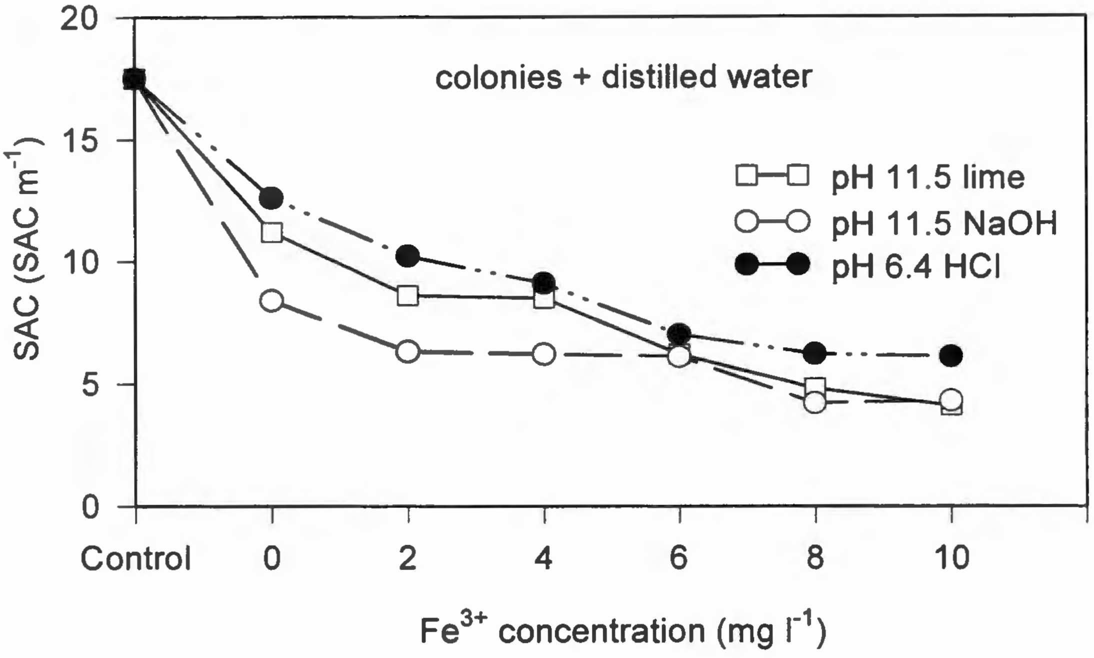


Figure 4.40 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. *Pandorina morum* colonies, suspended in distilled water, were added to Vaal River water.

When distilled water was added to Vaal River water (Fig. 4.43), the removal of DOC was slightly less effective than when *P. morum* colonies (suspended in culture medium or in distilled water) or culture medium (in which colonies did or did not grow) were added. When pH was adjusted to 6.4, only high iron concentrations (i.e. > 8 mg l^{-1} of Fe^{3+}) removed DOC effectively (Fig. 4.43).

The following conclusions can be drawn to summarise observations on the removal of DOC when *P. morum* colonies were added to Vaal River water. When *P. morum* colonies (suspended in culture medium or distilled water) were added to Vaal River water (Figs 4.39 and 4.40), DOC was more effectively removed at pH 6.4 than when culture medium in which the colonies grew (Fig. 4.41) or distilled water (Fig. 4.43) was added and the pH was adjusted to 6.4. The removal was possibly enhanced by added *P. morum* colonies. At pH 11.5 the removal of DOC was almost the same for all the treatments designed to investigate excreted organic material, except when colonies were added together with culture medium (Fig. 4.39) in which case better removal was observed. The slight improvement in removal when colonies were added together with culture medium in which colonies grew, indicated that algal colonies possibly assisted in the removal of DOC or the colonies had a lot of DOC attached to the surface of the colonies, which could possibly be removed together with the colonies.

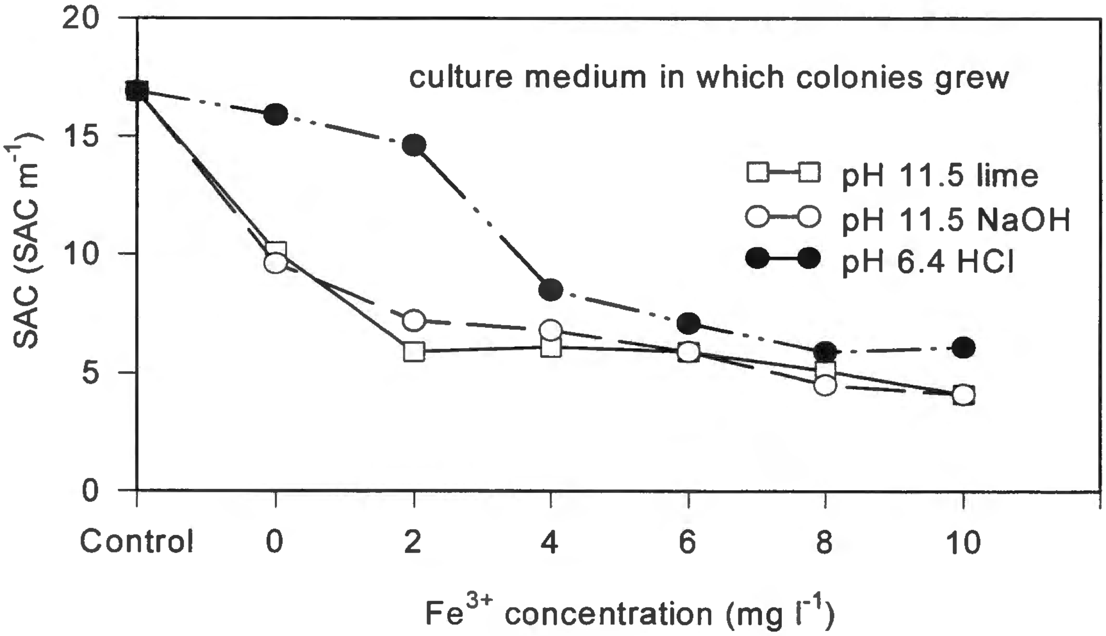


Figure 4.41 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. Culture medium, in which *Pandorina morum* colonies grew, was added to Vaal River water.

When culture medium in which no colonies grew was added to Vaal River water (Fig. 4.42), removal of DOC was slightly better than when distilled water only (Fig. 4.43) was added to Vaal River water. Therefore, inorganic components of the nutrient medium assisted in the removal of DOC.

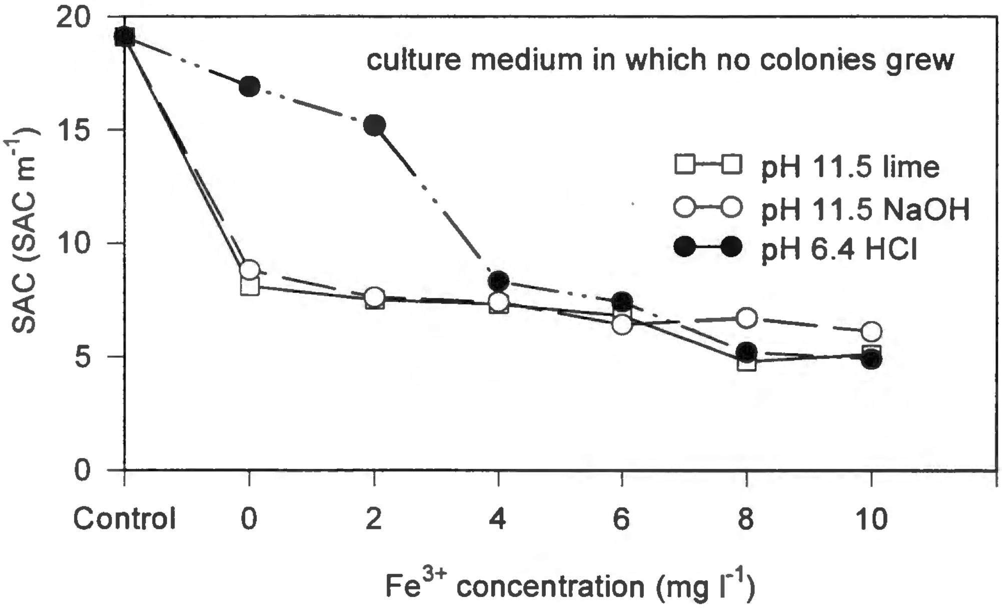


Figure 4.42 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. Culture medium, in which no colonies grew, was added to Vaal River water.

When culture medium in which colonies grew was added to Vaal River water (Fig. 4.41), similar removal of DOC was observed than when culture medium in which no colonies grew (Fig. 4.42) was added to Vaal River water. Therefore, organic substances excreted by the algal colonies apparently did not affect the removal of DOC.

At pH 6.4 ineffective removal of DOC occurred when colonies (suspended in culture medium or distilled water), culture medium in which colonies grew, culture medium in which no colonies grew or distilled water only were added to Vaal River water. At pH 11.5 effective removal of DOC was observed.

When culture medium in which colonies grew (Fig. 4.41) or no colonies grew (Fig. 4.42), were added to Vaal River water, removal of DOC was ineffective at pH 6.4 when low Fe^{3+} concentrations (< 4 mg l^{-1}) were added. When distilled water was added (Fig. 4.43), removal was ineffective at pH 6.4 when low Fe^{3+} concentrations (<8 mg l^{-1}) was added. At pH 11.5 effective removal of DOC occurred.

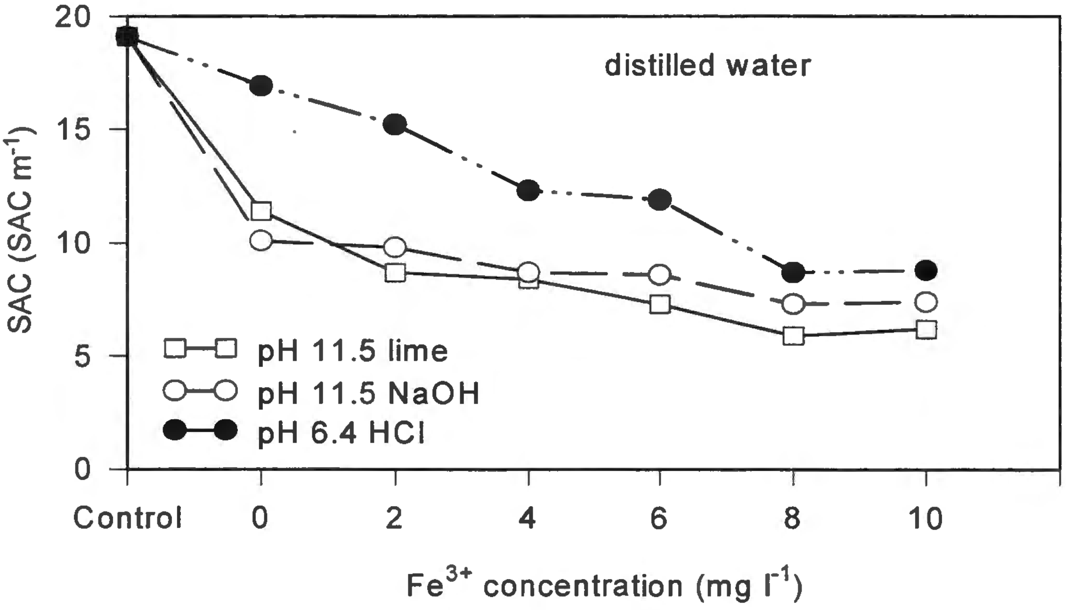


Figure 4.43 Variation in spectral absorption coefficient (SAC m^{-1}) with increased Fe^{3+} concentrations. Distilled water only was added to Vaal River water.

4.6 Summary of the effect of excreted organic material on the coagulation process

The following conclusions can be drawn to summarise the effect of the three algal species, and the organic substances excreted by them, on the removal of algal biomass, suspended solids and dissolved organic matter present in Vaal River water:

1. *Monoraphidium minutum* cells addition reduced the removal of algal biomass (algal cells). The organic matter excreted by the cells possibly improved the removal of algal biomass at pH 11.5, but inhibited removal at pH 6.4. Organic matter excreted by *M. minutum* apparently assisted in the removal of other than algal suspended matter present in Vaal River water. The organic substances excreted by *M. minutum* cells reduced or had no effect on the removal of DOC. The culture medium in which *M. minutum* grew contained higher concentrations of monocarboxylic, fatty and aromatic acids in addition to glycerol than the culture culture media of the other algae (Table 3).
2. *Cyclotella meneghiniana* cells were more effectively removed than *Monoraphidium minutum* cells in terms of chlorophyll-*a*. Organic substances excreted by *Cyclotella meneghiniana* reduced the removal of algal biomass (algal cells) at pH 6.4. The culture medium in which *C. meneghiniana* grew had higher concentrations of dicarboxylic acids than the culture media of the other algae (Table 3). *C. meneghiniana* cells apparently enhanced the removal of suspended particles at pH 6.4. When the pH was adjusted to 11.5, *C. meneghiniana* apparently enhanced the removal of DOC.
3. Although *Pandorina morum* colonies are motile by means of flagellar movements, the colonies were more effectively removed when compared with the removal of *Monoraphidium minutum* cells. Organic substances excreted by *Pandorina morum* colonies apparently reduced the removal of algal biomass (algal colonies). *P. morum* colonies, and the organic substances excreted by them, did not affect the removal of suspended particles in Vaal River water. *P. morum* colonies apparently enhanced the removal of DOC slightly. The organic substances excreted by

P. morum did not affect the removal of DOC. The culture medium of *P. morum* had less monocarboxylic and aromatic acids in solution than the culture media of the other algae (Table 3).

4. Although the extracellular substances of the different algae differed markedly, and while it is tempting to suggest that the differences in effect might have been attributable to the differences in concentration and relative ratios of the extracellular organic substances, the present investigation cannot provide conclusive answers.

CHAPTER 5: CONCLUSIONS

The results obtained in the experiments showed that the methods and conditions used here were generally effective for coagulation, flocculation and sedimentation of algal cells, algal colonies and suspended solids. The removal of dissolved organic substances occurred at specific conditions, i.e. low pH (pH 5) and high pH (pH 11.4).

The experimental procedures followed in the experiments allowed comparison between different variables measured in untreated and chemically treated water from the Vaal River. With the experiments it was possible to establish how much material had been removed by flocculation and what effect pH and different flocculants had on the different processes.

Results, on the water from the Vaal River used in this study, showed that it is not necessary to dose Fe^{3+} at higher concentrations than 8 mg l^{-1} because of the small additional effect that 10 and 22 mg l^{-1} Fe^{3+} had on flocculation.

Low Fe^{3+} concentration (lower than approximately 4 mg l^{-1}) dosages gave lower flocculation and removal efficiencies. These results apparently show the effect of too small amounts of Fe^{3+} available to form positive charged Fe-hydroxo complexes for charge neutralisation. With higher Fe^{3+} concentrations, flocculation and removal occurred more efficiently.

The main factor responsible for the formation of flocs on the surface of the water could be flocculant overdose, because it occurred only when higher Fe^{3+} concentrations were used (> 12 mg l^{-1}). The optimum flocculant concentration, based on the results of the experiments, was between 8 and 10 mg l^{-1} Fe^{3+} for a pH range between 5 and 11 after pH adjustment.

The formation and concentration of flocs or aggregates on the surface of the treated water could be an indication that the phytoplankton cells, colonies and filaments in the water produce gas bubbles or vacuoles (in the case of blue-green algae) which enable the cells in the flocs to float on the water surface. In addition, the concentration at the surface could also be due to mucilage material present around some algal cells which entraps gasses such as O_2 and CO_2 released by the cells. Differences in densities between the mucilage material and the water could also have resulted in the concentration of material at the surface of the water.

The results showed that the flocculation and removal of DOC decreased with an increase in pH (Fig. 3.1 – 3.4). The most efficient removal of DOC occurred at pH 5, which is an indication that $FeCl_3$ as sole flocculant was insufficient in the removal of DOC at pH conditions between 7 and 11. However, when the pH was raised to 11.5, the removal of DOC improved. This increase in removal of DOC was enhanced with the addition of iron.

Ca^{2+} ions added (to represent the Ca^{2+} concentration present in lime) apparently did not participate directly in flocculation processes when the pH was unchanged (Fig. 3.19). When the pH was adjusted to 11.5, algal cells and suspended particles were effectively removed even when no Ca^{2+} was added. The removal was, therefore, the result of high pH conditions. It is, however, possible that a pH of 11.5, in conjunction with the added Ca^{2+}, resulted in the formation of precipitates (calcium carbonate and magnesium hydroxide) which can possibly assist in flocculation. It was shown in other studies that suspended particles can be adsorbed onto these precipitates (Parker *et al.*, 1975; Dziubek and Kowal, 1984 and Steynberg, 1994).

The use of high Fe^{3+} concentrations (> 6 mg l^{-1}) under low pH conditions (< 7) for the water used in this study, may have contributed to the relatively efficient removal of DOC. Investigations by Black and Christman (1963), Hall and Packham (1965), Dempsey *et al.* (1984), Edwards and Amirtharajah (1985) and Sinsabaugh *et al.* (1986) showed that coagulation of DOC was dependent primarily on pH conditions, the coagulant dose, and the concentration of DOC. Effective removal of DOC occurred at lower pH conditions than the pH conditions necessary for turbidity removal. The optimum pH conditions for coagulation of organic matter was around pH 4 by Fe^{3+} salts. Gray (1988) reported that below pH 6, removal occurs by co-precipitation of ferric-organic matter and ferric hydroxide precipitates and concluded that effective removal of DOC with Fe^{3+} occurs primarily by the formation of an iron-organic precipitate rather than the formation of ferric hydroxide. This investigation showed that DOC was also effectively removed at pH 11.5 in conjunction with increased Fe^{3+} concentrations (> 8 mg l^{-1}).

The experiments demonstrated that low Fe^{3+} concentrations (< 6 mg l^{-1}) gave lower flocculation and removal efficiencies of algal cells, suspended solids and DOC. With an increase in Fe^{3+} concentration (i.e. 8 to 18 mg l^{-1}), an increase in the efficiency of flocculation and removal of algal cells, suspended solids and DOC were observed. It was possible to determine the optimum Fe^{3+} concentration (approximately 8 mg l^{-1}) necessary for efficient flocculation and removal of algal biomass (indicated by chlorophyll-*a*), DOC and total suspended solids for the water and conditions of the present study.

The experiments enable the determination of the optimum pH for flocculation of suspended material (biotic and abiotic) with $FeCl_3$ as flocculant. Except for the removal of DOC, the removal of total suspended solids in the water was optimal at pH 11. Therefore, it is possible to conclude that optimal conditions for flocculation with $FeCl_3$ as flocculant is at pH 11 at a Fe^{3+} concentration of 8 mg l^{-1}. Ferric chloride was more efficient in affecting the coagulation process than ferric sulphate.

The results obtained from the comparison between lime and sodium hydroxide showed that lime was more efficient in affecting flocculation. This can be due to the increase in suspended particles and possibly calcium concentrations which may possibly assist in flocculation as a result of calcium carbonate precipitates which forms at pH conditions higher than 10.5 (Ronen, 1981).

Monoraphidium minutum cells were ineffectively removed at pH 6.4, but effective removal occurred at pH 11.5. *M. minutum* cells are elongated and cresent-shaped which can be responsible for the possibility that the compactness of the flocs in which they were included, was low and that sedimentation would be affected. *Cyclotella meneghiniana* cells were effectively removed when added to Vaal River water, except when pH was adjusted to 6.4 and low iron concentrations (< 4 mg l^{-1}) were added. The areolae in the frustules, which can capture oxygen during photosinthesis, could possibly be responsible for the ineffective removal at pH 6.4 when low Fe^{3+} concentrations (< 4 mg l^{-1}) was added. *C. meneghiniana* cells apparently enhanced the removal of suspended matter at pH 6.4.

Pandorina morum colonies were effectively removed when the pH was adjusted to 11.5 and Fe^{3+} concentrations higher than 4 mg l^{-1} were added. Ineffective removal occurred at pH 6.4 when low Fe^{3+} concentrations (< 2 mg l^{-1}) were added. The ineffective removal at pH 6.4 can possibly be due to the presence of the mucilage sheath around the colonies. Mucilage density is lower than the density of water (Reynolds, 1975) and, therefore, sedimentation should be slower. The ineffective removal can also be due to the movement of the colonies by means of flagella.

The organic substances excreted by *Monoraphidium minutum* improved the removal of cells (indicated by chlorophyll-*a*) at pH 11.5, but algal biomass removal was inhibited at pH 6.4. Organic matter excreted by *M. minutum* possibly improved the removal of suspended matter present in Vaal River water, but reduced the removal of DOC.

Organic substances excreted by *Cyclotella meneghiniana* cells apparently reduced the removal of cells (indicated by chlorophyll-*a*) at pH 6.4, but the removal of cells was not affected at pH 11. 5 by excreted organic matter. The organic substances excreted by *Pandorina morum* colonies also apparently reduced the removal of chlorophyll-*a*.

The results showed that algal cells, especially of *Monoraphidium minutum* and *Cyclotella meneghiniana* (at pH 11.5) and *Pandorina morum* colonies themselves, possibly assist in the removal of DOC.

It can in general be concluded that increase in biomass together with the excreted organic matter from the algal cells, affect the removal of biomass, suspended matter and dissolved organic carbon. However, more detailed studies are needed to determine the exact effect of the algal cells and colonies as well as the specific excreted organic substances on coagulation and sedimentation.

Future studies should focus on extracellular organic substances by more algal species that occur in the raw water. The positive or negative effect of specific extracellular substances on coagulation and sedimentation of algal cells, colonies and filaments as well as colloidal particles and other dissolved organic substances must also be investigated. Should any extracellular organic substance enhance flocculation and sedimentation, the possibility of using the substance in water purification should be investigated.

ACKNOWLEDGEMENTS

My sincere gratitude and appreciation to the following persons and institutions for their indispensable contributions to the successful completion of this study:

- Water Research Commission, for initiating and financially supporting this research project.

- The Potchefstroomse Universiteit vir Christelike Hoër Onderwys (PU vir CHO) for the opportunity to do this study and especially for the Division Botany, School of Environmental Sciences and Development, for the use of their research facilities.

- Sedibeng Water for their assistance during the study and for granting me study leave for completion of this work.

- Professor Dr. A.J.H. Pieterse, as my supervisor, acted as a source of inspiration by continually striving for excellence. Through his interest in the work, his inspired leadership, guidance and patience he has fulfilled much more than the word promoter could ever implicate. It was an honour to work with someone so dedicated to his subject and with so much valuable experience to share.

- My parents, sister and brothers, for their genuine interest, motivation and assistance both morally and financially.

- A very special thanks to A.P. de Wet, Lennof Reynecke, Sanet van Vuuren, Antoïnette Jacobs and Annelie Swanepoel for their faith in me, their encouragement and interest during the study.

God Almighty who has blessed me with the opportunity and talents to complete this work.

REFERENCES

AMIRTHARAJAH, A. and O'MELIA, C.R. 1990. *Coagulation processes: destabilisation, mixing, and flocculation.* Water Quality and Treatment. Fourth Edition. American Water Works Associaton. McGraw - Hill, Inc. pp.269 - 367.

AMIRTHARAJAH, A., DENNETT, K.E. and STUDSTILL, A. 1993. Ferric chloride coagulation for removal of dissolved organic matter and trihalomethane precursors. *Wat. Sci. Tech.*, **27**:113-121.

AWWA, 1990. *Water quality and treatment. A handbook of community water supplies.* American Water Works Association. McGraw-Hill Inc., N.York.

BASSON, N. & PIETERSE, A.J.H. 1993. The occurrence and origin of THM substances in a full-scale plant purifying water from the middle Vaal river. Proceeding of the 1993 WISA Conference.

BERNHARDT, H. 1967. Uber die Entfernung von Oscillatoria rubescens aus einem Telsperrenwasser durch Flockung und Filtration. *Arch. Hydrobiol.*, **61**:311-327.

BERNHARDT, H. and CLASEN, J. 1967. Probleme der Entfernung von Mikroorganismen durch Flockung in der Trinkwasseraufbereitung. *Veröff. d. Abt. u. d. Lehrstuhls für Wasserchem. d. Univ. Karlsruhe*, **3**:294-338.

BERNHARDT, H. and WILHELMS, A. 1972. Einfluβ von Algenstoffwechsel- und Abbauprodukten auf den Flockungsprozeβ. *Gewässerschutz-Wasser-Abwasser,* **8**:111-138.

BERNHARDT, H. 1982. Investigation of algogenic organic substances and their effect on water treatment. *Proceedings of the Japanese-German Workshop on Waste Water and Sludge Treatment,* pp. 583-637.

BERNHARDT, H., HOYER, O. and LÜSSE, B. 1985a. Untersuchungen zur Beeinflussung der Flockung und Flockenabtrennung durch algenbürtige organische Substanzen. *Z. Wasser- Abwasser- Forsch.*, **18**:6-17.

BERNHARDT, H., HOYER, O., SCHELL, H. and LÜSSE, B. 1985b. Reaction mechanisms involved in the influence of algogenic organic matter on flocculation. *Z. Wasser- Abwasser- Forsch.,* **18**:18-30.

BERNHARDT, H., LÜSSE, B. and HOYER, O. 1986. The addition of calcium to reduce the impairmant of flocculation by algogenic organic matter. *Z. Wasser- Abwasser- Forsch.,* **19**:219-228.

BERNHARDT, H. and CLASEN, J. (1995) Investigation into the flocculation mechanisms of small algal cells. Aqua. Submitted for publication.

BLACK, A.P. and CHRISTMAN, R.F. 1963. Characteristics of colored surface waters. *Jour. Amer. Water Works Assoc.*, **55**:753-770.

BLACK, A.P. and THOMPSON, C.G. 1971. Magnesium carbonate, a recycled coagulant for water treatment. *EPA Reports,* 12120 ESW.

BLACK, A.P. and THOMSON, C.G. 1975. Plant scale studies of the magnesium carbonate water treatment process. *EPA Reports,* 660/2-75-006.

BRUWER, C.A., VAN VLIET, H.R., SARTORY, D.P. and KEMPSTER, P.L. 1985 An assessment of water-related problems of the Vaal River between Barrage and Douglas Weir. Technical Report TR 121, HRI, Department of Water Affairs, Pretoria.

DEMPSEY, B.A. et al., 1984. The coagulation of Humic Substances by means of Aluminium Salts, *Journal AWWA*, Vol 6 No. 4, p 141.

DEMPSEY, B.A. 1989. *Reactions between fulvic acid and aluminium: Effects on the coagulation process. Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants.* I.H. Suffet and P. MacCarty, Editors. American Chemical Society, Washington, D.C., pp.409-424.

DOLEJS, p. 1993. influence of algae and their removal of humic substances and optimal dose of coagulant. *Water Science and Technologies*, **27**:123-132

DWA 1986. *Management of water resources of the Republic of South Africa.* Department of Water Affairs (DWA), CTP Book Printers, Cape Town.

DZIUBEK, A.M. and KOWAL, A.L. 1984. Effect of magnesium hydroxide on chemical treatment of secondary effluent under alkaline conditions. *Proc. Water Reuse Symp. III*, AWWA Res. Foundation, San Diego, pp. 1428-1436.

DZIUBEK, A.M. and KOWAL, A.L. 1989. High-pH coagulation-adsorption: A new technology for water treatment and reuse. *Wat. Sci. Tech.*, **21**:511-517.

EDWARDS, G.A. and AMIRTHARAJAH, A. 1985. Removing color caused by humic acids. *Jour. Amer. Water Works Assoc.*, **77**:50-57.

EDZWALD, J.K., BECKER, W.C. and WATTIER, K.L. 1985. Surrogate parameters for monitoring organic matter and the precursors. *J. Am. Water Works Assoc.*, **77**:122-132.

FLENTJE, M.E. 1927. Calcium and magnesium hydrates. *J. Am. Water Works Assoc.*, **17**:253-260.

FOGG, G.E. 1962. Extracellular products. In: LEWIN, R.A. (Ed.) Physiology and Biochemistry of Algae. Academic press, New York and London. pp 475-492.

GRABOW, W.O.K., GRABOW, N.A. and BURGER, J.S. 1969. The bactericidal effect of lime flocculation/flotation as a primary unit process in a multiple system for advanced purification of sewage works effluent. *Wat. Res.*, **3**:943-953.

GRAY, K.A. 1988. The preparation, characterisation, and use of inorganic iron (III) polymers for coagulation in water treatment. Doctoral Dissertation. Johns Hopkins University, Baltimore, Maryland.

GREGORY, JOHN. 1989. Fundamentals of flocculation. *Critical Reviews in Environmental Control.* **19**:185-230.

HALL, E.S. and PACKHAM, R.F. 1965. Coagulation of organic color with hydrolysing coagulants. *Jour. Amer. Water Works Assoc.*, **57**:1149-1166.

HELLEBUST, J.A. 1974. Extracellular Products. In. STEWART, W.D.P. (E.d.) Algal Physiology ND biochemistry. Blackwell Scientific publications, Oxford, London.

HOYER, O., LÜSSE, B. & BERNHARDT, H. 1985. Isolation and characterization of extracellular organic matter from algae. Z. Wasser-Abwasser-Forsch., **18**:76-90.

HOYER, O., LÜSSE, B. & BERNHARDT, H. 1987. The effect of ozonation on the impairment of flocculation by algogenic organic matter. Z. Wasser-Abwasser-Forsch., **20**:123-131.

HUNTER, K.A. and LISS, P.S. 1979. The surface charge of suspended particles in estuarine and coastal waters. *Nature*, **282**:823-825.

JANSE VAN VUUREN, M.S. 1996. Environmental variables, abundace and seasonal succession of phytoplankton populations in the Vaal River at Balkfontein. Unpublished M.Sc Thesis, Potchefstroomse Universiteit vir Chriselike Hoër Onderwys, Potchefstroom, South Africa.

KONNO, H. 1993, Settling and coagulation of slender type diatoms. *Wat. Sci. Tech.*, **27**:231-240.

KRÜGER, G.H.J. and ELOFF, J.N. 1978. The effect of temperature on specific growth rate and activation energy of *Microcystis* and *Synechococcus* isolates revelant to the onset of natural blooms. *J. Limnol. Soc. Sth. Afr.*, **4**:9-20.

KUNIKANE, S. MAGARA, Y., and AIZAWA, T. 1986. The adverse effect of extracellular products of algae on coagulation process. *Journal of Japan Water Works Association*, **55**:33-39.

LEENTVAAR, J. and REBHUN, M. 1982. Effect of magnesium and calcium precipitation on coagulation-flocculation with lime. *Water Res.*, **16**:655-662.

LEPRINCE A., FIESSINGER, F. and BOTTERO, J.Y. 1984. Polimerised iron chloride: An improved inorganic coagulant, *Journal AWWA*, **76**:93-95.

LETTERMAN, R.D. and VANDERBROOK, S.G. 1983. Effect of solution chemistry on coagulation with hydrolysed Al: significance of sulphate ion and pH. *Water Research*, **17**:195-204.

LÜSSE, B., HOYER, O. and SOEDER, C.J. 1985. Mass cultivation of planktonic freshwater algae for the production of extracellular organic matter (EOM). *Z. Wasser- Abwasser- Forsch.,* **18**:67-75.

LÜSSE, B. 1988. Physiologically caused changes of the separability of algal cells by flocculation and filtration. *Vom Wasser,* **70**:11-20.

MAGARA, Y., KUNIKANE, S. and AIZAWA, T. 1986. The adverse effect of extracellular products of algae on coagulation process. *Journal of Japan Water Works Association*, **55**:33-39.

MOUCHET, P. and Bonnèlye, V. (1998) Solving algae problems: French expertise and world-wide applications. *Journal Water SRT – Aqua* **47** (3) 125-141.

NIEHOF R.A. and LOEB G.I. 1972. The surface charge of particulate matter in sea water. *Limnology Oceanography*, **17**:7-11.

O'COLLA, P.S. 1962. Mucilages. In: LEWIN, R.A. (E.d.) Physiology and Biochemistry of Algae. Academic Press, New York and London. pp 337-356.

OLDHAM, W.K. and RUSH, R.J. 1978. Color removal in kraft mill wastewaters with magnesium. *J. Water Poll. Control Fed.*, **50**:875-883.

ORLITA, A. (1960). Chemical flocculation and its influence on microorganisms (in Czech). *Vodni Hosp.*, **10**:69-71.

PACKHAM R.F. 1965. Some studies of the coagulation of dispersed clays with Hydrolysing Salts. *Journal Colloid Interface Science*, **20**:81-89.

PALMER, C.M. 1980. Algae and pollution. Castle House, England.

PARKER, D.S., DE LA FUENTE, E., BRITT, L.O., SPEALMAN, M.L. STENQUIST, R.J. and ZADICK, F.J. 1975. Lime use in wastewater treatment: design and cost data. *EPA Reports,* 600/2-75-038.

PIETERSE, A.J.H. 1986 Aspects of the ecology and significance of algal populations of the Vaal River. In: The Vaal ecosystem: status and problems. Proceedings of a

Joint Symposium corvened by the Foundation for Research Development and the Vaal River Catchment Association. Occasional Report No. 5, CSIR, Pretoria.

PIETERSE, A.J.H., LANGENEGGER, O. & FOUCHÈ, L. 1993 The penetration of purification processes by algal species in a full-scale water treatment plant. Proceeding of the 1993 WISA Conference 269 – 284 pp.

REYNOLDS, C.S. 1975. *The ecology of freshwater phytoplankton,* Cambridge University Press.

RONEN, M. 1981. *Lime clarification In: Manual for water renovation and reclamation.* 2nd Ed., CSIR Technical Guide K 42.

SARTORY, D.P. 1982. *Spectophotometric analysis of chlorophyll a in freshwater phytoplankton.* Technical report TR 115, Dept. of Environmental Affairs, HRI, Pretoria.

SVORCOVA, L. 1970. Biological evaluation of laboratory coagulation experiments (in Czech). *Vodni Hosp.*, **20**:1-6.

SINSABAUGH III, R.L., HOEHN, R.C., KNOCKE, W.R. and LINKINS, A.E. 1986. Removal of dissolved organic carbon by coagulation with iron sulphate. *Jour. Amer. Water Works Assoc.*, **78**:74-82.

STANIER, R.Y., KUNISAWA, R., MANDEL, M. and COHENBAZIRE, G. 1971. Purification and properties of unicellular blue-green algae (Order Chroococcales) *Bact. Rev.*, **35**:171-205.

STEYNBERG, M.C. 1994. Pre-Oxidant. An Unit process to limit Algal-Related Water Quality and Purification Problems. Unpublished Ph.D. Thesis, University of the Orange Free State, Bloemfontein, South Africa.

STUMM, W. and MORGAN, J.J. 1981. *Aquatic chemistry.* 2nd Ed., John Wiley & Sons, New York.

TENNEY, M. and STUMM, W. 1965. Chemical flocculation of microorganisms in biological waste treatment. *J. Water Poll. Conf. Fed.*, **37**:1370-1388.

TEBUTT, T.H.Y. 1977 Principals of water quality control. Pergamon press, London.

VAN ROOYEN, J.P.G., MIENIE, C.J., ERASMUS, E., DE WET, W.J., DURAN, M. and WADMAN, I.C. 1994.. Urinary excretions of homocitric acid in propionic acidaemia: minor metabolic products of the citrate synthase aldol condensation reaction. *Clinica Chimica Acta*, **230**:91-99.

VERWEY E.J.W. and OVERBEEK, J. TH. G. 1984. *Theory of the stability of lyophobic colloids.* Elsevier, Amsterdam.

VISSER, R. 1996. Algal species penetrating unit processes in the Balkfontein Water Purification Plant. Unpublished M.Sc. Thesis, Potchefstroomse Universiteit vir Christelike Hoër Onderwys, Potchefstroom, South Africa.

WEBER, W.J., JR. 1972. *Physicochemical Processes for Water Quality Control.* Wiley-Interscience New York, p. 86.

ZOBELL C.E. 1946, *Marine Microbiology.* Waltham, Massachusets.