BIODEGRADABLE COMPOUNDS AND MICROBIAL REGROWTH IN WATER

Report to the

WATER RESEARCH COMMISSION

on the Project "Comparison of methods for determining microbiological degradable organic compounds in drinking water and the effect of different treatment procedures on the potential of water to maintain microbial growth"

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EXECUTIVE SUMMARY

1. GENERAL BACKGROUND AND MOTIVATION OF PROJECT

Rand Water supplies drinking water to more than 9 million people in an area of more than 17000 square kilometres within the Provinces of Gauteng and parts of the Free State, North West and the Mpumalanga. Rand Water abstracts almost all its raw water from the Vaal River system (Vaal Dam (78,49%), Vaal River at Lethabo (21,26%). The remaining 0.25% is abstracted from ground sources at Zuurbekom.

During the treatment/purification process, most (± 15%), but not all, of the organic compounds contained in the raw water is removed. Part of the organic compounds are present in the form of dissolved organic carbon (DOC),a portion of which, namely biodegradable organic carbon (BDOC), can be mineralised by heterotrophic micro-organisms. Assimilable organic carbon (AOC) is that part of the biodegradable organic carbon that can be converted into cell mass (bacterial growth).

The presence of BDOC and/or AOC in drinking water can give rise to bacterial regrowth in the distribution network. This bacterial growth can cause deterioration of the water quality. Bacteria from the coliform group of organisms, like *Escherichia coli*, are associated with regrowth. The presence of these organisms raise suspicion about the efficiency of the treatment processes, particularly disinfection and change in water quality, i.e. pollution, higher disinfection demands or the presence of biodegradable organic compounds.

Aeromonas and *Pseudomonas* species are also associated with bacterial regrowth. Like some coliforms, these organisms are opportunistic pathogens and if present, serious problems can occur in the industry, *e.g.* food processing, cosmetic, pharmaceutical companies.

To be able to predict bacterial regrowth in the distribution network and to recommend possible adjustments to the treatment process, it is essential to make use of reliable methods in determining the quality and quantity of biodegradable and/or assimilable organic compounds in the water.

No clarity exists on direct methods for the determination of these compounds. All the methods are based on indirect measurements which are a function of bacterial growth (the activity of the micro-organisms).

2. OBJECTIVES OF THE STUDY

2.1 Evaluation of some of the available methods to choose the most suitable method(s) to determine biodegradable organic carbon present in water.

2.2 Determination of the extent to which results, obtained with these methods, are comparable.

2.3 Evaluation of different treatment processes, with respect to the removal of biodegradable organic carbon, by using the most suitable method(s).

2.4 Determination of a possible minimum concentration of biodegradable organic carbon at which no growth of heterotrophic or coliform bacteria, especially *Aeromonas* and *Pseudomonas* species would be expected.

2.5 Investigation into the relationship between the presence of biodegradable organic carbon and the concentration of easily measurable determinands.

3. SUMMARY OF MAJOR FINDINGS AND CONCLUSIONS REACHED

3.1 **Suitable methods to determine the biodegradable organic carbon in water:**

- The methods of Van der Kooij, (using both *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX) and Werner are both suitable for use with different kinds of water, like raw water with a high concentration of biodegradable organic carbon (AOC-P17 ≈ 362 μg acetate C eq/ℓ; AOC-NOX ≈ 138.1 μg acetate C eq/ℓ; f-Factor ≈ 27.66) to low concentrations as obtained after sedimentation (AOC-NOX ≈ 33.3 μg acetate C eq/ℓ) and sand filtration (AOC-P17 ≈ 10.5 μg acetate C eq/ℓ; f-Factor ≈ 1).

- The Van der Kooij method is cheaper to perform but more labour intensive and results are only available after two to four weeks. *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX must both be used during experiments to ensure utilisation of most of the carbon sources available. If only one strain is used, valuable information can be lost and a false impression obtained about the growth potential of that specific type of water or the efficiency of a treatment process.

- The AOC analyser used for the Werner method is expensive, but analyses are less labour intensive and results are available within three to five days. The inoculum obtained from the water sample is a mixed culture and has therefore already been adapted to utilise most of the available carbon sources in that water.

- The BDOC methods of Joret-Lévi and Billen-Servais are easy to perform. Care should however be taken to work in a "DOC-free" environment to prevent DOC contamination via the air. The instrument used for the DOC analyses should be sensitive enough to record changes in μg/ℓ units when work is done at very low DOC concentrations (BDOC ≈ 0.2 mg/ℓ).

- The method that relies on ATP measurements proposed by Jago-Stanfield was found to be unreliable and is not recommended for use.

- The methods of Van der Kooij and Werner were found to be reliable and were used for the remaining part of the project.

3.2 **Possible seasonal effects on biodegradable organic carbon in water during the conventional treatment process at Rand Water:**

- Increases in the concentration of biodegradable organic carbon, measured as AOC-P17, f-Factor and DOC-Begin, occurred around the beginning of autumn (April) and during spring (October/November) in the raw water through the treatment process until after chlorination.

- The difference in trend between the AOC-P17 concentration and the f-Factor, is possibly due to the fact that only *Pseudomonas fluorescens* strain P17 was used and not *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX.

3.3 **Effect of different treatment processes on the concentration and availability of biodegradable organic carbon:**

Silica/lime vs lime/ferric chloride, high ferric chloride/low lime vs low ferric chloride/high lime and pre-chlorination vs pre-ozonation were evaluated.

- Both the concentration and availability of the biodegradable organic carbon in the raw water, were increased by each of these different treatment processes under evaluation. Although the silica/lime treatment resulted in a higher percentage (549%) increase in AOC-P17 in comparison with the other treatment processes, it was of low quality, only 1.15% increase in the μ-value of the raw water after treatment.

- Pre-ozonation resulted in an increase of up to 243% for the availability (μ-value) of the biodegradable organic carbon.

3.4 **Biodegradable organic carbon present directly after treatment vs the possible formation in the distribution network:**

Primary vs secondary disinfection, ozonation as primary disinfection and chloramination as secondary disinfection were investigated.

- Both ozonation and chloramination caused an increase in the concentration of biodegradable organic carbon in water directly after the treatment process (Total AOC $\approx$ 144.7% and 94.8%; f-Factor $\approx$ 54.4% and -3.3% respectively).

- Although ozonation as primary disinfection increased the concentration (Total AOC $\approx$ 144.7%; f-Factor 54.4%) and availability (μ-value $\approx$ 45.2%) of the biodegradable organic carbon, chlorination as secondary disinfection reduced the biodegradability of the carbon source (μ-value $\approx$ -9.6%).

- The increase caused by chloramination directly after treatment (Total AOC ≈ 94.8% and 90.6%; f-Factor ≈ -3.3% and 4.4%; μ-value ≈ 38.5% and 37.9% respectively for chlorine followed by ammonia vs ammonia followed by chlorine) seemed to be reduced as water moved through the distribution system (AOC-P17 ≈ -1.21%; f-Factor ≈ 3.3%; μ-value ≈ -4.47% after secondary disinfection to the distribution endpoint).

3.5 **Activated carbon for the removal of biodegradable organic carbon:**

- Although the concentration of biodegradable organic carbon decreased during the treatment process with GAC, the availability increased after each GAC column (μ-value ≈ 4.14% - GAC_1, 7.23% - GAC_2 and 10.21% - GAC_3). This observation may be the result of changes that took place in the GAC columns due to biological activity.

- As most of the biodegradable organic carbon was removed by the first GAC column, irrespective of its operational age (12 months in this case), a logical conclusion may be to use fresh GAC, in the first column.

3.6 **A possible minimum AOC value at which no regrowth would be expected:**

- It will be difficult to determine a possible minimum concentration of biodegradable organic carbon at which no bacterial growth can be expected, because the indigenous bacterial population present in water consists of a wide variety of species, each with its own nutritional requirements.

- Depending on what kind of species present, a specific carbon source will have different effects.

- Regarding the two organisms tested, it is clear that with both being present in the water, more regrowth problems can be expected with *Pseudomonas* than with *Aeromonas* under the same nutritional conditions.

- Organic carbon concentrations may vary by such a small margin that differences may not be detectable using standard TOC or DOC methods of analysis.

3.7 **Water treatment methods for the removal of biodegradable organic carbon:**

- Whether pre-chlorination was practised or not, the same trend was observed in treatment plants - a decrease in the concentration of biodegradable organic carbon up till sand filtration, with an increase after chlorination.

- Although chlorination resulted in an increase of the concentration, a decrease in the availability (μ-value) of the biodegradable organic carbon took place. Therefore, less bacterial regrowth should be expected.

4. REVIEW OF PROJECT IN TERMS OF OBJECTIVES

4.1 Evaluation of some of the available methods to choose the most suitable method(s) to determine biodegradable organic carbon present in water:

The methods of Van der Kooij and Werner are suitable to determine the concentration and availability of the biodegradable organic carbon compounds present in water. Changes in the concentration and availability of the carbon compounds may be detected with these methods, while it is not always the case with DOC analyses.

4.2 Determination of the extent to which results, obtained with these methods, are comparable:

The results obtained with both methods follow the same trend during the evaluation of treatment processes in practice , but not necessarily when bench tests were performed. It is also important that both cultures (*Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX) should be used in the method of Van der Kooij, to obtain a total true AOC value.

4.3 Evaluation of different treatment processes with respect to the removal of biodegradable organic carbon by using the most suitable method(s):

The effect of the different treatment processes on the removal and availability of biodegradable organic carbon, can be determined and evaluated by using either or both of these methods (Methods of Van der Kooij and Werner).

4.4 Determination of a possible minimum concentration of biodegradable organic carbon at which no growth of heterotrophic or coliform bacteria, especially *Aeromonas* and *Pseudomonas* species would be expected:

It will be difficult to determine a minimum concentration of biodegradable organic carbon that will not support bacterial growth, because the indigenous bacterial population present in water consists of a wide variety of species, each with its own nutritional requirements.

4.5 Investigation into the relationship between the presence of biodegradable organic carbon and the concentration of easily measurable determinands:

No firm relationship between biodegradable organic carbon and an easily measurable determinand could be found.

5 RECOMMENDATIONS FOR FUTURE RESEARCH

5.1 Both the BDOC methods should be evaluated, with the assurance of continuous DOC analysis being done on a sensitive instrument capable of detecting low concentrations DOC (BDOC $\approx$ 0.2 mg/ℓ).

5.2 A continuous monitoring programme should be initiated to evaluate the concentration and availability of biodegradable organic carbon in the water from the raw water intake through the treatment process at different stages, taking into consideration parameters like rainfall, temperature and water source. The methods of Van der Kooij and Werner should be used, making use of both cultures for the Van Der Kooij method.

- These results may give an answer to the problem of increasing bacterial counts during summer and after heavy rainfall.

- Depending on the changes following each unit treatment process, modifications to that process may be recommended to improve the removal of, or decrease the formation of biodegradable organic compounds at that point of treatment.

5.3 The effect of the different unit treatment processes and chemicals (silica/lime, lime/ferric chloride, pre-chlorination, pre-ozonation) was evaluated by means of bench tests, without being able to use the same retention time as in practise. Therefore analyses should be done on an operational plant where these treatment processes are in use to determine the actual effect of these treatment processes on the concentration and/or availability of biodegradable organic carbon present in the water.

5.4 The effect of pre-ozonation on the concentration and availability of biodegradable organic carbon should be investigated under operational conditions in a water treatment plant.

5.5 A long term investigation should be initiated to determine the relation between the potential of GAC in removing biodegradable organic carbon during the water treatment process and the frequency of reactivation required to obtain maximum removal.

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LIST OF SYMBOLS AND ACRONYMS AND NOTES ON TERMINOLOGY

AOC Assimilable organic carbon (AOC) is that portion of the biodegradable organic carbon that can be converted to cell mass and expressed as a carbon concentration by means of a conversion factor or calibration.

DOC Dissolved organic carbon - the fraction of TOC that passes through a 0,45-μm-pore-diam filter.

TOC Total organic carbon - all carbon atoms covalently bonded in organic molecules.

BDOC Biodegradable dissolved organic carbon (BDOC) is that portion of organic carbon in water that can be mineralized by heterotrophic microorganisms.

Regrowth Bacterial regrowth is commonly used to describe the phenomenon of bacterial growth in treated water, typically in the distribution system.

Van der Kooij method Determination of AOC in water samples by measuring the growth of *Pseudomonas fluorescens* strain P17 and a *Spirillum* sp. strain NOX.

Werner method The basis of this method is the demonstrated correlation between turbidity and total bacterial cell number, despite changes in cell morphology and size during different growth phases.

μ-value Is the rate of increase in cells/time unit. It is calculated from the slope of the (logarithmic) growth curve during the logarithmic growth phase of the bacteria. It is a parameter for substrate quality (biodegradability).

f-Factor It is the ratio of biomass (=turbidity) at the end of an experiment to that of the beginning. It is a parameter for substrate quantity.

RDOC Refractory dissolved organic carbon (RDOC) is that portion of the organic carbon in water that is resistant to chemical or biological oxidation, therefore it cannot be utilized by the micro-organisms.

AOC-P17 AOC concentration when using only the *Pseudomonas fluorescens* strain P17 in the Van der Kooij method.

AOC-NOX AOC concentration when using only the *Spirillum* strain NOX in the Van der Kooij method.

TOTAL AOC AOC-P17 + AOC-NOX - both strains were used simultaneously in the Van der Kooij method (AOC [P17 plus NOX]).

AOC [ ] (μg acetate C eq./ℓ) Assimilable organic carbon concentration expressed as microgram acetate carbon equivalents per litre.

DOC-Begin DOC value at the beginning of the experiment, before inoculation (Werner method).

DOC-End DOC value at the end of the experiment (Werner method).

d-DOC d-DOC = DOC-Begin - DOC-End ---> that part of the DOC that is assimilable or biodegradable.

Initial DOC DOC value of samples as been taken at the sample points.

GAC Granular activated carbon.

cfu/mℓ A bacterial plate count expressed as colony forming units per milliliter.

Nmax Maximum colony count reached by an organisms during logarithmic growth.

Δ DOC See d-DOC.

Min Minimum value for a set of results.

Max Maximum value for a set of results.

Avg Average value for a set of results.

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Rand Water supplies drinking water to more than 9 million people in an area of more than 17000 square kilometres within the Provinces of Gauteng and parts of the Free State, North West and the Eastern Transvaal. Rand Water abstracts almost all its raw water from the Vaal River system (Vaal Dam (78,49%), Vaal River at Lethabo (21,26%) and from the Barrage reservoir 0,25%).

During the treatment/purification process, most (± 15%), but not all, of the organic compounds contained in the raw water is removed. Part of the organic compounds are present in the form of dissolved organic carbon (DOC). A part of the DOC, namely biodegradable organic carbon (BDOC), can be mineralised by heterotrophic micro-organisms. Assimilable organic carbon (AOC) is that part of the biodegradable organic carbon that can be converted into cell mass (bacterial growth).

The presence of BDOC and/or AOC in drinking water can give rise to bacterial regrowth in the distribution network. This bacterial growth can cause deterioration of the water quality. Bacteria from the coliform group of organisms, like *Escherichia coli*, are associated with regrowth. The presence of these organisms raise suspicion about the efficiency of the treatment processes, particularly disinfection and change in water quality, i.e. pollution, higher disinfection demands or the presence of biodegradable organic compounds.

Aeromonas and *Pseudomonas* species are associated with bacterial regrowth. Like some coliforms, these organisms are opportunistic pathogens and thus serious problems can occur in the industry, *e.g.* food processing, cosmetic, pharmaceutical companies.

To be able to predict bacterial regrowth in the distribution network and to recommend possible adjustments to the treatment process, it is essential to make use of reliable methods in determining the quality and quantity of biodegradable and/or assimilable organic compounds in the water.

No clarity exists on direct methods for the determination of these compounds. All the methods are based on indirect measurements which are a function of bacterial growth (the activity of the micro-organisms).

The main objective of this study was to find the most suitable method(s) for determining BDOC and/or AOC in water and the applicability of those methods. By using the chosen methods, different water treatment processes were evaluated with respect to the removal of, or change, in these biodegradable compounds. Determinations were done to try and find the minimum concentration of biodegradable or assimilable compounds at which level no growth of heterotrophic and/or coliform bacteria will be expected. The possible

relationship between easily measurable determinants (*e.g.* DOC) and the presence of biodegradable and/or assimilable compounds was also investigated.

1.2 LITERATURE STUDY

1.2.1 Bacterial regrowth and the link to AOC

Under certain conditions the quality of drinking water may change to such an extent during its stay in the distribution system of water mains that the water works are faced with technical problems and with possible consumer complaints (Van der Kooij, 1978). Micro-organisms play an important role in this quality change, especially when they multiply considerably inside the distribution system (Van der Kooij, 1978), the so-called after growth or regrowth. The problems associated with microbial regrowth in the distribution system include:

- development of organisms visible with the naked eye,
- interference with bacteriological quality control,
- intensified degradation of the materials from which the water mains are constructed, particularly cast iron, by creating anaerobic conditions and reducing pH in a limited area,
- and deterioration of taste, odour and colour of drinking water, (Van der Kooij *et al.*, 1982a; Kaplan and Bott, 1988; Van der Kooij and Hijnen, 1990; Kaplan and Bott, 1990; Van der Kooij, 1992) and increased chlorine demand (Kaplan and Bott, 1990).

Regrowth is largely associated with heterotrophic bacteria, some of which are opportunistic pathogens (Kaplan and Bott, 1988 and Kaplan and Bott, 1990), which may pose a threat to public health - *e.g. Pseudomonas aeruginosa, Mycobacterium kansassi* and *Klebsiella pneumoniae* (Van der Kooij *et al.*, 1982a). Recent findings indicate that *Legionella pneumophila* proliferates in hot water systems (Van der Kooij *et al.*, 1982a).

More than half a century ago it was already argued that regrowth of bacteria in drinking water should be limited by reducing or controlling conditions that may support microbial regrowth or by maintaining a free chlorine residual in the distribution system (Van der Kooij *et al.*, 1982a; Van der Kooij and Hijnen, 1990). These conditions to be controlled, include the retention time of the water in a distribution system, temperature, disinfection efficiency and the nature and concentration of biodegradable compounds in freshly prepared drinking water, the release of biodegradable compounds from sediments and from materials used to construct the distribution system (Van der Kooij *et al.*, 1982a; Kaplan and Bott, 1988; Kaplan and Bott, 1990).

Although free chlorine represses regrowth, considerable chlorine dosage is required to maintain a residual throughout the system, resulting in the formation of chlorinated organic compounds (trihalomethanes) (Van der Kooij *et al.*, 1982a; Van der Kooij and Hijnen, 1990; Van der Kooij, 1992). Therefore, reducing the concentration of biodegradable compounds in water to sufficiently low levels, as well as using construction materials that do not release biodegradable compounds into the water, is important in controlling regrowth (Van der Kooij *et al.*, 1982a).

Micro-organisms can only multiply in the water if the compounds required for their growth are present in sufficient concentrations. These compounds should provide all the elements (*e.g.* C, H, O, N, P, S) needed in synthesising biomass and to satisfy the energy requirements of the cells (Van der Kooij, 1978; Van der Kooij *et al.*, 1982a). The organisms can be classified by the type of compounds they use as a source of energy (inorganic or organic hydrogen), the carbon source (inorganic or organic carbon) and the type of hydrogen acceptor (oxygen [O_2]; nitrate [NO_3^-]; sulfate [SO_4^{2-}]; carbon dioxide [CO_2] or organic carbon) (Van der Kooij, 1978; Van der Kooij *et al.*, 1982a). The bacteria responsible for most of the regrowth in the distribution system are heterotrophic, meaning that they use organic carbon compounds as carbon and energy sources and make use of oxygen as the hydrogen acceptor (Van der Kooij, 1978; Van der Kooij *et al.*, 1982a).

The ratio derived from the gross composition of their biomass (dry matter) gives an indication of the variety of nutrients needed by micro-organisms. This gross composition is : $C_5H_7NO_2P_{1/30}$; in which C : N : P = 60 : 14 : 1. Approximately 50% of the organic carbon taken up during growth of micro-organisms, is converted into CO_2 (dissimilation) to supply energy to the cells. The remaining 50% is bound into new cellular material (assimilation). Therefore the ratio in which compounds are needed, is approximately 100 C : 10 N: 1 P (Van der Kooij, 1978; Van der Kooij *et al.*, 1982a; Le Chevallier *et al.*, 1991).

The distribution system is continuously supplied with water containing organic and inorganic compounds (Van der Kooij, 1992). The dissolved organic matter is most often quantified by the measurement of carbon as DOC, a relatively easy measurement (Kaplan and Bott, 1988). The heterogeneous mixture of DOC in water supplies ranges in complexity from large molecules of humic, fulvic and hydrophilic acids, to relatively simple compounds such as carbohydrates, carboxylic acids, amino acids, and hydrocarbons (Kaplan and Bott, 1990). These simple compounds are the part that is most susceptible to microbial decomposition and have been referred to as biologically labile DOC. This concept of labile DOC has been applied to drinking water studies as the AOC concept (Kaplan and Bott, 1990). This AOC is also known as that portion of the total organic carbon (TOC) which can be metabolised rapidly by micro-organisms (*i.e.* is biodegradable) and converted to cell mass (Le Chevallier *et al.*, 1991; Huck *et al.*, 1990; Huck *et al.*).

According to Kaplan and Bott (1990) the need to quantify AOC has arisen partly because an easily quantified chemical parameter (TOC) has not been found to be a good predictor of bacterial regrowth. This failure is understandable because the ratio of AOC to TOC is not constant. AOC must be viewed as only one variable in a complex regrowth equation. The ability to quantify AOC will be one step in the process of understanding and eventually predicting when and where regrowth will occur.

1.2.2 Available methods

Huck (1990) has given the following definitions:

- Biodegradable organic carbon (BDOC) is that portion of the organic carbon in water that can be mineralized by heterotrophic micro-organisms. With the test procedures used, this biodegradation period could last up to about one month.

- Assimilable organic carbon (AOC) is that portion of the biodegradable organic carbon that can be converted to cell mass and expressed as a carbon concentration by means of a conversion factor or calibration.

- The term bacterial regrowth is commonly used to describe the phenomenon of bacterial growth in treated water, typically in the distribution system. Although it might be preferable to use the term bacterial growth, the terms regrowth and regrowth potential have become established in the literature.

The available methods can be classified according to the purpose for which the measurement is being made, namely (1) to assess the removal of dissolved organic compounds by a treatment process, or (2) to predict the potential of a finished water to support the growth of a microorganism. It may also not be the same parameter that will be measured. If bacterial regrowth is of concern, bacterial biomass should be the parameter to be measured. AOC will be an appropriate term for the organic matter producing this growth. On the other hand, DOC will be a more closely related parameter if the reduction in chlorine demand or disinfection by-product formation potential is of concern. In this case the appropriate term would be BDOC (Huck, 1990). Table 1.1 presents the classification of the methods by objective.

Table 1.1: Classification of methods by objective

	Objective	Parameter	Term
1.	Bacterial regrowth	Biomass	AOC
2.	Chlorine demand, or disinfection by-product formation potential	DOC	BDOC

A further classification can be made in terms of the parameter measured in the assay - biomass or DOC.

1.2.2.1 Biomass - based methods

All the biomass-based methods are based on the assumption of BDOC being the limiting nutrient for growth. A further classification can be made on these methods according to the type of inoculum been used - either one or more known organisms, or the indigenous bacteria from the water being tested. According to Huck (1990), the methods of Van der Kooij, Kemmy *et al.*, and the US Environmental Protection Agency (USEPA) use known organisms, whereas those of Werner, Jago-Stanfield and Billen-Servias use indigenous (unknown) organisms. The advantages of a known organism are that the inoculum does not change over time, the results may be compared with those of other workers, and in some cases inferences may be made about the type of substrate (Huck, 1990). Against this, an inoculum of indigenous organisms provides an assay that is more representative of conditions existing in practice.

Colony forming units (cfu) is the most commonly used measure of biomass. In all the assays, except those of Werner and USEPA, biomass is converted to an organic carbon concentration by means of a factor that is either measured or assumed, depending on the method (Huck, 1990).

In Table 1.2 are some of the biomass-based methods listed with the applicable parameter being measured.

Table 1.2: Measured of biomass (Huck, 1990)

Parameter	Method
Colony forming units	Van der Kooij, Kemmy *et al.*, USEPA
Turbidity	Werner
ATP	Jago-Stanfield
Cell numbers and volume	Billen-Servais

Table 1.3 summarises the principal test conditions of each of the biomass-based methods (Huck, 1990).

Table 1.3. Comparison of procedures - Biomass based methods (HUCK, 1990)

Method	Sample Preparation	Source of Inoculum	Incubation Time *Days*	Temp. *°C*	Parameter Measured	Calibration	Expression of Results
van der Kooij	Pasteurization	Pure cultures (P17 and NOX)	Up to 20	15	cfu/mℓ	Sodium acetate	AOC (μg/ℓ) acetate equivalents
Kemmy *et al*	Filter sterilization	Four species (*P.fluorescens Curtobacterium* sp., *Corynebacterium* sp., unidentified coryneform)	6	20	cfu/mℓ	Mixtures of organic compounds	AOC (μg/ℓ)
USEPA (coliform growth response)	Filter sterilization	Three coliform organisms. (*E. coli, Enterobacter cloacae,* and *Klebiella oxytoca*)	5	20	cfu/mℓ	None	CGR = log (N_5/N_0)
Werner	Filter sterilization	Sample	2.5 or 5	Approx. 20	Slope and height of curve	None	μ (growth rate); log (y/y_0)(amount of substrate)
Jago-Stanfield	Filter sterilization	Raw water of treatment plant or distribution system	Until maximum ATP concentration reached	20	ATP	Standard conversion factor (assumed)	AOC (μg/ℓ)
Billen-Servais	Filter sterilization	Treatment plant	10-30	Approx. 20	Bacterial number and size	See text	BDOC (mg/ℓ)

From the comparison done by Huck (1990), it is clear that some of the biomass-based methods are quite similar in one or more procedural aspects. With respect to the difference regarding the inoculum, there was insufficient information available to conclude whether one approach is superior to the other. The methods of Kemmy *et al.*, (1989) and the USEPA (coliform growth response) is almost similar to the Van der Kooij method, except that more species or different organisms were used from that in the Van der Kooij method. The other main difference between these three methods is that in sample preparation, pasteurization is used in the Van der Kooij method, while filter sterilization is used for the other two methods.

The Jago-Stanfield method is conceptually similar to that of Werner, although each uses a different measure of bacterial growth (Table 1.3) (Huck, 1990). The Billen-Servais method represents a completely different approach from the other biomass-based methods. Although it potentially offers more information than the other assays, it is more labour intensive and expression of the final results relies on several coefficients that must either be assumed or measured experimentally (Huck, 1990; Servais *et al.*, 1987).

Concerning the major procedural difference between the Van der Kooij method and the other methods (*i.e.* pasteurization *vs* filter sterilization in sample preparation), Huck (1990) was not aware of data that demonstrated the possible changes to AOC due to pasteurization. As contamination due to leaching of AOC can occur during filter sterilization, cellulose-based filters should therefore not be used, while polycarbonate filters should be copiously rinsed with AOC-free deionized water. Difficulty in filtering turbid raw water samples is confirmed by the same experience of Huck (1990).

Time required for the assay is one of the procedural differences that exhibits the greatest variation among the methods. Although the purpose for which biodegradable measurements is used does not require an extremely rapid test, an assay needing only a few days, is obviously more attractive than one requiring much longer periods of time (Huck, 1990).

Capital cost and labour requirements must also be considered for the methods. Although cost data is not readily available for any of the methods, methods with the lowest capital cost (*i.e.* those requiring the least specialized equipment) are those that use colony forming units as measured parameter. These methods can be used by any utility with a microbiology laboratory. The Werner method requires a "mAOC-Analyzer" consisting of a computer with hard disk and disk drive, printer, data acquisition and control unit (DACU), control unit for stirring apparatus and a turbidimeter unit containing four turbidimeters (Link, Hartmann, Eberhagen, Hambsch, 1992). The Jago-Stanfield method requires a luminometer (Stanfield and Jago, 1989) to measure ATP concentrations and the Billen-Servais method, an

epifluorescence microscope. Sample preparation time for the Werner method is very short. Even the time required for ATP measurements (Jago-Stanfield method) should be considerably less than that for determinations of colony forming units.

1.2.2.2 DOC-based methods

In Table 1.4 a comparison of the DOC-based methods is given by Huck (1990).

The Billen-Servais method uses the change in DOC concentration over time (28 days) to give an indication of the BDOC content of the water (Servais *et al.* 1989). The Joret-Lévi method also uses the change in DOC concentration to give the BDOC value of the water, but over a shorter period (3 to 5 days) (Joret, *et al.*, 1991). In both methods the measured parameter is DOC and was measured with a Dohrman 80 Total Carbon Analyser (Servais, *et al.*, 1987 and 1989, Joret *et al.*, 1991). The main difference between the two methods is in the use of a support medium in the Joret-Lévi method, which greatly increases the amount of biomass present (Huck, 1990). Although this shortens the test period, it raises the possibility of release of organic matter from this biomass (despite pre-washing) or adsorption of organic matter from solution by the biomass. At that stage, according to Huck (1990), this was the only assay with published results that deliberately attempts to use a biofilm. Huck (1990) also stated that an assay incorporating a biofilm is more representative of treatment processes and perhaps of distribution systems than the suspended growth methods are.

The long duration of the Billen-Servais (BDOC) test could allow for the development of a succession of bacterial populations that would not occur in a treatment plant, or at any given point in a distribution system (Huck, 1990).

An important limitation of these DOC-based methods are their applicability to water with low BDOC levels. According to Servais *et al.*, (1989) their method will be helpful for BDOC concentrations greater than 0.2 mg/ℓ. The accuracy and precision of low level DOC measurements is dependent on factors such as the type of instrument and possible contamination in the laboratory. A difference in DOC concentration of approximately 0.1 or 0.2 mg/ℓ could not be detected reliably by many laboratories (Huck, 1990).

At a later stage another method for measuring BDOC in water was published by Frias, *et al.*, (1992). This method differs from the Joret-Lévi method in the sense that a column with inert support medium was used and colonisation of the medium was induced by recirculation of a mixture of $^1/_3$ river water plus $^2/_3$ of granular activated carbon filtered water. After the colonisation has taken place, the actual experiment can be started by circulating the test water sample through the column and measuring the DOC values on a daily basis, until a constant minimum DOC is reached.

- BDOC = initial DOC - minimum DOC.

As this method was not available beforehand, it was not taken into consideration when choosing methods in the planning phase of this project.

Table 1.4: Comparison of procedures - DOC based methods (HUCK, 1990)

Method	Sample Preparation	Source of Inoculum	Incubation Time *Days*	Temp. *°C*	Parameter Measured	Calibration	Expression of Results
Billen-Servais	Filter sterilization	Water from same environment as sample	28	20 $\pm$ 0.5	DOC	None	d-DOC=BDOC
Joret-Lévi	None	Biologically active sand from a water treatment plant that does not use prechlorination	Until no change in DOC	Approx. 20 (room)	DOC	None	d-DOC=BDOC

1.2.2.3 Choice of methods used in the project

It would be ideal to have an automated method where a calibrated instrument could be used to perform the desired analysis.

Therefore, although each method has its own advantages and disadvantages, it was decided mainly to use the Werner method and to compare it to some of the other methods for this study. The other methods were those of Van der Kooij and Jago-Stanfield (Biomass-based) and the two DOC based methods of Joret-Lévi and Billen-Servais.

1.2.3 Field of application

It is clear from the previous discussions that water with levels of biodegradable organic matter below approximately 0.2 mg/ℓ, will require a biomass based approach to obtain sufficient sensitivity. It is important to keep in mind that the biomass and DOC based assays measure different parameters. Although the parameters are related, the use of both types of assays will provide complementary information. If only one assay is to be used the choice will be based on the objectives of the measurement (Huck, 1990).

In using the Werner method, bacterial growth curves are measured and with that information available, it is possible to make a statement on the substrate quality of a water. The growth registered, is shown by the amount of biomass built up by the specific substrate. It is also possible to take samples for DOC analysis which will reveal the amount of substrate in the water by means of the $\triangle$DOC values. Using the described method, the assessment of water quality depends on biomass increase and DOC decrease (Hambsch and Werner, 1990). For the distribution system, biomass increase is more important (giving an indication of the regrowth potential in that system), whereas the DOC decrease helps to evaluate treatment steps (Werner and Hambsch, 1986). Since this method allows both possibilities, it guarantees the maximum information (Hambsch and Werner, 1990). The high sensitivity of the turbidity measurements is of great importance since the growth can be seen, even if the DOC is not measurably degraded. This method can , therefore, be of great help especially in controlling denitrification plants, because traces of a substrate like acetate would have a strong effect on the growth.

With the van der Kooij method it is possible to establish the effect of a water treatment process on the AOC concentration. Making use of that knowledge, the purification process can be optimised (Van der Kooij, 1978) to produce a water with a low AOC concentration.

The Jago-Stanfield method, using ATP as a parameter to determine the AOC concentration in water, can also be used to evaluate different treatment procedures (*e.g.* ozonation, slow sand filtration) and the treatment process as a unit (Stanfield and Jago, 1989). According to Le Chevallier *et.al.* (1993), care should be taken when intensely coloured water is analysed, for interference can occur with the ATP assay. By using an internal ATP standard, compensation for this effect can be obtained.

According to Servais *et al.* (1989), their method will be helpful for BDOC concentrations higher than 0.2 mg/ℓ. This method can also be used in the study of natural aquatic ecosystems, especially river water and for treatment plant design, more particularly for studying various operating conditions of biological granular activated carbon filters.

The BDOC method of Joret-Lèvi can also be used to evaluate treatment steps and the process. According to Joret *et al.* (1991), it is sufficiently sensitive even for distributed water. The main problem in using these BDOC methods, lies with the sensitivity of the DOC analysis.

1.2.4 Effect of water treatment on AOC or BDOC

In a water treatment plant, the removal of dissolved organic matter is controlled by chemical addition (oxidants and coagulants), rapid and slow mixing processes, particle deposition, sorption and biodegradation in the settling tanks and sand filtration. The type and dose of any pre- or intermediate oxidants and the types and doses of coagulants affect the nature of dissolved and particulate organic substances, their incorporation into particles which may be removed by filtration and their removal by sorption or biodegradation (Tobiason *et al.*, 1993).

According to work done by Kaplan and Bott (1990), the AOC concentrations were generally lower in ground water than in surface water, ranging from 48 to 607 μg C/ℓ. In work done by Kaplan and Bott (1990) (Van der Kooij - AOC method - only P17 used) and Gibbs *et al.*, (1993) (ATP method of Jago and Stanfield), no seasonal patterns were found in the AOC concentration of raw surface waters. In contrast, Huck *et al.*, (1991) found that the AOC levels (as determined by the AOC method of Van der Kooij with strains P17 and NOX added simultaneously) of raw water, varied seasonally with average values just above 100 μg acetate C eq/ℓ in summer, approximately 40 μg/ℓ in the autumn - winter period and more than 200 μg/ℓ in spring. These seasonal differences had measurable effects on the AOC concentrations that were observed after various treatment processes (Huck *et al.*, 1991).

Reduction in the AOC level can be achieved by coagulation, flocculation and sedimentation (Van der Kooij and Hijnen, 1984 and Huck *et al.*, 1991). Huck *et al.* (1991), found a removal of AOC that varied between 80% and zero %, with a median removal of approximately 38%. They also found that the percentage removal of AOC-P17 was slightly higher than that of the total AOC (Total AOC = AOC-P17 + AOC-NOX).

It was observed that filtration (rapid sand, slow sand and granular activated carbon [GAC] filtration) caused a reduction in AOC levels (Van der Kooij and Hijnen, 1984). This reduction is caused by the consumption of biodegradable compounds by bacteria present in these filters (AOC removal) or adsorption to the filter media (DOC removal). Free chlorine in the influent to sand filters, affects these bacteria, resulting in a poor AOC reduction (Van der Kooij and Hijnen, 1984; Van der Kooij, 1987).

With fresh filter media (slow sand filters or GAC filters), most of the organic carbon removal takes place by adsorption, therefore most of the DOC and little of the AOC are removed in this stage. At a later stage when colonization of the filter media has taken place, more AOC is removed due to this higher biological activity. AOC reduction with GAC filtration decreased after extended periods of use (Van der Kooij, 1987).

Bonnet *et al.* (1992), observed that combined contact coagulation, coagulation on the filter and slow sand filtration allow the removal of an average 40% of DOC from the raw water and 84% of BDOC from the raw water by coupling pre-treatment (pre-ozonation) and slow sand filtration. Ozonation, after the slow sand filtration, increased the BDOC content. These BDOC values were very low and a combination of ozonation and GAC filtration, resulted in very small BDOC amounts (0.1 - 0.2 ppm) which could hardly be detected (Bonnet *et al.*, 1992). In work done by Servais *et al.*, (1992) in a pilot study, using biological GAC as a filter between the sand filtered ozonated water and the final chlorination step, BDOC reduction of more than 50% from the inlet to the outlet of the GAC filter was achieved. It was also shown that as far as design is concerned, deep GAC filters (3m) with a rapid filtration velocity (up to 18 m/h) seem to be as efficient as most GAC filters with a depth of 1 m and a filtration velocity around 6 m/h.

Ozone is used in water treatment to remove colour, taste, odour and as a disinfectant (Volk, *et al.*, 1993.b). Ozonation during water treatment increases the biodegradability of the organic compounds present in the water (Van der Kooij *et al.*, 1989, Volk *et al.*, 1993a).

The fractionation of the large molecules into low molecular weight compounds by O_3 is the main reason for this increased biodegradability, therefore distribution of ozonated water results in an increased number of bacteria in that water (after growth), even when it was chlorinated at the final stage (Van der Kooij *et al.*, 1989). The observation was made that ozonation in water treatment caused a significant increase of the AOC concentration (Van der Kooij *et al.*, 1989; Van der Kooij and Hijnen, 1984; Van der Kooij, 1987; Van der Kooij *et al.*, 1982; Hijnen and Van der Kooij, 1992; Janssens *et al.*, 1984; Tobiason *et al.*, 1993).

In a comparison study done by Bonnet *et al.*, (1992) between an existing treatment plant and a pilot plant (see diagram of both systems), it was found that pre-ozonation increased the BDOC level in the pilot plant.

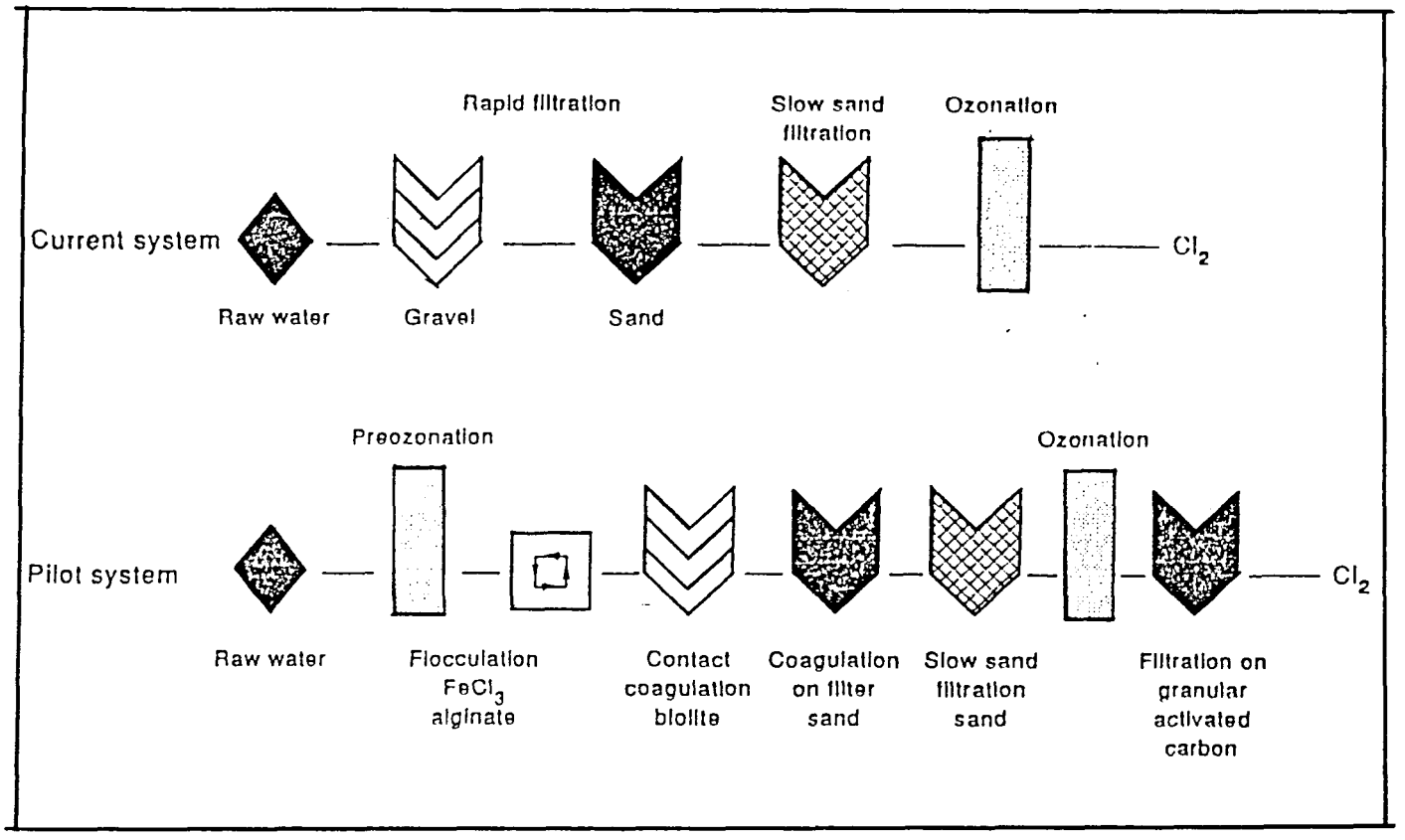


Diagram 1: Process of both systems (Bonnet *et al.*, 1992).

During laboratory experiments done by Van der Kooij *et al.*, (1989), it was found that an increasing ozone dosage caused an increase in the AOC concentration. With an ozone dosage of up to about 3 mg/ℓ, a linear relationship exists between AOC concentration and ozone dosage (Van der Kooij, 1987). Volk *et al.*, (1993a) showed in dosage/time studies done with ozone, that the highest concentration of BDOC was produced with a high dosage and a short contact time (5 minutes with an applied ozone dose of 0.25 - 0.5 mg O_3/mg C). Their results also show that higher dosages have little effect on the biodegradability of the organic matter.

In the AOC method of Van der Kooij, two different types of bacteria can be used, namely *Pseudomonas fluorescens* strain P17 and *Spirillum* strain NOX. Ozonation causes an increase in the AOC concentration as determined with strain P17 (AOC-P17), while strain NOX was still able to multiply in the ozonated water after P17 had reached its maximum colony count. This observation by Van der Kooij (1987) shows that certain ozonation by-products (*e.g.* oxalic, glyoxylic and formic acids - Van der Kooij and Hijnen, 1984) were not utilized by P17. Oxalate is relatively resistant to further oxidation by ozone and may be one of the compounds responsible for growth of strain NOX (Van der Kooij, 1987).

Chlorination may affect the AOC concentration of water by inhibition of microbiological processes in filters and by the production of biodegradable compounds from large molecules, which is particularly important when chlorination is the last treatment step, *e.g.* post chlorination (Van der Kooij, 1987). Data concerning the effect of post chlorination on the AOC concentration indicate that AOC values were doubled by this treatment in a number of situations (Van der Kooij and Hijnen, 1984; Van der Kooij, 1987). According to Van der Kooij (1987), the formation of assimilable carboxylic acids by chlorination, is much less than the formation of such compounds by ozonation.

Van der Kooij and Hijnen (1984) found that despite the doubling in AOC concentration caused by chlorination, there was, in most cases, a reduction in the average relative biodegradability. It was also found that after dosage of chlorine and chlorine dioxide, strain P17 did not multiply within a period of one month. Thereafter, growth was observed with higher Nmax values than before disinfection. Chlorine dioxide or its reaction products seem very persistent and may therefore be effective in repressing regrowth in water during distribution.

A decrease was observed in the AOC concentration of ozonated water during distribution, even with chlorination at the final stage of treatment (Van der Kooij, 1989). Kaplan and Bott (1990), LeChevallier *et al.* (1991), and Huck (1990) also found that the AOC level remained relatively constant, or declined, as the water moved through the distribution system. Although it was expected that AOC concentration would decrease through the distribution system, Gibbs *et al.* (1993), could not find any relationship between AOC concentration (as was determined by the ATP method of Jago and Stanfield) and retention time. According to Van der Kooij (1989) the greatest reduction in AOC levels was observed with the highest initial AOC value and took place in the first part of the distribution system.

CHAPTER 2

SUITABLE METHODS TO DETERMINE THE BIODEGRADABLE ORGANIC CARBON IN WATER

Water samples from different the stages in the purification process were used to compare the available five methods with regard to,

- the feasibility of the method,
- the reproducibility of the method,
- and the suitability to use a specific method for AOC determination on water samples of varying quality

The water quality varied from raw water with a turbidity of 150 NTU, DOC content of 3.2 mg/ℓ and chlorophyll a of 7.28 to drinking water with a turbidity of 0.43 NTU, DOC of less than 2.0 mg/ℓ and chlorophyll a of 0.12 (Appendix F, Table F1).

2.1 WATER SAMPLES

Water samples were collected at different positions as been indicated on Diagram 2.

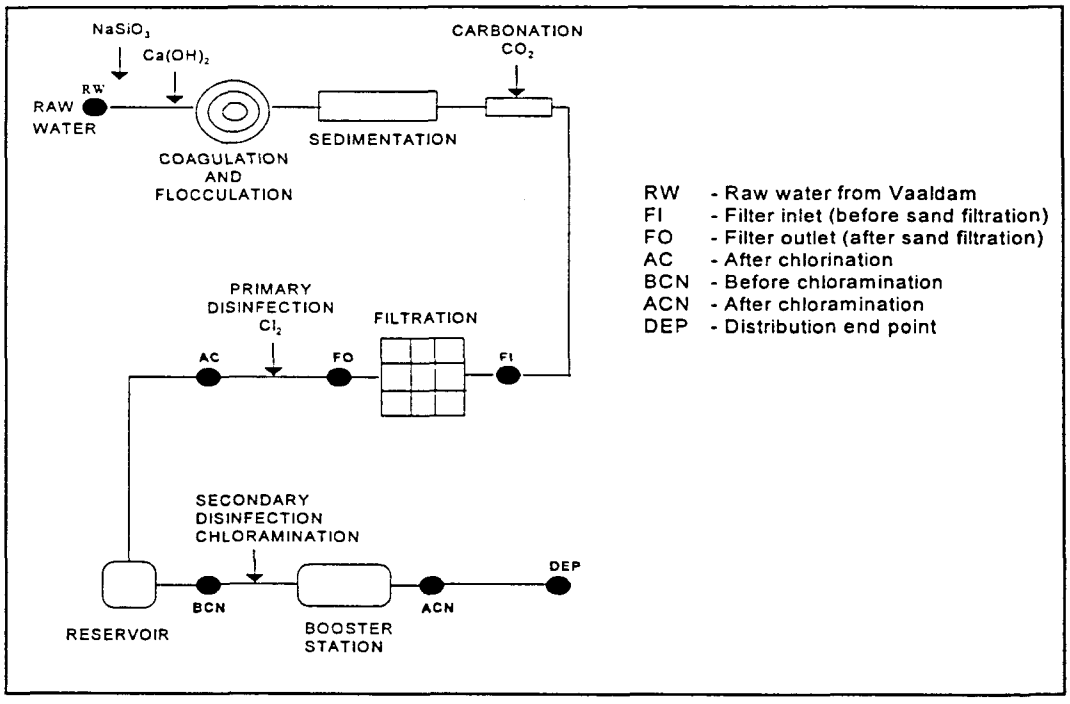


Diagram 2: Flow diagram of the treatment process and sample points.

The following samples were analysed:

RW - raw water from Vaal Dam
FI - water after flocculation and sedimentation before sand filtration (Filter inlet)
FO - water after sand filtration (Filter outlet)
AC - water after chlorination
ACN - water after chloramination

One sample was taken at a time, split into the specific volumes required for each method and analysed according to the method as been described. (Apendix A to E).

2.2 METHODS

The methods were compared by analysing the different samples successively over a period of five months from 05-07-93 to 15-11-93. Each sample was analysed in triplicate with each test run.

The following methods were used:

A. Method of Werner: Determination of bacterial dynamics - growth rate (μ) and growth factor (f).
B. Method of Van der Kooij: AOC concentration as μg acetate C equivalents/ℓ. Only *Pseudomonas fluorescens* strain P17 was used and the inoculum was prepared in tap water with acetate added.
C. Method of Jago-Stanfield: ATP determination was used as an indication of the concentration of assimilable organic carbon in water.
D. Method of Joret-Lévi: BDOC
E. Method of Billen-Servais (dDOC): BDOC

2.3 RESULTS

Correlation coefficients were calculated between methods on each of the different samples tested.

In Table 2.1, the actual results are given with the calculated correlation coefficients in Table 2.2. Correlation coefficients ≥ 0.5 are typed in bold digits.

The minimum/maximum range for the results obtained during the test period, is summarised in Table 2.3.

In Figure 2 a comparison of the average measured parameters from each method at the different sample points are illustrated. The method of Jago-Stanfield was not taken into account, due to problems experienced.

Table 2.1 : COMPARISON OF METHODS
Results obtained during the test period.

SAMPLE	DATE	AOC-P17	f-FACTOR	µ-VALUE	B/S-BDOC	J/L-BDOC
RW	05/07/93	34.81	3.84	0.1028	-0.7	-0.3
		127.63	7.31	0.1965	-0.4	0.3
		171.72	1.85	0.082	-0.8	0.4
	09/08/93	44.09	2.45	0.0701	1.4	0.4
		41.77	2.38	0.0742	1.4	0.2
		53.37	2.35	0.0686	1.4	0.3
	13/09/93	97.46	19.2	0.23	1.6	-0.3
		90.5	20.46	0.2682	2.6	1.4
		153.15	26.01	0.2771	2.7	1.3
	18/10/93	111.39	7.18	0.1913	-1.2	0.6
		116.03	4.99	0.149	-0.1	0.4
		141.55	4.3	0.1587	0.4	0.3
FI	12/07/93	20.19	2.58	0.0668	1.3	1.3
		14.39	2.78	0.078	1.2	1.3
		14.62	2.94	0.0818	1.2	0.9
	16/08/93	37.13	2.35	0.0516	0.4	0.1
		37.13	2.38	0.0576	0.9	-0.3
		34.81	2.47	0.0666	0.9	-0.2
	20/09/93	220.45	2.51	0.1334		1.3
		153.15	2.65	0.1544	NRA	-0.1
		139.23	2.67	0.1622		-0.1
	25/10/93	67.3	21.66	0.1497	-0.1	1.2
		69.62	3.31	0.1975	-0.3	0.2
		83.54	5.33	0.1013	-0.9	1.2
FO	19/07/93	12.76	2.59	0.0817	0.2	1
		17.4	2.62	0.0683	0.8	1.1
		17.17	3.26	0.0807	0.7	0.8
	23/08/93	10.44	1.88	0.0539	-1.8	-0.2
		8.82	1.91	0.0566	-2.9	-0.3
		12.99	1.83	0.0597	-1.6	-0.6
	27/09/93	4.87	1.75	0.1161	-0.3	-0.4
		2.78	1.62	0.105	-0.8	0.2
		3.25	1.59	0.1262	-0.6	-0.6
	01/11/93	48.73	4.01	0.1134	1.1	2
		90.5	4.66	0.174	1	-0.5
		67.3	4.13	0.1573	1	-0.2
AC	26/07/93	27.85	1.75	0.0513	0.1	0
		39.45	1.73	0.0567	-0.3	1
		16.48	1.71	0.0659	0.3	0.9
	30/08/93	14.39	2.33	0.0907	1.6	-0.6
		12.53	2.15	0.0717	1.4	-0.4
		11.14	2.87	0.0795	1.9	-0.2
	04/10/93	9.51	4.83	0.1696	2.3	-1.1
		6.73	5.01	0.1942	1.6	-0.1
		7.66	5.08	0.204	2.2	-0.9
	18/11/93	85.86	2.83	0.1304		1.7
		60.33	3.19	0.1598	NRA	1.3
		92.82	2.78	0.1476		2.1
ACN	02/08/93	37.13	1.42	0.0337	-3.1	1.8
		32.49	1.23	0.0303	0.1	1.4
		25.53	1.47	0.046	-0.2	0.6
	06/09/93	64.97	1.93	0.1018	-0.8	0.8
		27.85	2.07	0.1067	0.7	-1
		30.17	2.06	0.0932	-1.1	1.9
	11/10/93	104.42	3.16	0.1167	0	1.1
		125.31	15.68	0.3841	-1.1	3.8
		118.35	3.15	0.1237	0.5	4.4
	15/11/93	55.69	7.42	0.2201		-0.9
		143.87	6.48	0.2078	NRA	-0.4
		97.46	5.51	0.1684		0.3

AOC-P17 = Method of Van der Kooij
f-Factor & µ-Value = Method of Werner
B/S-BDOC = Method of Billen-Servais
J/L-BDOC = Method of Joret-Levi

NRA = No results available

Table 2.2 : COMPARISON OF METHODS
Corresponding correlation coefficients

(a)

RW	AOC-P17	f-FACTOR	µ-VALUE	B/S-BDOC	J/L-BDOC
AOC-P17	1				
f-FACTOR	0.30692	1			
µ-VALUE	0.46733	*0.90685*	1		
B/S-BDOC	(0.17577)	*0.63965*	0.37148	1	
J/L-BDOC	0.37159	*0.54028*	*0.53766*	0.47740	1

(b)

FI	AOC-P17	f-FACTOR	µ-VALUE	B/S-BDOC	J/L-BDOC
AOC-P17	1				
f-FACTOR	(0.03160)	1			
µ-VALUE	*0.61103*	0.28961	1		
B/S-BDOC	(0.97322)	(0.40238)	(0.62167)	1	
J/L-BDOC	(0.01894)	0.34201	(0.04998)	(0.05993)	1

(c)

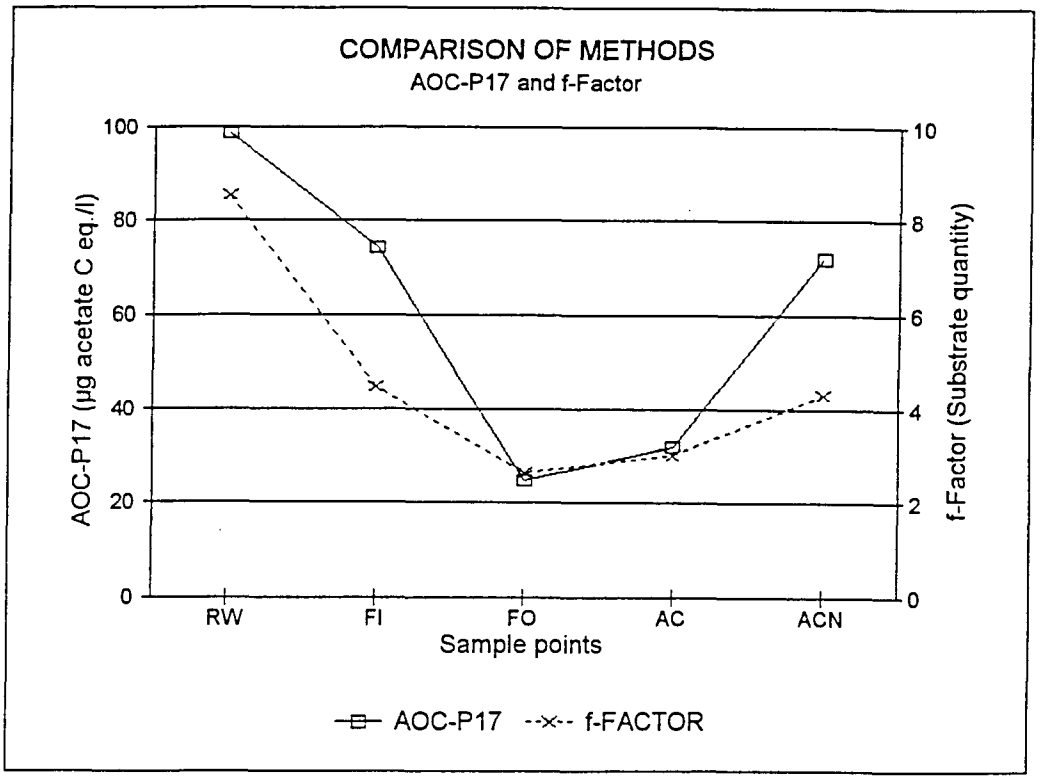
FO	AOC-P17	f-FACTOR	µ-VALUE	B/S-BDOC	J/L-BDOC
AOC-P17	1				
f-FACTOR	*0.93080*	1			
µ-VALUE	*0.73079*	*0.61739*	1		
B/S-BDOC	*0.60658*	*0.75712*	*0.61484*	1	
J/L-BDOC	0.04413	0.34152	(0.17409)	*0.51855*	1

(d)

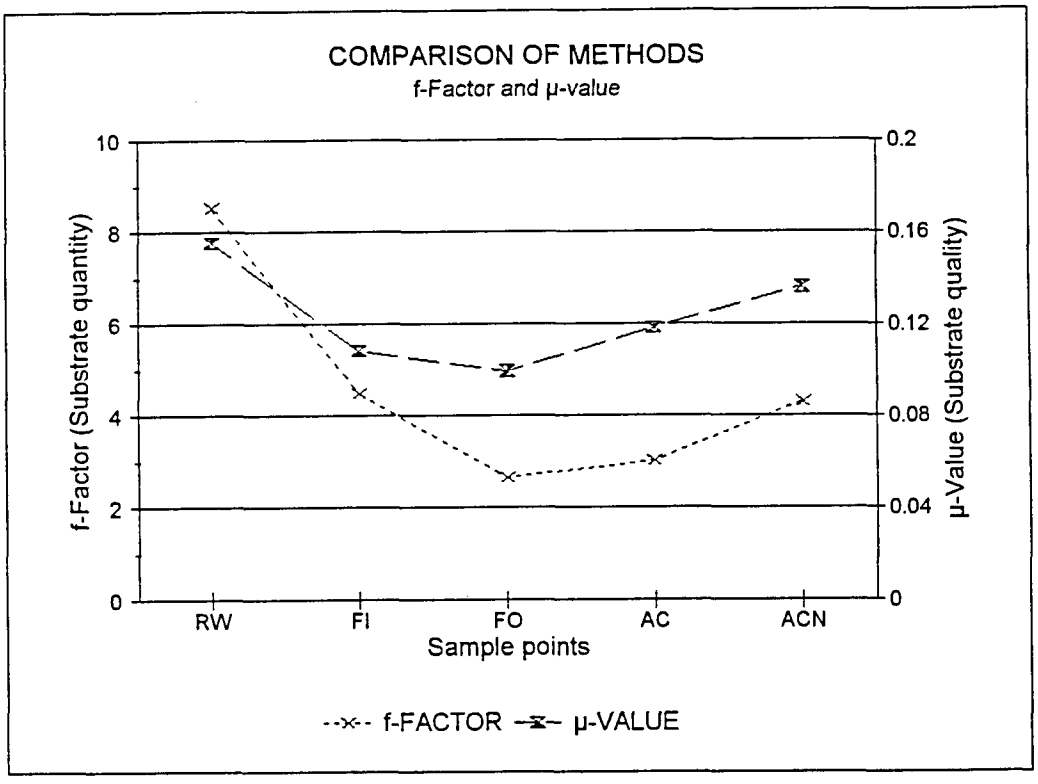
AC	AOC-P17	f-FACTOR	µ-VALUE	B/S-BDOC	J/L-BDOC
AOC-P17	1				
f-FACTOR	(0.25991)	1			
µ-VALUE	0.06792	*0.92096*	1		
B/S-BDOC	(0.88210)	*0.77212*	*0.72185*	1	
J/L-BDOC	*0.89197*	(0.42829)	(0.11083)	(0.88078)	1

(e)

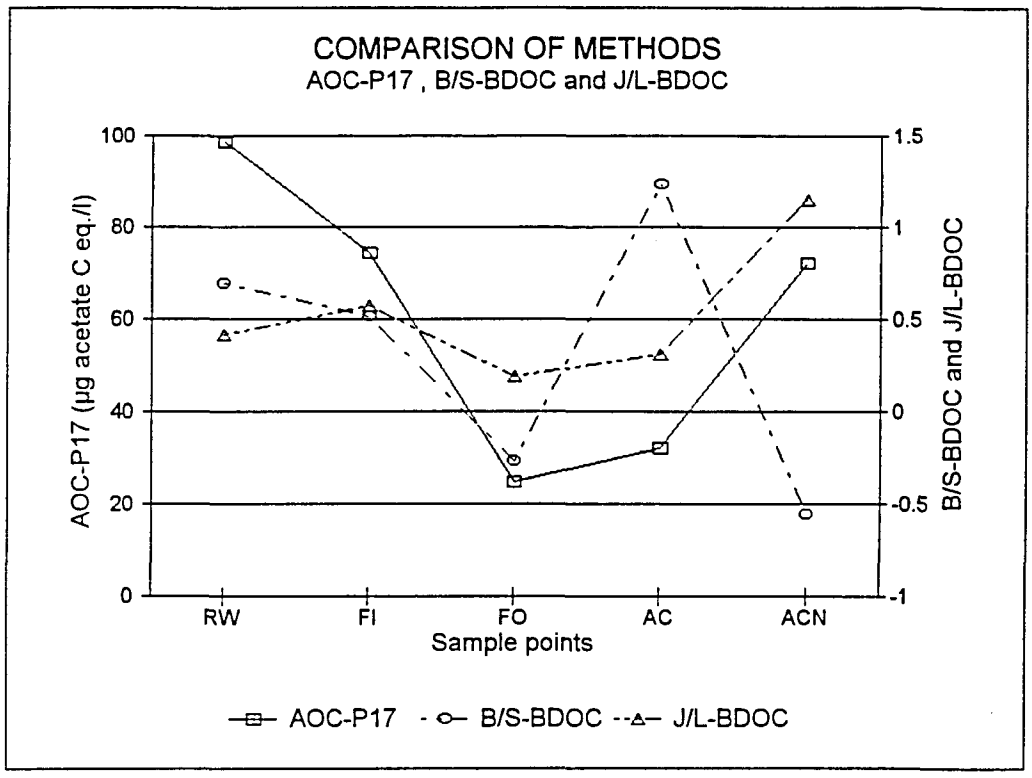
ACN	AOC-P17	f-FACTOR	µ-VALUE	B/S-BDOC	J/L-BDOC
AOC-P17	1				
f-FACTOR	*0.61207*	1			
µ-VALUE	*0.66988*	*0.97599*	1		
B/S-BDOC	0.13784	(0.11153)	(0.00495)	1	
J/L-BDOC	0.31088	0.24183	0.16684	(0.18139)	1


COMPARISON OF METHODS
AOC-P17 and f-Factor
AOC-P17 (µg acetate C eq./l)
f-Factor (Substrate quantity)
100
80
60
40
20
0
10
8
6
4
2
0
RW
FI
FO
AC
ACN
Sample points
AOC-P17
f-FACTOR

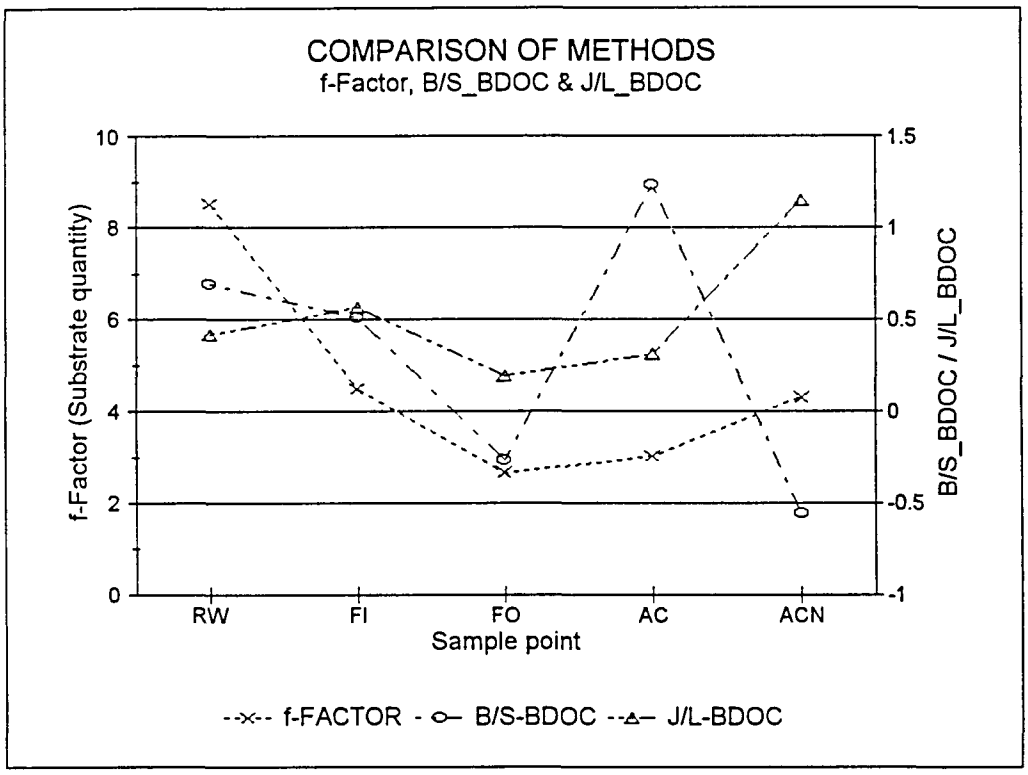
(a)


COMPARISON OF METHODS
f-Factor and µ-value
f-Factor (Substrate quantity)
µ-Value (Substrate quality)
10
8
6
4
2
0
0.2
0.16
0.12
0.08
0.04
0
RW
FI
FO
AC
ACN
Sample points
f-FACTOR
µ-VALUE

(b)



(c)



(d)

Figure 2: **Comparison of the methods at the different sample points - (a) AOC-P17 and f-Factor, (b) f-Factor and μ-value, (c) AOC-P17, B/S-BDOC and J/L-BDOC, and (d) f-Factor, B/S-BDOC and J/L-BDOC**

2.4 DISCUSSION

i) Method of Van der Kooij (AOC-P17)

No major problems were experienced in the implementation of the method of Van der Kooij. The one and only disadvantage was that only the *Pseudomonas fluorescens* strain P17 culture was used at this stage. According to Kaplan and Bott (1988) it was shown that working with *Pseudomonas fluorescens* strain P17 alone, an underestimation of the total AOC by 8 to 44% might occur. Despite the fact that this method is very labour intensive, it can easily be performed in any microbiology laboratory with the specified basic equipment and staff trained in the basic microbiological skills. Triplicate sets of results show reproducibility. Unfortunately the AOC concentration can vary in the water samples and therefore a major fluctuation can occur in samples from the same point but taken on different dates.

ii) Method of Werner (μ-value and f-Factor)

No problems were experienced in the implementation of the method of Werner. A disadvantage was that the number of samples were restricted to four. Good reproducibility was obtained with the triplicate sets of data. The instrument used for the analyses is very expensive and may not be affordable by smaller laboratories. Staff with the basic microbiological background skills will be able to perform the analysis. A major advantage is the short time in which results can be available, usually within three days, at most five days.

iii) Method of Jago-Stanfield (ATP)

Major problems were experienced in performing the method of Jago-Stanfield where ATP determinations had to be carried out. In spite of aseptic working procedures to prevent carry over of ATP during the reading process on the instrument, results fluctuated and background readings were higher than the readings on the actual samples. The supplier also could not solve the problem. Thus no standardization curve could be compiled where a known amount of ATP was added to samples. Therefore, this method was not investigated further.

iv) Methods of Joret-Lévi and Billen-Servais (BDOC)

With both the methods of Joret-Lévi and Billen-Servais where the change in DOC was monitored, no problems were experienced in the sample preparation or set up of the experiment. Regular contrasting results were, however, obtained in the sense that the final DOC values that should theoretically be lower than the DOC values at the beginning, were higher, resulting in a negative value for the calculated BDOC. This means that there

was actually a "production" of DOC, instead of a reduction as the bacteria utilised the carbon. From literature it is clear that the instruments used for DOC analysis, are not always sensitive enough (Huck, 1990) to record changes in $\mu g/\ell$ units. Another problem that could have occurred was contamination from the air, especially with the method of Joret-Lévi where the sample bottles were not covered. According to Dr Dick van der Kooij, it is almost essential to conduct the method of Joret-Lévi in a "DOC-free" laboratory, where no other microbiological work like plating, media preparation, etc is done (personal communication). With the method of Joret-Lévi, the possibility also does excist that organic matter may be released by the biological activated sand (BAS), despite of the pre-washing, or adsorption of organic compounds from the sample by the BAS could also take place.

The results obtained for both these methods are given in Table 2.

v) From the calculated correlation coefficients, it can be seen that as the organic carbon content varied during the treatment process, so did the correlation between the methods. With the FO sample (Table 2.2c), a good correlation was obtained for almost all methods. The fact that only *Pseudomonas fluorescens* strain P17 was used, can possibly explain the poor correlation between the methods of Van der Kooij and Werner.

vi) In Figure 2a the same trend can be seen between AOC-P17 and f-Factor. The μ-value followed the same trend as the f-Factor (Figure 2b). B/S-BDOC showed an increase at AC, much higher than the value at RW, and a decrease at ACN in contrast with the AOC-P17, f-Factor and J/L-BDOC (Figures 2c and 2d). J/L-BDOC followed the same trend as the AOC-P17 and f-Factor, except at ACN, where it increased to a higher value than that at RW (Figure 2c and d).

2.5 CONCLUSIONS

The methods of Van der Kooij (using *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX) and Werner, seem to be suitable for use with any kind of water, especially where small changes in the concentration of available carbon substrate may occur. Both methods were easy to perform and reproducibility was obtained with the triplicate sets of data. Due to variation in the AOC concentration of the water, fluctuation in results obtained from the same sample point, but taken on different dates, should be expected.

An advantage of the Werner method is the short time in which results can be available. The natural mixed inoculum obtained from the water samples will give a better indication of the regrowth potential of that specific type of water than a pure cultured inoculum, like *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX. Although both of these cultures can utilise a variety of carbon sources and at

low concentrations, their ability is still more limited than that of the natural inoculum.

Availability of resources and the urgency to obtain results, may play the major role in the choice between these two methods. The Van der Kooij method is cheaper but more labour intensive and results are only available after about two to four weeks, while the AOC analyser used for the Werner method, is expensive, but analyses are less labour intensive and results are available within three to five days from the reception date of samples.

Both BDOC methods are easy to perform. Care should however be taken to work in a "DOC-free" laboratory to prevent DOC contamination via the air. When working at very low DOC concentrations, the available instruments used for DOC analysis, should be sensitive enough to record changes in $\mu g/\ell$ units as the DOC analyses are the most important part of the experiment. At this stage more work has to be done on a continuous basis to evaluate both the BDOC methods, with the assurance of continuous DOC analysis being done on a sensitive instrument capable of detecting low concentrations DOC.

With the abovementioned methods available, there was no need to pay any further attention to the method of Jago-Stanfield (ATP determination).

CHAPTER 3

THE POSSIBLE SEASONAL EFFECTS ON BIODEGRADABLE ORGANIC CARBON IN WATER DURING THE CONVENTIONAL TREATMENT PROCESS AT RAND WATER.

In this part of the project, the possible seasonal effects on biodegradable organic carbon in water were investigated. Water samples taken from different stages in the conventional treatment process, used at Rand Water, were analysed for the AOC concentration and the F-factor, an indication of substrate quantity.

3.1 WATER SAMPLES

Water samples used: (See Diagram 2).

- Raw water (RW) from the Vaal Dam
- After sedimentation (FI) before sand filtration
- After sand filtration (FO) before chlorination
- Drinking water after chlorination (AC)
- Drinking water after chloramination (ACN)

Appendix F gives a summary of the treatment process.

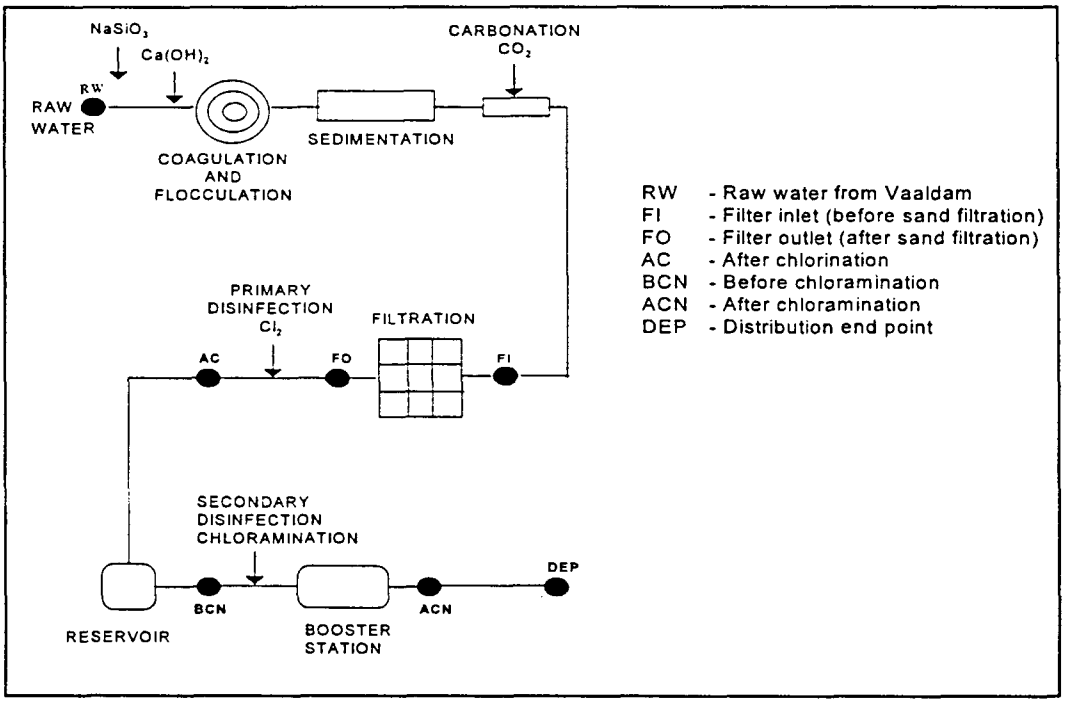


Diagram 2: Flow diagram of the treatment process and sample points

The water samples were divided into two groups which were alternately examined biweekly for six to eight weeks during each season:

Group 1: RW; RI: FO and AC
Group 2: FI; FO; AC and ACN

3.2 SEASONAL INVESTIGATION

Samples were examined during the following seasons:

3.2.1 **Summer** - January/February: 17-01-94 to 22-02-94; 5-12-94 and 13-12-94

3.2.2 **Autumn** - April/May: 11-04-94 to 31-05-94

3.2.3 **Winter** - July/August: 12-07-94 to 23-08-94

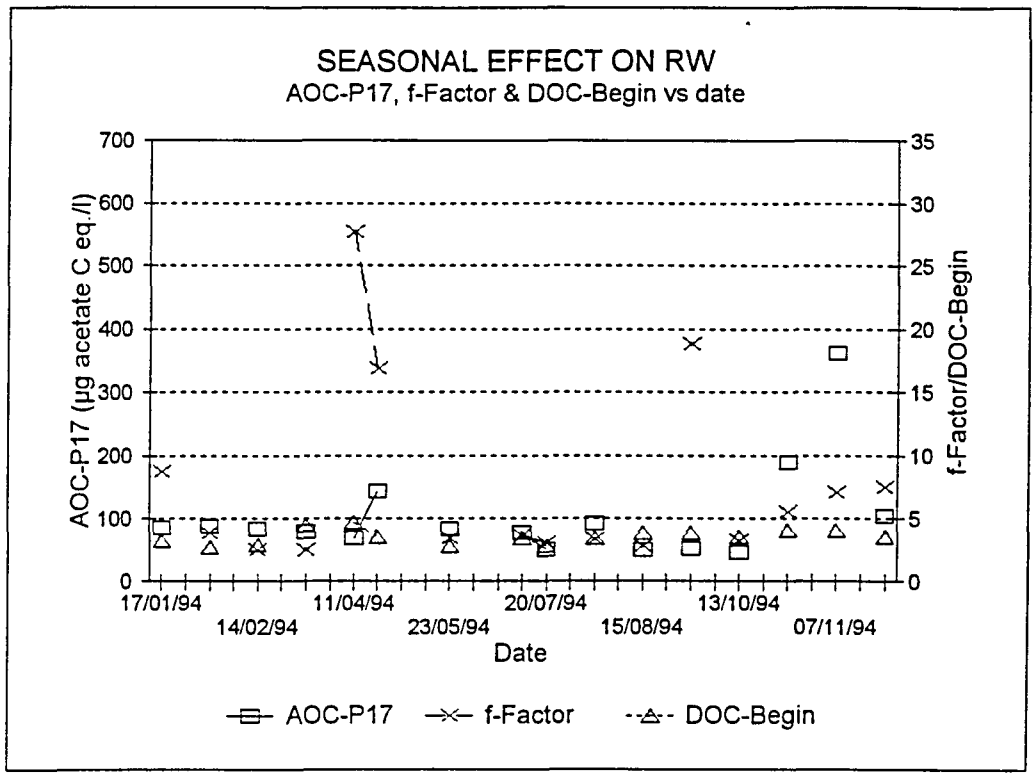
3.2.4 **Spring** - October/November: 26-09-94 to 15-11-94.

The Van der Kooij - (measurement of AOC concentration) and Werner methods (measurement of the μ-value and f-Factor) were performed on the samples. Only *Pseudomonas fluorescens* strain P17 was used in the Van der Kooij method. Chemical analysis including DOC measurements were done as well.

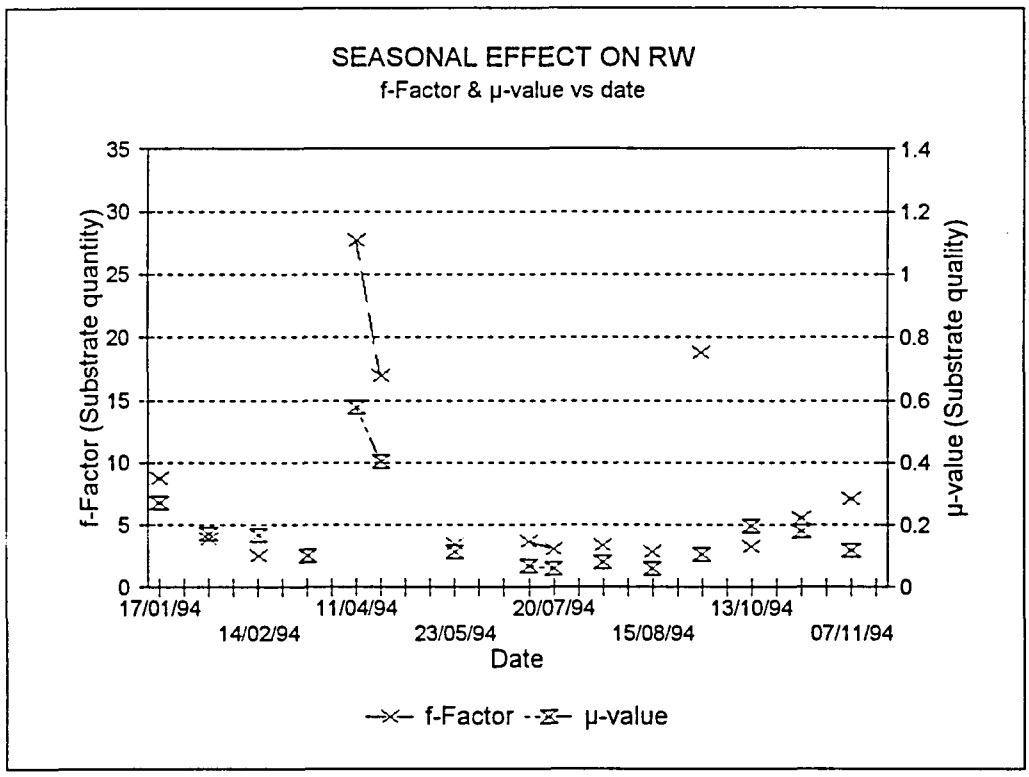
3.3 RESULTS

The results obtained with the investigation into the possible seasonal effect on biodegradable organic carbon in water during the treatment process are reflected in Figures 3.1 to 3.5.

A summary of the water quality during each season of the test period, is indicated in Table F2 (Appendix F).

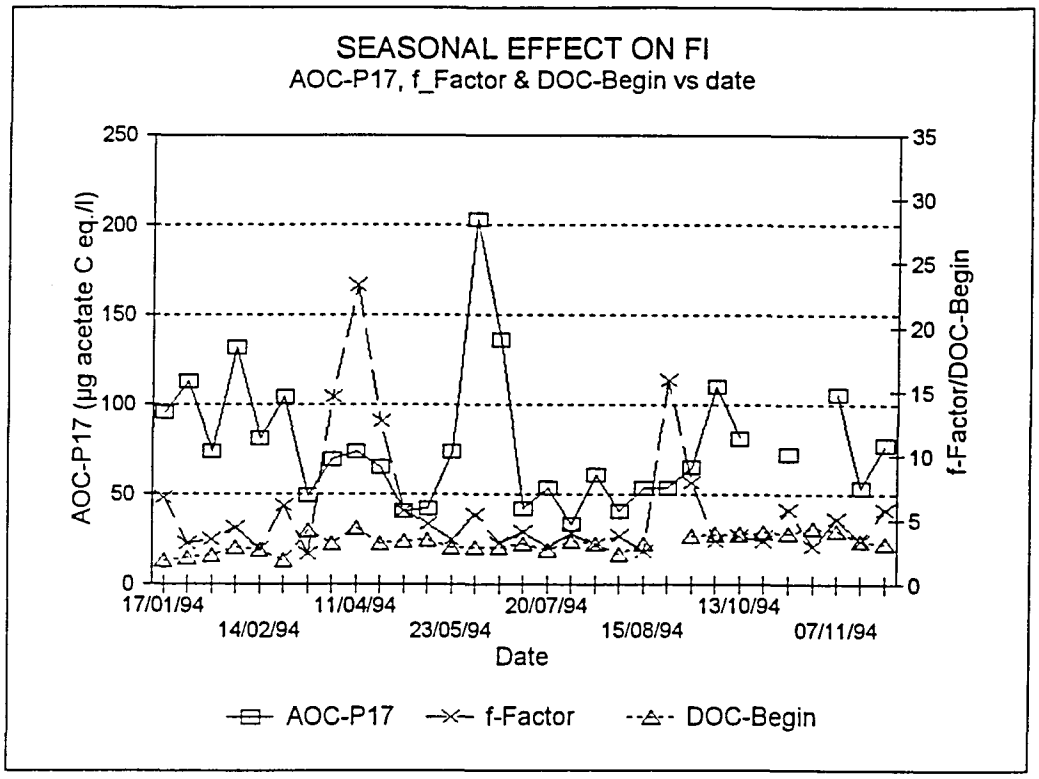


(a)

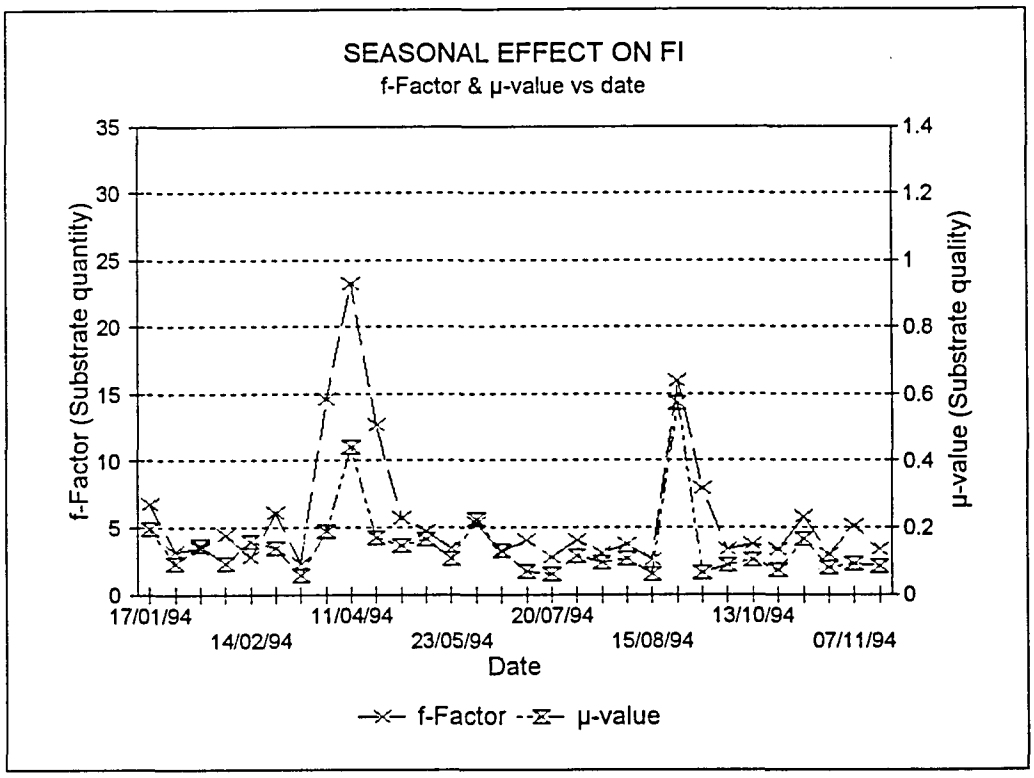


(b)

Figure 3.1: **Seasonal effect on RW for (a) AOC-P17, f-Factor and DOC-Begin vs date and (b) f-Factor and μ-value vs date.**

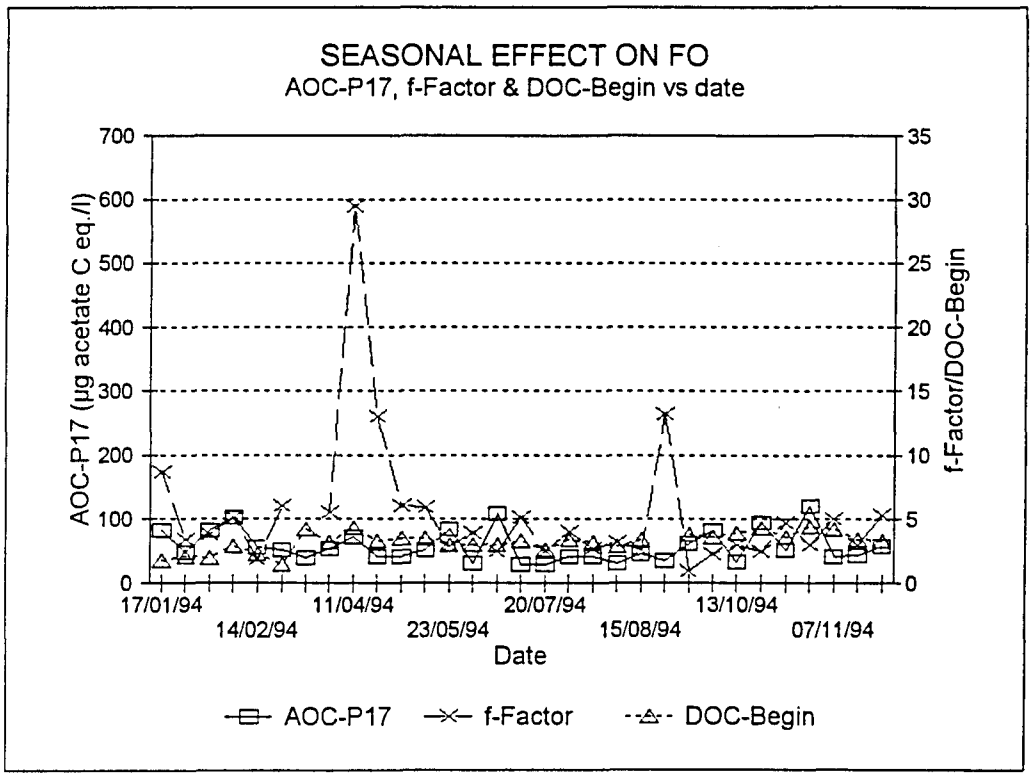


(a)

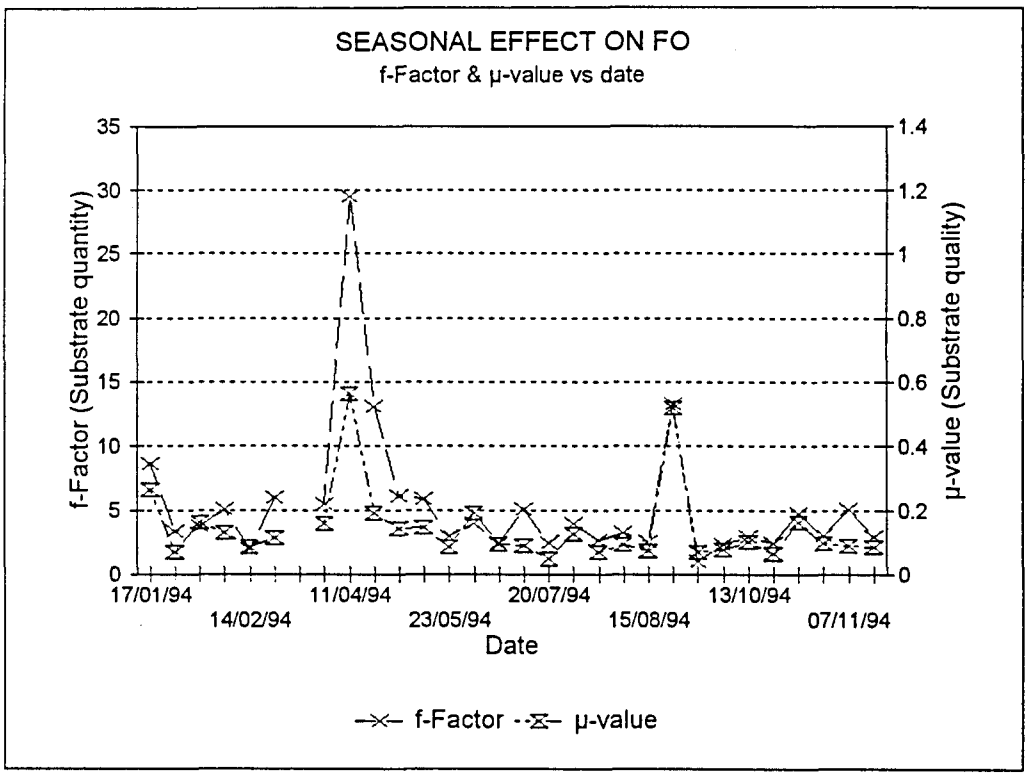


(b)

Figure 3.2: **Seasonal effect on FI for (a) AOC-P17, f-Factor and DOC-Begin vs date and (b) f-Factor and μ-value vs date.**

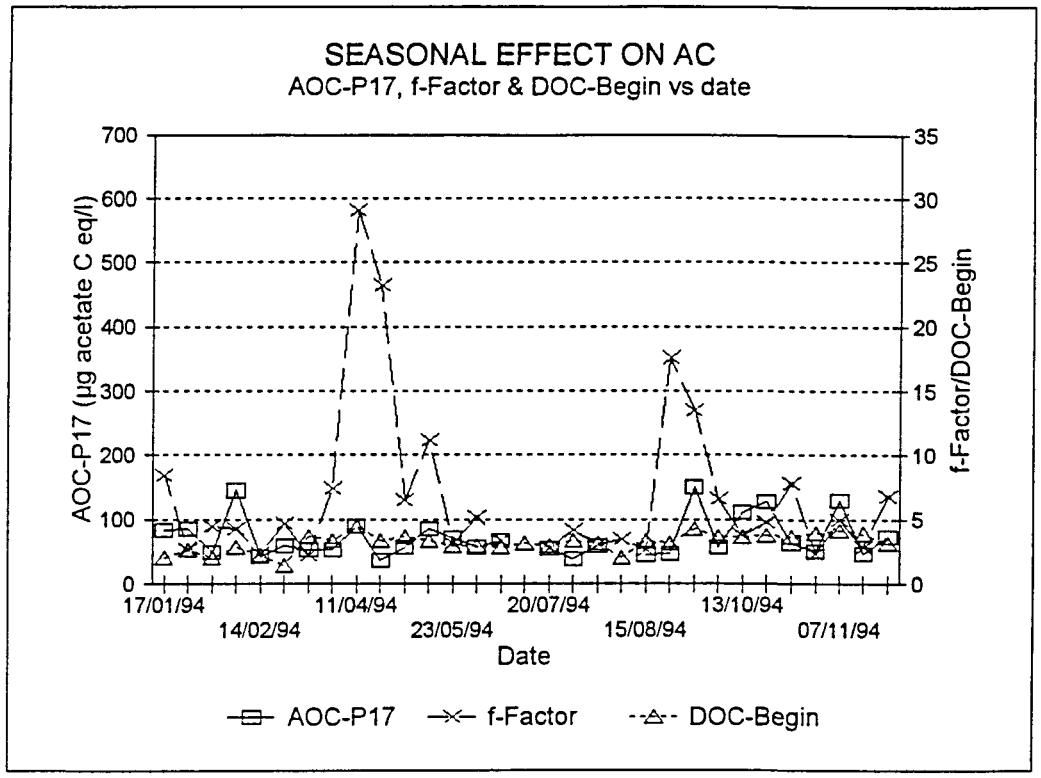


(a)

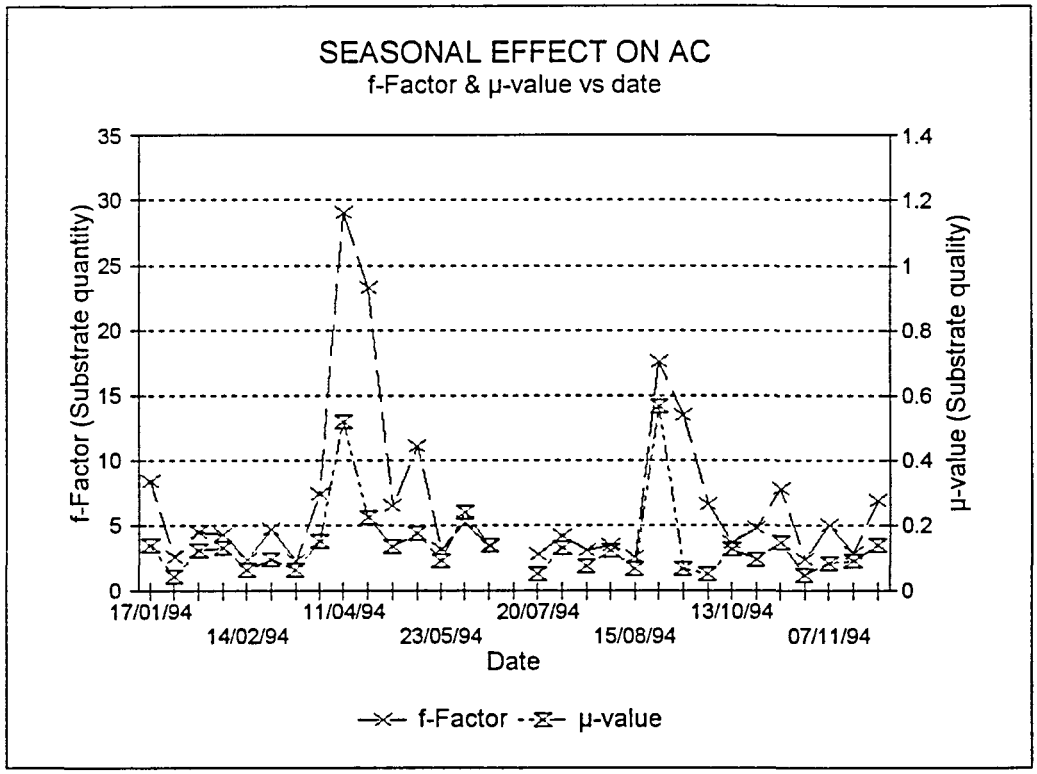


(b)

Figure 3.3: Seasonal effect on FO for (a) AOC-P17, f-Factor and DOC-Begin vs date and (b) f-Factor and μ-value vs date.

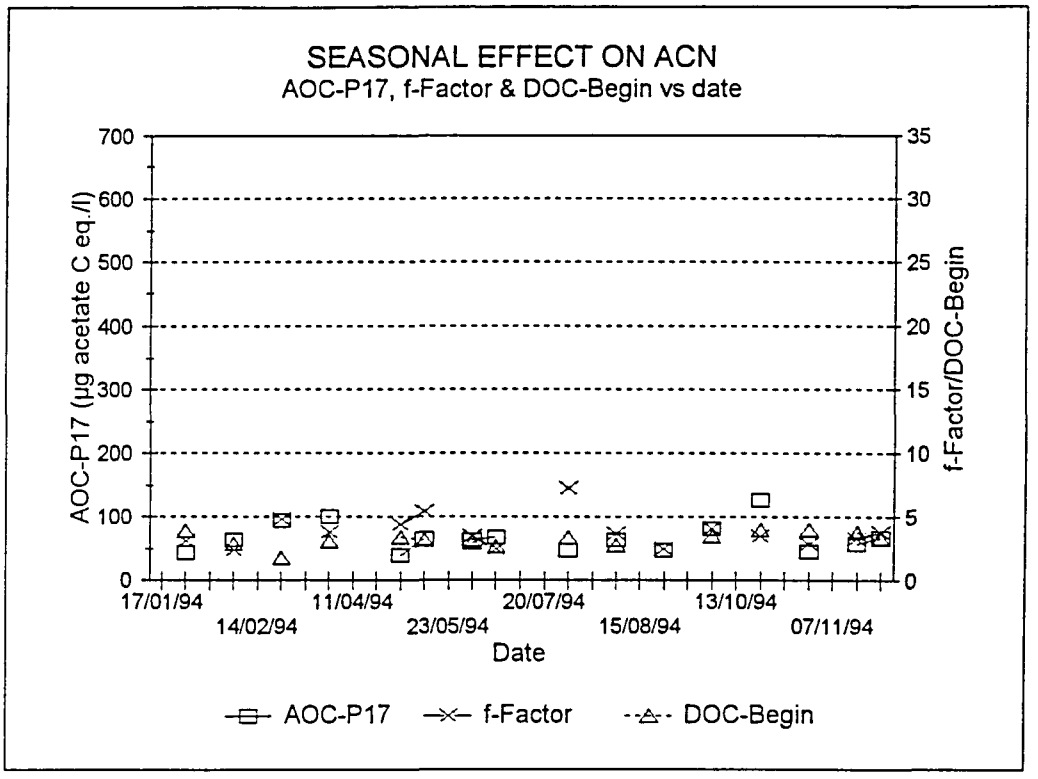


(a)

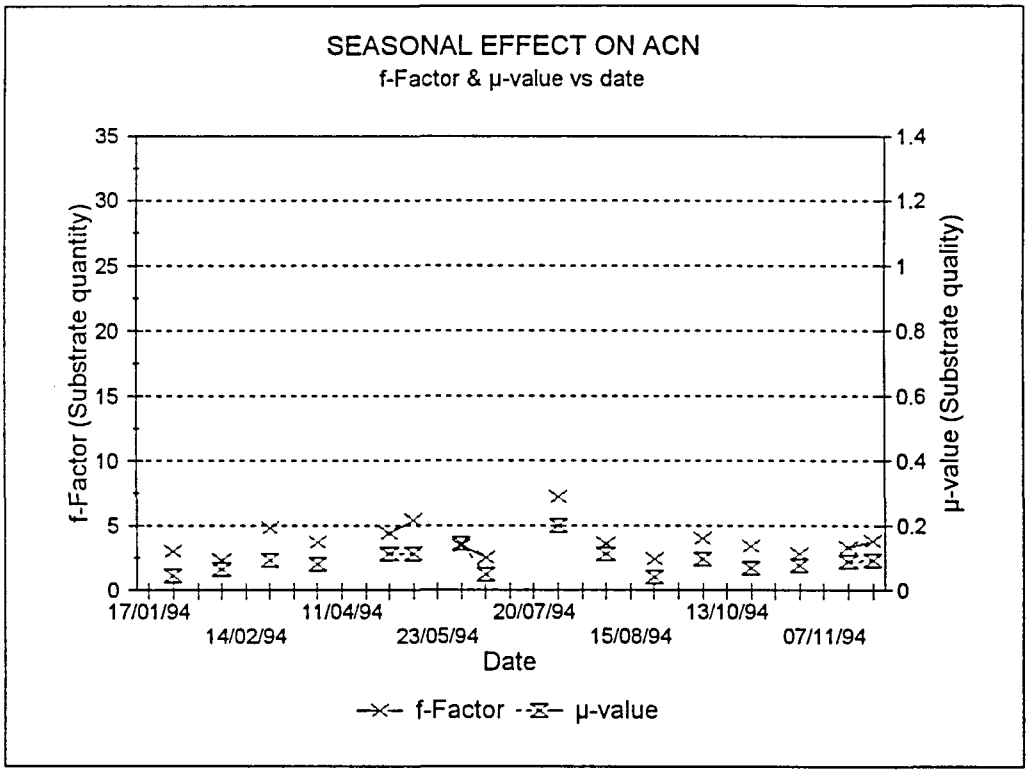


(b)

Figure 3.4: Seasonal effect on AC for (a) AOC-P17, f-Factor and DOC-Begin vs date and (b) f-Factor and μ-value vs date.



(a)



(b)

Figure 3.5: Seasonal effect on ACN for (a) AOC-P17, f-Factor and DOC-Begin vs date and (b) f-Factor and μ-value vs date.

3.4 DISCUSSION

Results shown in the graphs (Figures 3.1 to 3.5) are the actual results over the experimental period. In Figure 3.1a where AOC-P17 concentration, f-Factor and DOC-Begin were plotted over time for RW, an increase in all the parameters occurred at the beginning of autumn and from middle spring. Because DOC analyses are not that sensitive for very small changes, it showed a more stable trend with little variance. In Figure 3.1b the same trend was observed for the f-Factor and μ-value versus time, but the μ-value showed a decrease, instead of an increase from middle spring to the beginning of summer.

In Figure 3.2a. The f-Factor for FI showed a peak around the beginning of autumn and at the end of winter, while the AOC-P17 concentration showed a peak at the end of autumn and at the end of spring. The DOC-Begin showed a little increase around the beginning of autumn. In Figure 3.2b, the μ-value followed the same trend as the f-Factor, thus showing an increase in both concentration and availability of the substrate at the same time.

In Figure 3.3a, the AOC=P17 concentration and DOC-Begin for FO, stayed quite constant with a mean value of 57 μg acetate C eq/ℓ and 3.23 mg/ℓ respectively. No obvious peaks occurred. Small peaks occurred at the end of summer and again at the beginning of winter and during spring. In contrast, the f-Factor showed definite increases at the beginning of autumn and end of winter with more fluctuating values over the rest of the period. The μ-Value showed the same trend as the f-Factor as can be seen in Figure 3.3b, except at the end of spring and beginning of summer when it seemed as if the availability decreased.

In Figure 3.4a, the f-Factor for AC showed increases at the beginning of autumn and again at the end of winter and during spring. One major increase could be observed at the beginning of winter for AOC-P17 and some smaller increases during spring. Although the DOC-Begin values stayed quite constant over the whole period a slight increase could be observed at the beginning of autumn and during the spring period. The μ-Value followed also the same trend as the f-Factor and a gradual increase at the end of spring to the beginning of summer was noticed.

In both Figures 3.5a and 3.5b, not much variance was observed for any of the parameters, indicating that both concentration and availability stabilised after chloramination (ACN).

Chemical analyses done on the RW sample showed the following (Appendix H : Figure [i] to [iv]):

- Turbidity showed an increase at the beginning of autumn, with a gradual decrease over the rest of the year (Figure [i]).

- Conductivity was relatively constant except for a decrease during winter and late summer (Figure [i]).

- Alkalinity increased gradually over the whole year (Figure [ii]).
- Calcium (Ca) gave higher values during autumn and late spring (Figure [ii]).
- Magnesium (Mg) fluctuated slightly during the year (Figure [ii]).
- Sodium (Na) increased during mid-winter and spring (Figure [iii]).
- Potassium (K) fluctuated slightly during the year (Figure [iii]), with higher values ($\pm$ 5 mg/ℓ) during spring.
- pH fluctuated slightly between pH 7.6 and 8.0 during the year (Figure [iii]).
- Ammonia as N (Amm.N) and Nitrite as N (Nitrite) were most of the time below the detection limits of 0.05 and 0.10 mg/ℓ respectively. Therefore, these points were not indicated on Figure [iv].
- Nitrate as N (Nitrate) gave peak values at mid-winter and at the beginning of spring, but with an overall decrease from January to December during 1994.
- Orthophosphate as P (Ortho.P) fluctuated between 0.17 mg/ℓ and 0.26 mg/ℓ during the year with a slight increase at mid-autumn, followed by a decrease.
- Sulphate as SO_4 gradually increased during the year with peaks at the end of autumn and again at the beginning of spring (Figure [iv]).

The DOC values indicated in Table F2, show an increase from summer to autumn and another increase from winter to spring/summer. This observation confirms the trend observed with the AOC-P17 concentration and f-Factor values where increases are seen during autumn and from middle spring to the beginning of summer.

3.5 CONCLUSIONS

When summarised, it seemed as if increases of biodegradable organic carbon measured as AOC-P17 concentration, f-Factor and DOC-Begin, occurred around the beginning of autumn and during spring. This trend was noticed from the raw water (RW), through the treatment process (FI and FO) until the after chlorination (AC) point. It seemed as if this trend was not carried over to the after chloramination (ACN) point. Some of the chemical analysis that were done on RW also showed trends of increases during autumn and spring. DOC analysis confirm this trend of an increase in the organic carbon content of the water, during autumn, spring and the beginning of summer. The difference in trend between the AOC-P17 concentration and the f-Factor may possibly be due to the fact that only *Pseudomonas fluorescens* strain P17 was used and not both *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX in the Van der Kooij method.

A continuous monitoring of the water quality from the raw water intake and through the system at specified stages, will confirm the above mentioned results. Parameters like rainfall, temperature and water sources *e.g.* abstraction mainly from Vaal Dam or mixing with Barrage Reservoir, where the source water quality may differ to a great extent, should also be taken into consideration with further investigations.

CHAPTER 4

EFFECT OF DIFFERENT TREATMENT PROCESSES ON THE CONCENTRATION OR AVAILABILITY OF BIODEGRADABLE ORGANIC CARBON

Bench tests were performed to evaluate the effect of different treatment processes on the concentration and/or availability of biodegradable organic carbon. Coagulant/flocculant combinations at approximately the same or different concentrations, as used in practise, were tested.

The retention time during these bench tests, was not exactly the same as that during the actual treatment processes. The following combinations of coagulant/flocculant were tested:

- silica/lime vs lime/ferric chloride at concentrations of 3 mg SiO_2/ℓ and 65 mg CaO/ℓ as lime vs 65mg CaO/ℓ and 3 mg Fe^{3+}/ℓ as $FeCl_3$.
- High concentration ferric chloride/low concentration lime vs low concentration ferric chloride/high concentration lime (15 mg CaO/ℓ as lime and 30 mg $FeCl_3/\ell$ vs 65 mg CaO/ℓ as lime and 4 mg $FeCl_3/\ell$).
- Pre-chlorination (± 0.64 mg Cl_2/ℓ) vs pre-ozonation (±2.8 mg O_3/ℓ).

4.1 SILICA/LIME VS LIME/FERRIC CHLORIDE

4.1.1 Water samples

A raw water (RW) sample was collected from the purification plant (see diagram 2) and split samples were treated with the two different combinations of coagulant/flocculant (see diagram 4.1). The following samples were compared:

RW	-	untreated raw water sample
Silica/lime	-	raw water treated with silica/lime
Lime/Fe^{3+}	-	raw water treated with lime/ferric chloride

4.1.2 Experimental work

Bench tests were performed to evaluate the effect of silica/lime vs lime/ferric chloride as coagulant/flocculant on the concentration and/or availability of the AOC.

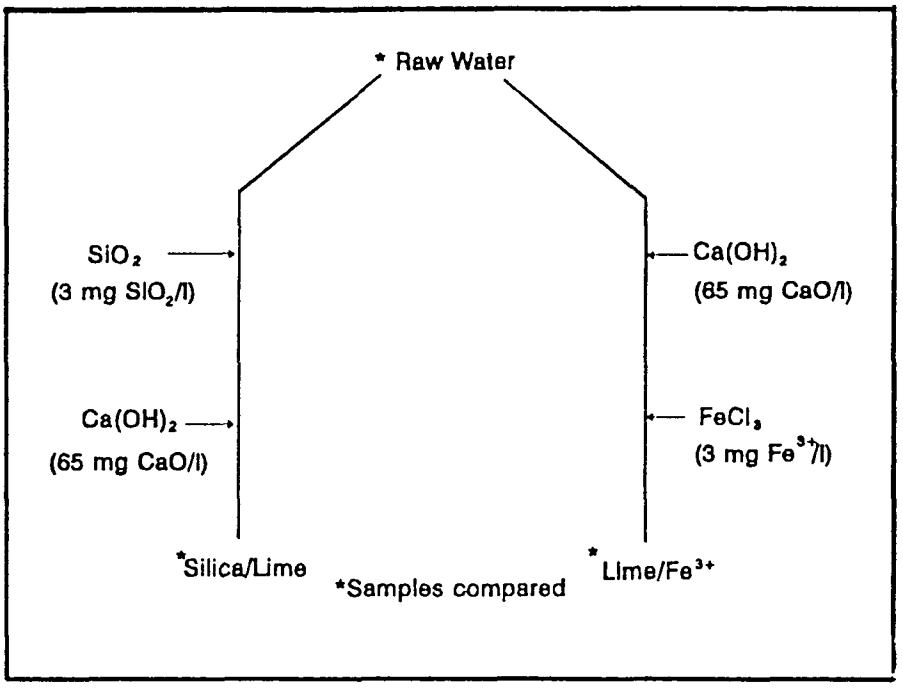


Diagram 4.1: **Flow diagram of the simulated treatment process to compare the effect of silica/lime vs lime/ferric chloride as coagulant/flocculant on the concentration and/or availability of AOC.**

A Leetech six paddle stirrer was used in the bench test to simulate the coagulation - flocculation - sedimentation processes. Duplicate samples of one litre each were mixed in the square beakers with the coagulant/flocculant combination as shown in the diagram and under conditions as set out in Table 4.1a.

Table 4.1a: Mixing conditions for coagulation and sedimentation

Pre-mixing	30 sec./300 rpm
Add 1st coagulant (see diagram)	within 15 sec.
Add 2nd coagulant	
Rapid mix	60 sec./300 rpm
Flocculation	30 sec./200 rpm
Gradual decrease of mixing speed over 30 sec.	From 200 to 60 rpm
Slow mix	420 sec./60 rpm
Final mix	90 sec./30 rpm

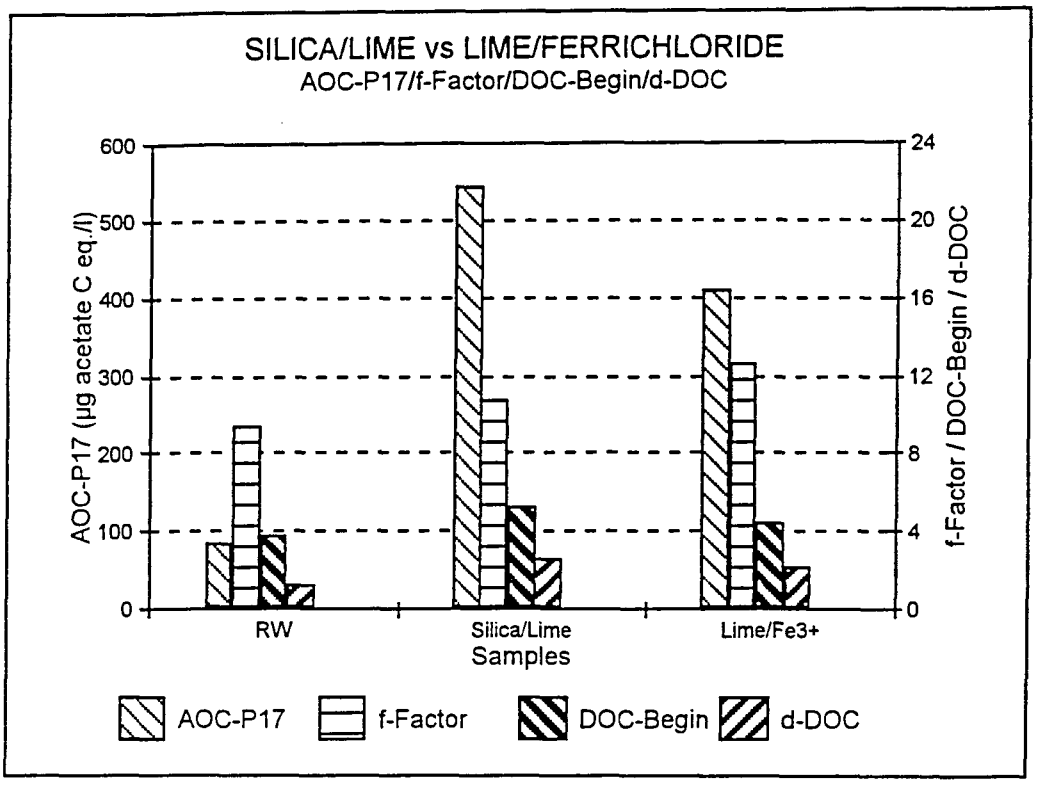
sec. - seconds rpm - revolutions per minute

After the flocculated matter was allowed to settle, the clear supernatant was siphoned off carefully into pre-cleaned glass beakers (the supernatant of each set combined). The pH was determined and adjusted to approximately 8.2 by bubbling CO_2 gas slowly through the water until the desired pH was reached. The water was filtered through a 0.8 μm nucleopore membrane filter to remove the flocculated material. Each treated sample was divided into two portions, one for the Werner and the other for the Van der Kooij method. For the Van der Kooij method a duplicate set of each sample was used and a single set of three dilutions from each flask were plated out. Only *Pseudomonas fluorescens* strain P17 was used for the Van der Kooij method at this stage. No chemical analysis, except DOC analysis with the Werner method, were done.

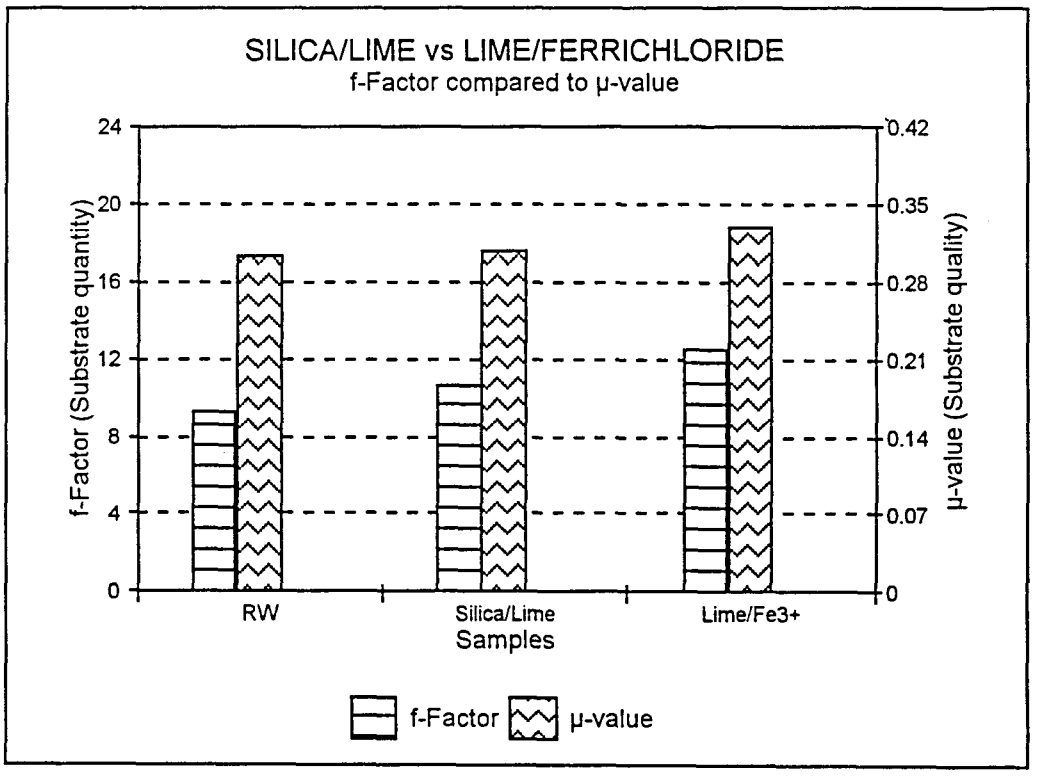
4.1.3 Results

Table 4.1b: Range for results obtained in the comparison of silica/lime vs lime/ferric chloride.

Sample		Parameters				
		AOC-P17 (μg acetate C eq/ℓ)	f-Factor	μ-value	DOC-Begin (mg/ℓ)	d-DOC (mg/ℓ)
RW	Min	52.2	2.14	0.113	2.1	-0.4
	Max	159.4	16.49	0.4911	5.1	2.1
	Avg	83.7	9.33	0.3042	3.8	1.2
Silica/Lime	Min	405.8	5.2	0.2524	2.5	0.2
	Max	637.7	14.83	0.4195	7.5	4.1
	Avg	543.5	10.68	0.3077	5.2	2.5
Lime/Fe^{3+}	Min	217.4	5.76	0.2612	2.7	0.8
	Max	608.7	18.84	0.3804	5.7	2.9
	Avg	409.4	12.56	0.3287	4.4	2.1



(a)



(b)

Figure 4.1: **The effect of silica/lime vs lime/ferric chloride treatment on the concentration or availability of biodegradable organic carbon as reflected in (a) AOC-P17/f-Factor/DOC-Begin/d-DOC and (b) f-Factor compared to μ-value. (Test period: 08-03-94 to 22-03-94, 23-04-96).**

Table 4.1c: Percentage increase in AOC-P17, f-Factor and μ-value of the raw water caused by the different treatment processes

Treatment Process	Parameter		
	AOC-P17 (μg acetate C eq/ℓ)	f-Factor	μ-value
Silica/Lime	549%	14.5%	1.15%
Lime/Fe^{3+}	389%	34.6%	8.1%

4.1.4 Discussion

A higher average (549%) increase in the concentration of AOC-P17 occurred with the silica/lime treatment, than with the lime/ferric chloride (389%) (Figure 4.1a). In contrast a higher average (34.6%) increase occurred in the f-Factor, with the lime/ferric chloride than with the silica/lime treatment (14.5%) (Figures 4.1a and b). According to the μ-value, there was little difference between the availability of the substrate in the raw water and that after silica/lime treatment (average 0.3042 and 0.3077 respectively), but it was observed that the lime/ferric chloride treatment gave a higher μ-value (0.3287 average), indicating a more available substrate. DOC analyses that were done on samples from the Werner method, showed the same trend as was observed with the Van der Kooij method (DOC-Begin - DOC-End = dDOC: RW = 1.2; silica/lime = 2.5 and lime/ferric chloride = 2.2 average).

The difference in trend between the AOC-P17 concentration and the f-Factor, may possibly be due to the fact that only the *Pseudomonas fluorescens* strain P17 culture was used and not both *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX in the Van der Kooij method. No comparison of this kind was found in the available literature.

4.1.5 Conclusions

Both treatments increased the concentration of biodegradable organic carbon present in the raw water. Although the silica/lime treatment increased the AOC-P17 by 549%, it was less biodegradable (μ-value increased by 1.15%) in comparison with the biodegradability of the carbon source with the lime/Fe^{3+} treatment (μ-value increased by 8.1%).

4.2 HIGH CONCENTRATION FERRIC CHLORIDE/LOW CONCENTRATION LIME VS LOW CONCENTRATION FERRIC CHLORIDE/HIGH CONCENTRATION LIME.

4.2.1 Water samples

A raw water (RW) sample was collected from the purification plant (see Diagram 2) and split samples were treated with the two different combinations of coagulant/flocculant (see Diagram 4.2). The following samples were compared:

RW	-	untreated raw water sample
L_$FeCl_3$	-	treated raw water sample with a low concentration ferric chloride/high concentration lime
H_$FeCl_3$	-	treated raw water sample with a high concentration ferric chloride/low concentration lime.

4.2.2 Experimental work

Bench tests were performed to evaluate the effect of the low concentration ferric chloride/high concentration lime vs the high concentration ferric chloride/low concentration lime on the concentration and/or availability of the AOC

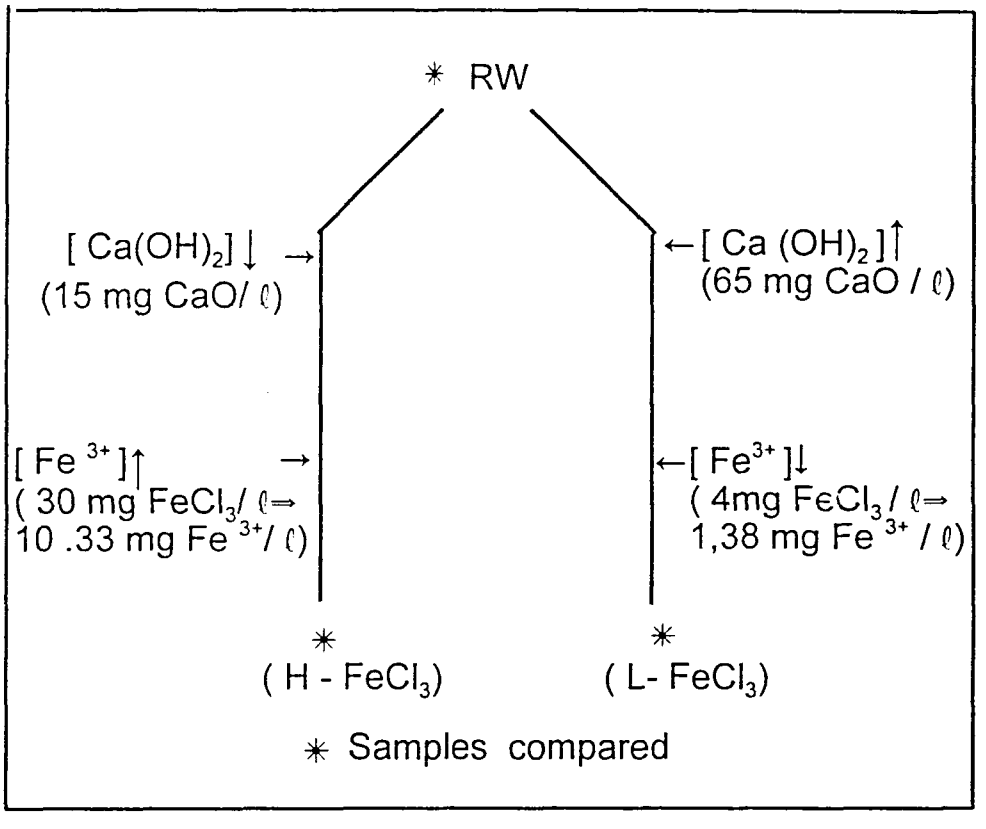


Diagram 4.2: **Flow diagram of the simulated treatment process used to compare the effect of low ferric chloride/high lime vs high ferric chloride/low lime on AOC.**

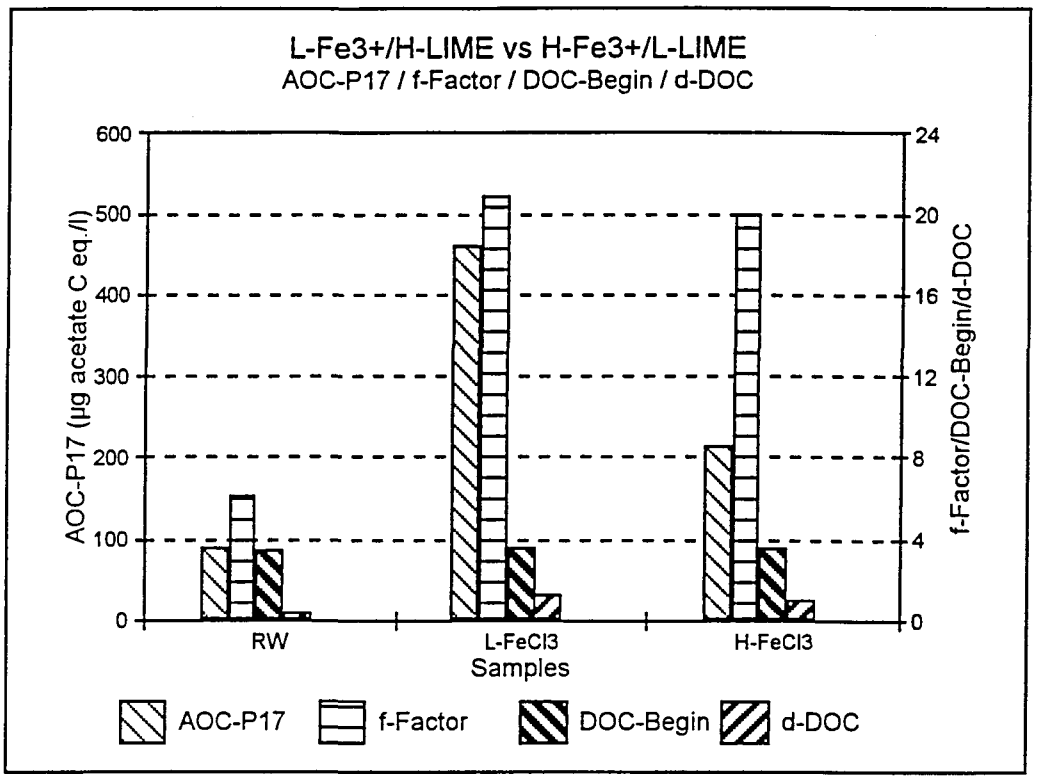
A Leetech six paddle stirrer was used in the bench test to simulate the coagulation - flocculation - sedimentation processes. Duplicate samples of one litre each were mixed in the square beakers with the coagulant/flocculant combination as shown in Diagram 4.2 and under conditions set out in Table 4.1a.

After the flocculated matter was allowed to settle, the clear supernatant was siphoned off carefully into pre-cleaned glass beakers (the supernatant of each set combined). The pH was determined and adjusted to approximately 8.2, by bubbling CO_2 gas slowly through the water, until the desired pH was reached. The water was filtered through a 0.8 μm nucleopore membrane filter to remove the flocculated material. Each treated sample was divided into two portions, one for the Werner method and the other for the Van der Kooij method. The sample for the Van der Kooij method was inoculated only with the *Pseudomonas fluorescens* strain P17 culture. Standard plate counts were done by the spread plate method on a triplicate set of three dilutions. No chemical analysis were done, except DOC analysis with the Werner method.

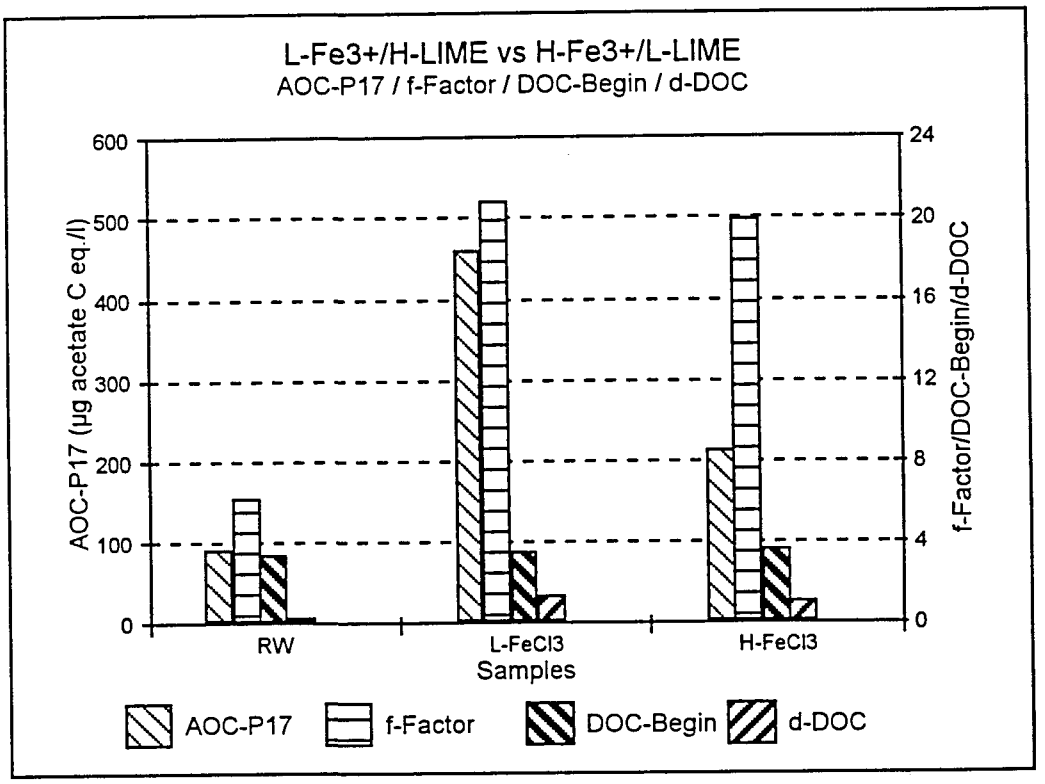
4.2.3 Results

Table 4.2a: Range for results obtained in the comparison of low ferric chloride/high lime vs high ferric chloride/low lime.

Sample		Parameters				
		AOC-P17 (μg acetate C eq/ℓ)	f-Factor	μ-value	DOC-Begin (mg/ℓ)	d-DOC (mg/ℓ)
RW	Min	37.7	3.06	0.1049	3.2	0.1
	Max	188.4	10.37	0.3829	3.5	0.4
	Avg	89.4	6.12	0.2414	3.4	0.3
L - $FeCl_3$	Min	420.3	16.37	0.3857	3.4	0.1
	Max	507.2	27.07	0.3929	3.6	3
	Avg	458.9	20.82	0.3891	3.5	1.3
H-$FeCl_3$	Min	105.8	15.56	0.3745	3.5	0.9
	Max	376.8	27.53	0.4438	3.7	1.1
	Avg	214.0	19.9	0.4123	3.6	1



(a)



(b)

Figure 4.2: **The effect of low ferric chloride/high lime vs high ferric chloride/low lime treatment on the concentration or availability of biodegradable organic carbon as reflected in (a) AOC-P17/f-Factor/DOC-Begin/d-DOC and (b) f-Factor compared to μ-value (Test period: 15-06-94 to 28-06-94).**

Table 4.2b Percentage increase in AOC-P17, f-Factor and μ-value of the raw water, caused by the different treatment processes.

Treatment Process	Parameter		
	AOC-P17 (μg acetate C eq/ℓ)	f-Factor	μ-value
L-$FeCl_3$	413%	240%	61%
H-$FeCl_3$	139%	225%	70%

4.2.4 Discussion

Both treatment combinations increased the concentration and availability of the biodegradable organic carbon (Figure 4.2a and b). Although the difference in the f-Factor (20.82 and 19.9 respectively) was not as clear as with the AOC-P17 value (488.9 and 214 respectively), the low concentration ferric chloride/high concentration lime caused a higher increase (413%) in the concentration of biodegradable organic carbon compared to the high ferric chloride/low lime treatment (139%). The same trend was noticed with the d-DOC values of the Werner method (1.3 mg/ℓ and 1 mg/ℓ respectively)

Although the low ferric chloride/high lime treatment caused a higher increase (418% vs 139%) in the concentration of biodegradable organic carbon, it was less available (61%) for microbial growth than with the high ferric chloride/low lime treatment (70%). (Figure 4.2a and b and Table 4.2a and b).

4.2.5 Conclusions

Both treatments caused an increase in concentration as well as availability of the biodegradable organic carbon. Although both treatment combinations were investigated, only the low ferric chloride/high lime combination is used during the treatment process. The low ferric chloride/high lime treatment process gave a higher concentration (413% AOC-P17 and 240% f-Factor) of biodegradable organic carbon, but of lower (61% μ-value) quality than that of the high ferric chloride/low lime treatment process (average μ-values = 0.3891 and 0.4123 respectively - Table 4.2a) This observation means that although the normal low ferric chloride/high lime treatment combination increases the concentration of biodegradable organic carbon more than that of the high ferric chloride/low lime treatment combination, it is less available to maintain bacterial regrowth (lower μ-value).

4.3 PRE-CHLORINATION VS PRE-OZONATION

4.3.1 Water samples

A raw water (RW) sample was collected from the purification plant (see Diagram 2). Two of the four split samples were pre-treated, one with chlorine (pre-chlorination) and the other with ozone (pre-ozonation). Afterwards these two samples and one of the untreated samples were flocculated with lime/ferric chloride (see Diagram 4.3), to give the following samples:

RW	-	untreated raw water sample
Fe^{3+}/Lime	-	raw water flocculated with lime/ferric chloride
Cl_2	-	pre-chlorinated raw water flocculated with lime/ferric chloride
O_3	-	pre-ozonated raw water flocculated with lime/ferric chloride

4.3.2 Experimental work

Bench tests were performed to evaluate the effect of the pre-chlorination vs pre-ozonation on the concentration and/or availability of the biodegradable organic carbon.

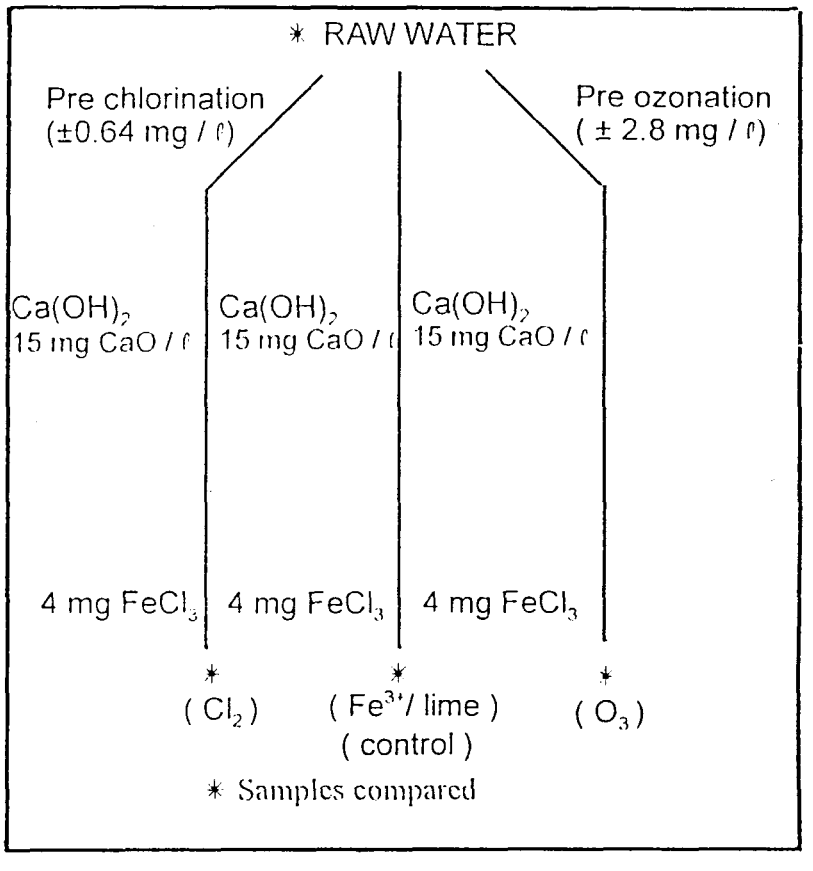


Diagram 4.3: **Diagram of the simulated treatment process used to compare the effect of pre-chlorination vs pre-ozonation on AOC.**

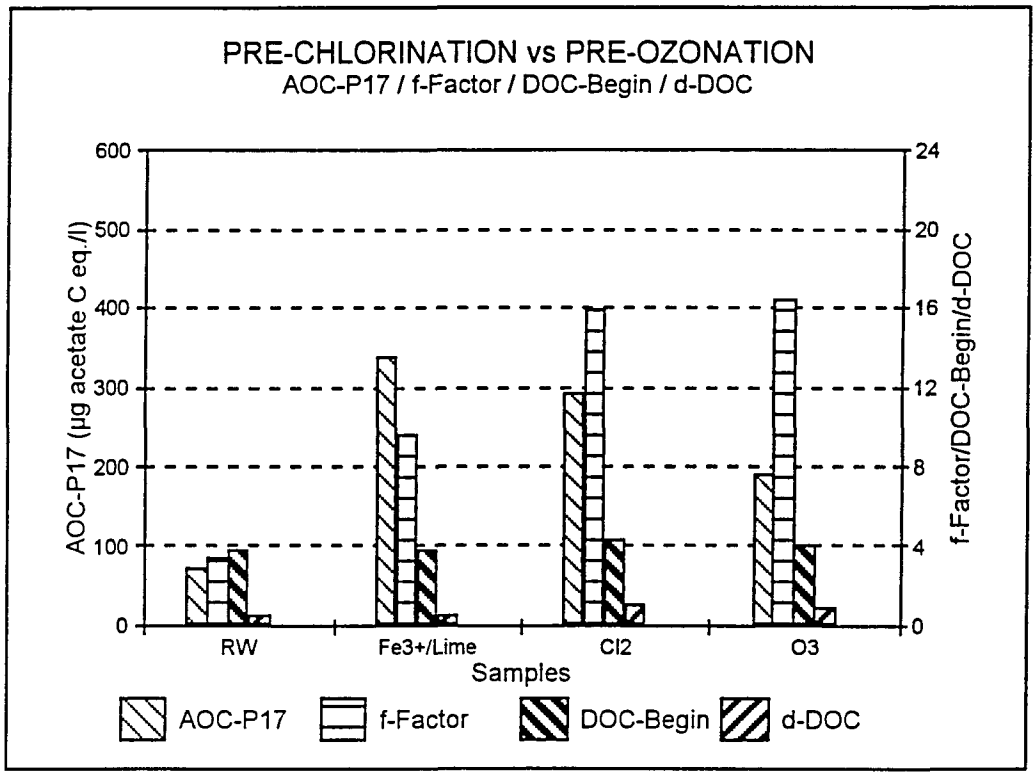
A Leetech six paddle stirrer was used in the bench test to simulate the coagulation - flocculation - sedimentation processes. Duplicate samples of one litre each (RW, pre-chlorinated and pre-ozonated raw water) were mixed in the square beakers with the coagulant/flocculant combination as shown in diagram 4.3 and under conditions set out in Table 4.1a.

After the flocculated matter was allowed to settle, the clear supernatant was siphoned off carefully into pre-cleaned glass beakers (the supernatant of each set combined). The pH was determined and adjusted to approximately 8.2, by bubbling CO_2 gas slowly through the water, until the desired pH was reached. The water was filtered through a 0.8 μm nucleopore membrane filter to remove the flocculated material. Each treated sample was divided into two portions, one for the Werner method and the other for the Van der Kooij method. The sample for the Van der Kooij method was inoculated only with the *Pseudomonas fluorescens* strain P17 culture. Standard plate counts were done by the spread plate method on a triplicate set of three dilutions. Chemical analysis were done, on the treated samples to be compared.

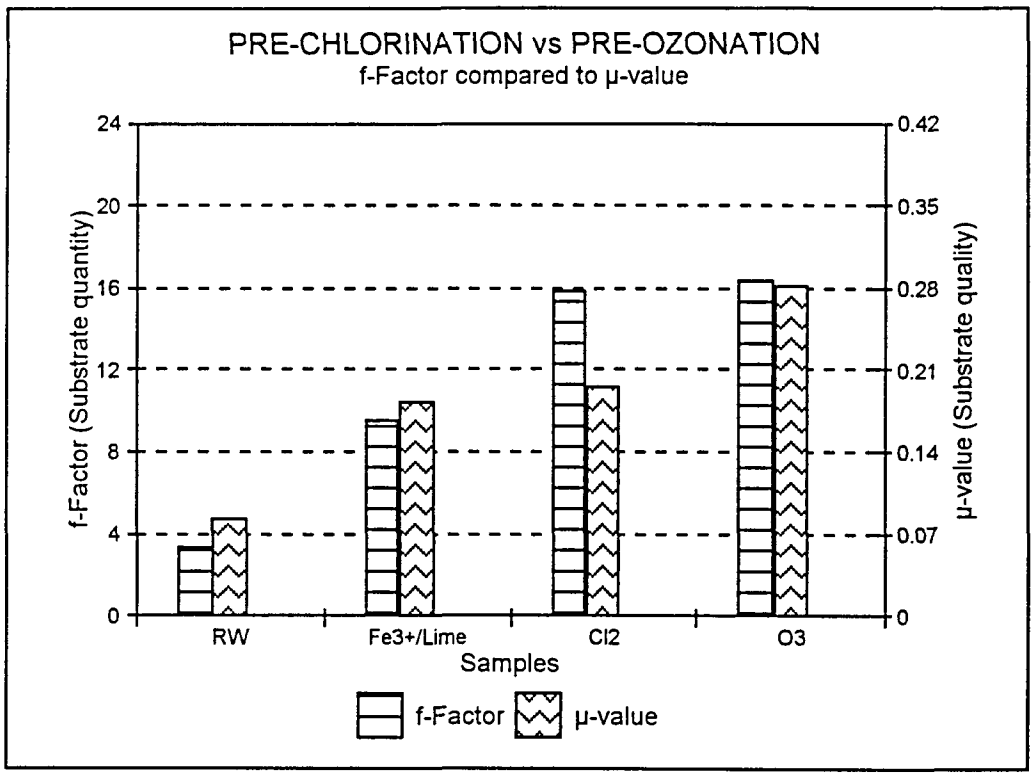
4.3.3 Results

Table 4.3a Range for results obtained in the comparison of pre-chlorination vs pre-ozonation.

Sample		Parameters				
		AOC-P17 (μg acetate C eq/ℓ)	f-Factor	μ-value	DOC-Begin (mg/ℓ)	d-DOC (mg/ℓ)
RW	Min	42.0	1	0.039	3.5	0
	Max	113.0	5.76	0.1071	3.9	1.4
	Avg	70.55	3.38	0.0821	3.75	0.475
Fe^{3+}/Lime	Min	151.7	2.92	0.0991	3.4	0.3
	Max	492.8	14.33	0.2382	4.2	1.2
	Avg	338.7	9.56	0.1830	3.8	0.575
Cl_2	Min	65.8	2.13	0.0781	4.1	0.5
	Max	488.4	22.36	0.2919	4.6	1.4
	Avg	290.7	15.93	0.1965	4.38	1.1
O_3	Min	108.7	8.93	0.1898	3.7	0.3
	Max	376.8	24.77	0.3587	4.8	1.7
	Avg	191.3	16.35	0.2816	4.05	0.95



(a)



(b)

Figure 4.3: **The effect of pre-chlorination/pre-ozonation on the concentration or availability of biodegradable organic carbon as reflected in (a) AOC-P17/f-Factor/DOC-Begin/d-DOC and (b) f-Factor compared to μ-value (Test period: 30-08-94 to 20-09-94).**

Table 4.3b Percentage increase in AOC -P17, f-Factor and μ-value in the raw water, caused by the different treatment processes.

Treatment Process	Parameter		
	AOC-P17 (μg acetate C eq/ℓ)	f-Factor	μ-value
Fe^{3+}/Lime	380%	183%	123%
Pre-Cl_2	312%	371%	139%
Pre-O_3	171%	384%	243%

4.3.4 Discussion

The ferric chloride/lime treatment caused an increase in both the concentration and availability of the biodegradable organic carbon present in the raw water (Figure 4.3a and b and Table 4.3b). An increase in both concentration and availability of the biodegradable organic carbon were observed for pre-chlorination as well as for pre-ozonation. A higher concentration increase in AOC-P17 was observed for the pre-chlorination (312%) than for the pre-ozonation (171%) (Figure 4.3a). An explanation for this observation may be the fact that only *Pseudomonas fluorescens* strain P17 was used and that some of the ozonation by-products cannot be utilized by *Pseudomonas fluorescens* strain P17.

The Werner method showed an increase in both the f-Factor and the μ-value (Figure 4.3b) caused by the pre-chlorination and pre-ozonation. The μ-value for the pre-ozonated sample was, however, higher than that of the pre-chlorinated sample (average of 0.2816 and 0.1965 respectively). There was no big difference between the f-Factor values (average 16.35 and 15.93 respectively) or the DOC values (average 0.95 and 1.1 respectively) for the pre-ozonation or the pre-chlorination (Table 4.3a).

4.3.5 Conclusions

Both the pre-chlorination and pre-ozonation increased the concentration as well as the availability of the biodegradable organic matter. The difference in concentration increase of the biodegradable organic carbon measured by the f-Factor, (371% for pre-chlorination and 384% for pre-ozonation), is less than that indicated by AOC-P17 (312% and 171% respectively). The discrepancy between the results (Table 4.3b) of the two methods, could be explained by the fact that only *Pseudomonas fluorescens* strain P17 was used and not *Sprillum* sp strain NOX too. It is known that *Pseudomonas fluorescens* strain P17 cannot utilize some of the ozonation by-products during treatment. It is clear from the μ-value that pre-ozonation produced a much more biodegradable carbon compound (243%) than the pre-chlorination (139%)

To clarify the discrepancy with the AOC concentration increase for the two treatment processes, an experiment should be run where both *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX are used.

4.4 GENERAL CONCLUSIONS

Both the concentration and availability of the biodegradable organic carbon in the raw water, was increased by each of the different treatment processes under evaluation (Figures 4.1, 4.2 and 4.3). Although the silica/lime treatment resulted in a higher percentage (549%) increase in AOC-P17 in comparison with the other treatment processes, it was of low quality, only 1.15% increase in the μ-value of the raw water after treatment. Some of the other treatment processes (pre-ozonation) resulted in an increase of up to 243% for the μ-value (Table 4.1c, 4.2b and 4.3b)

Where silica/lime and lime/ferric chloride are used in practice for the treatment process, it has less of an effect on the increase of the concentration and availability of the biodegradable organic carbon compounds if used without pre-chlorination and/or pre-ozonation.

These conclusions were drawn on results obtained from bench tests used to simulate the treatment processes, but without being able to used the same retention time as in practise. Therefore analyses should be done on an operational plant where these treatment processes are in use. In order to obtain better comparative results for the AOC concentration, both *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX should be used in the Van der Kooij method.

CHAPTER 5

BIODEGRADABLE ORGANIC CARBON PRESENT DIRECTLY AFTER TREATMENT VS THE POSSIBLE FORMATION IN THE DISTRIBUTION NETWORK

This evaluation was done to determine whether any difference exists in the concentration and/or availability of biodegradable organic carbon present in the water, directly after treatment and the part that could possibly be formed in the distribution system due to the action of the oxidising agents, like chlorine, chloramine and ozone.

The following investigations were done:

- Primary vs secondary disinfection in practice, where chlorination and chloramination were used respectively. Water samples from the treatment plant and distribution network were analysed.

- Ozonation as primary disinfection. Bench tests were performed to evaluate ozone and a surplus dosage of ozone as primary disinfection and chlorination as secondary disinfection.

- Chloramination as secondary disinfection. Bench tests were performed to evaluate the effect of chloramination as secondary disinfection. The possible effect of the order in which chlorine and ammonia were added during the chloramination process, was also investigated.

5.1 PRIMARY VS SECONDARY DISINFECTION

5.1.1 Water samples

The following water samples were taken from the treatment plant and distribution network:

AC	-	after chlorination as primary disinfection
BCN	-	before chloramination as a distribution end point with primary, but without secondary disinfection
DEP	-	distribution end point with primary as well as secondary disinfection

The retention time between AC and BCN, as well as between BCN and DEP, were 6 to 8 h.
Refer to Diagram 2 for the complete flow diagram of the treatment process.

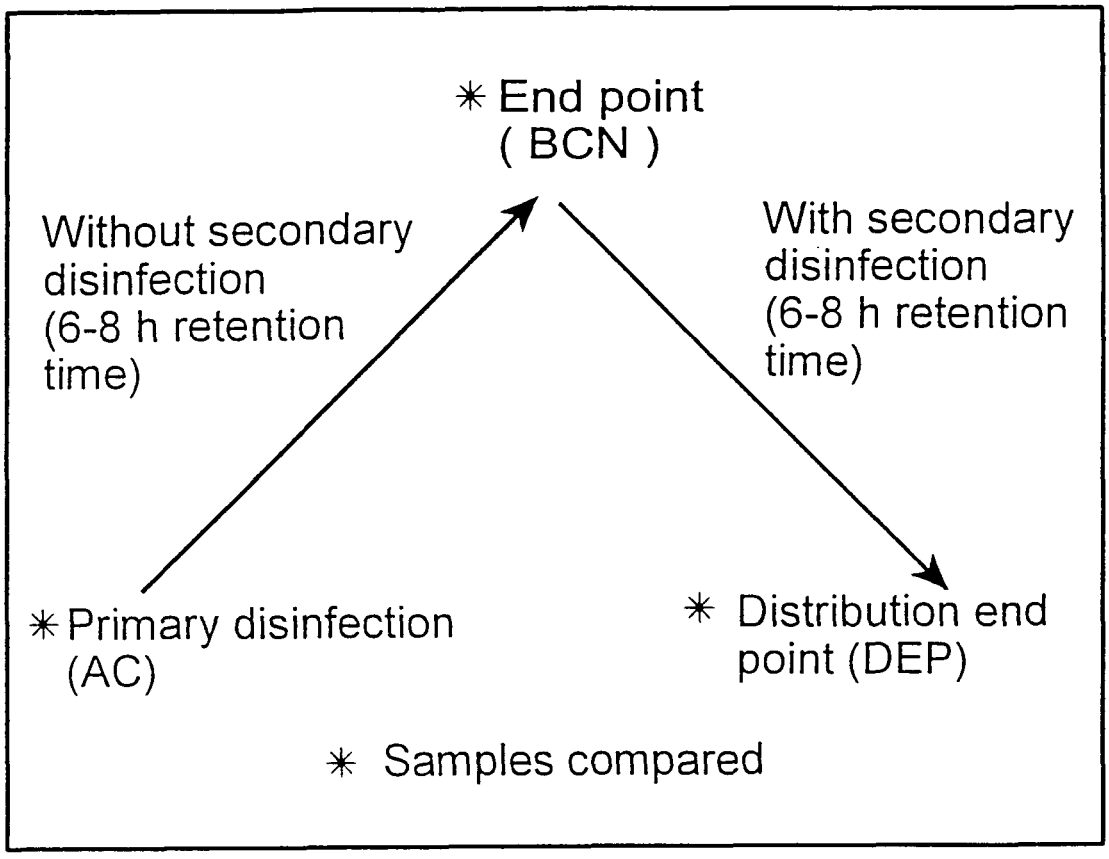


Diagram 5.1: **Sampling positions from primary disinfection, secondary disinfection through to a distribution endpoint**

5.1.2 Experimental work

All the water samples were treated with sodium thiosulphate to neutralise the effect of the chlorination and chloramination. The methods of Van der Kooij and Werner were used to evaluate the effect of primary and secondary disinfection on the concentration and availability of biodegradable organic carbon in the water. Only *Pseudomonas fluorescens* strain P17 was used as inoculum in the method of Van der Kooij. Chemical and DOC analyses were done on all the samples.

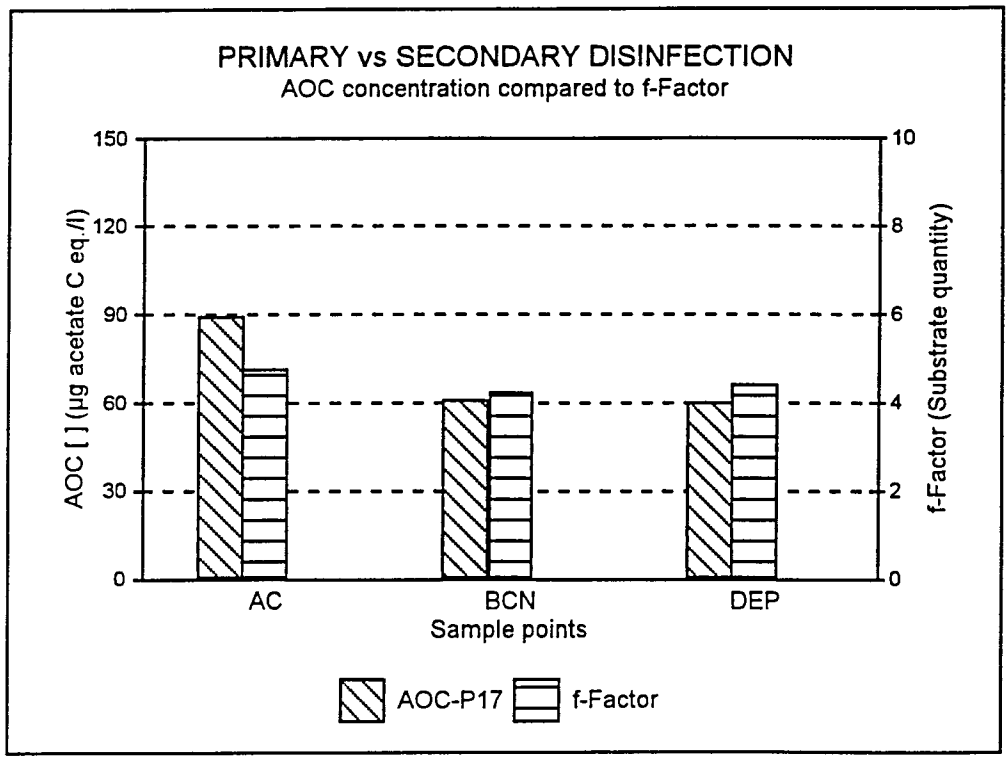
5.1.3 Results

Table 5.1a: Range for results obtained in the comparison of primary vs secondary disinfection.

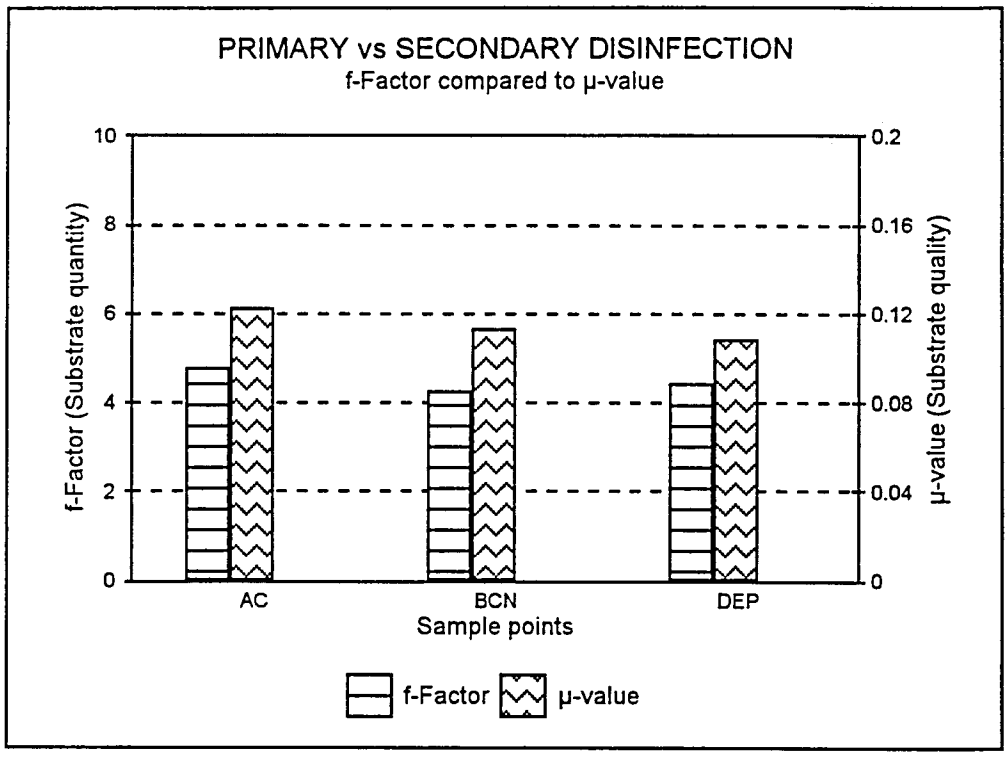
Sample		Parameter			
		AOC-P17 (μg acetate C eq/ℓ)	f-Factor	μ-value	Initial DOC (mg/ℓ)
AC	Min	36.36	3.09	0.0925	1.80
	Max	218.18	6.81	0.1815	4.1
	Avg	89.27	4.75	0.1220	2.63
BCN	Min	40.00	3.22	0.0380	1.70
	Max	103.64	5.72	0.1912	3.6
	Avg	60.55	4.24	0.1140	2.61
DEP	Min	36.36	2.49	0.0610	1.60
	Max	81.82	6.15	0.1592	3.7
	Avg	59.82	4.38	0.1089	2.38

Table 5.1b: Percentage change in AOC-P17, f-Factor, μ-value and Initial DOC caused by the different treatment processes towards a distribution end-point

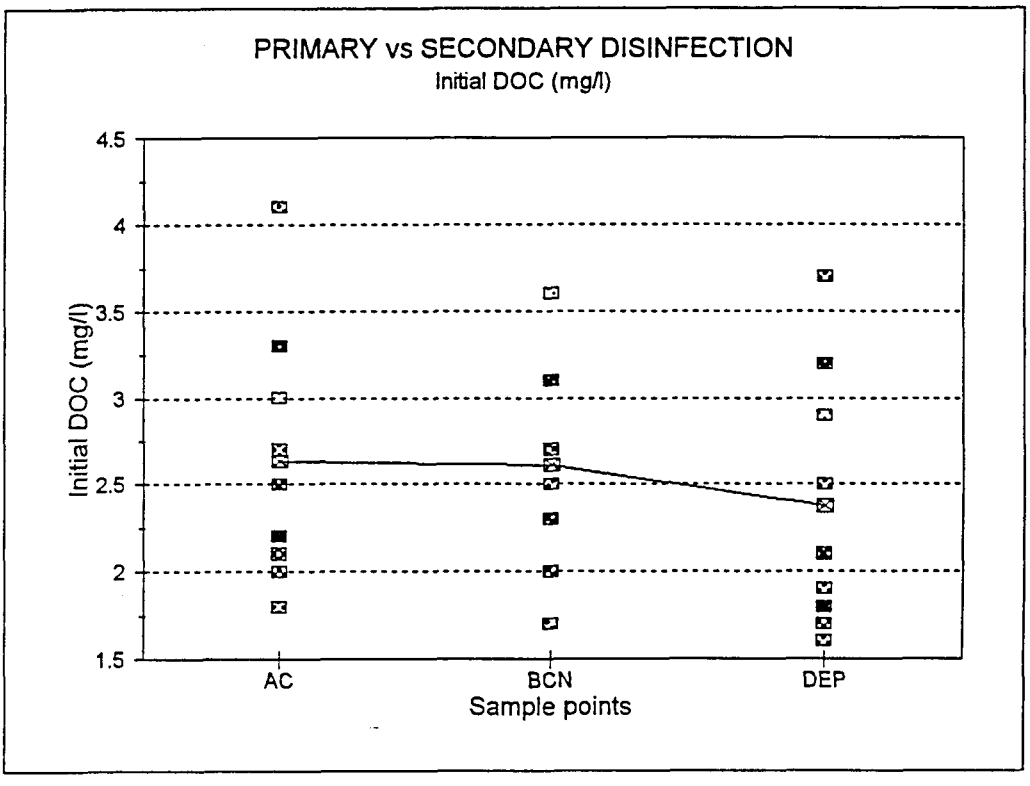
Source		Parameter			
		AOC-P17 (μg acetate C eq/ℓ)	f-Factor	μ-value	Initial DOC (mg/ℓ)
AC	BCN	-32.17%	-0.11%	-6.56%	-0.76%
	DEP	-32.99%	-7.79%	-10.74%	-9.51%
BCN	DEP	-1.21%	3.3%	-4.47%	-8.81%


PRIMARY vs SECONDARY DISINFECTION
AOC concentration compared to f-Factor
AOC [ ] (µg acetate C eq./l)
f-Factor (Substrate quantity)
150
120
90
60
30
0
10
8
6
4
2
0
AC
BCN
DEP
Sample points
AOC-P17
f-Factor

(a)


PRIMARY vs SECONDARY DISINFECTION
f-Factor compared to µ-value
f-Factor (Substrate quantity)
µ-value (Substrate quality)
10
8
6
4
2
0
0.2
0.16
0.12
0.08
0.04
0
AC
BCN
DEP
Sample points
f-Factor
µ-value

(b)



(c)

Figure 5.1: The effect of primary vs secondary disinfection on (a) AOC concentration compared to f-Factor (b) f-Factor compared to μ-value, and (c) Initial DOC value (Test period: 16-01-95 to 03-04-95).

5.1.4 Discussion

No significant formation of biodegradable organic carbon or change in the availability of the biodegradable organic carbon took place in the distribution system after primary or secondary disinfection (Table 5.1a and b and Figure 5.1a to c). A decrease in concentration (AOC-P17, f-Factor and Initial DOC) and availability (μ-value) was observed after primary (AC to BCN/DEP) and secondary (BCN to DEP) disinfection (Table 5.1b), except for a minor increase of 3.3% in the f-Factor after secondary disinfection. This increase may be due to the indigenous inoculum used for the Werner method, resulting in a better utilization of the available biodegradable organic carbon.

The DOC of the original samples (Initial DOC), showed a decrease (Table 5.1a and b and Figure 5.1c) after both primary (-0,76%) and secondary (-8,81%) disinfection.

5.1.5 Conclusions

It is clear that the AOC concentration either remains constant or declines in the distribution system. Thus the increases that may have taken place with primary disinfection (increase caused by chlorination - refer back to chapter 2 and 3) are not escalated through the distribution system. From this it is concluded that if any bacterial regrowth problems occured in the distribution system, it could be the result of biodegradable organic carbon formed during the treatment process and remaining present after primary disinfection.

5.2 OZONATION AS PRIMARY DISINFECTION

5.2.1 Water samples

A water sample was taken at the filter outlet, after sand filtration (FO) (See Diagram 2). The sample was split into sub-samples (Diagram 5.2) of which some were treated to give the following:

FO	-	sample directly after sand filtration, used as blank reference sample.
Surplus O_3	-	FO treated with a surplus dosage of ± 5mg O_3/ℓ.
O_3	-	FO treated with ± 2 mg O_3/ℓ.
$O_3 + Cl_2$	-	A split sample of O_3 treated with chlorine as secondary disinfectant to give a total residual chlorine value of ± 0.97 mg/ℓ after ± 4 h contact time.

A contact period of ± 24 h was used to simulate contact time as in a distribution network towards an endpoint consumer.

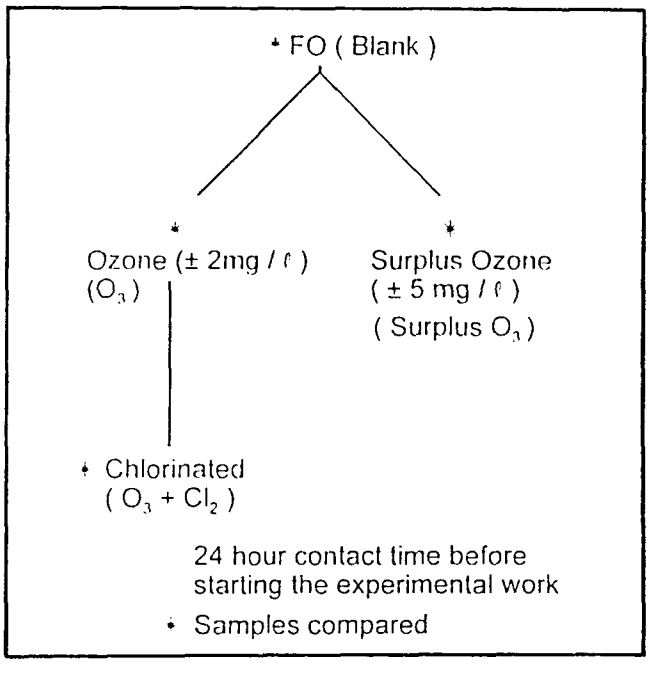


Diagram 5.2: Flow diagram of the simulated treatment process using ozonation as primary disinfection.

5.2.2 Experimental work

Water samples to be examined by the Van der Kooij (both *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX were used) and Werner methods were prepared in the laboratory. All the samples were treated with sodium thiosulphate to neutralise the ozone and chlorine. Chemical and DOC analysis were performed on these samples.

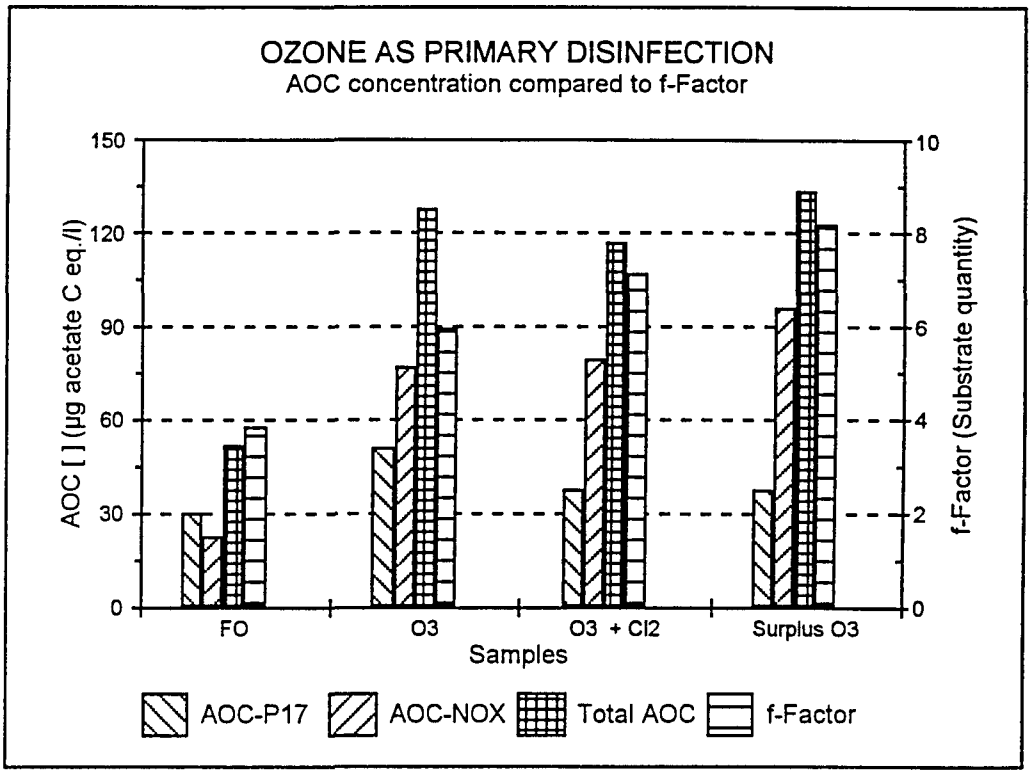
5.2.3 Results

Table 5.2a: Range for results obtained with ozonation as primary disinfection.

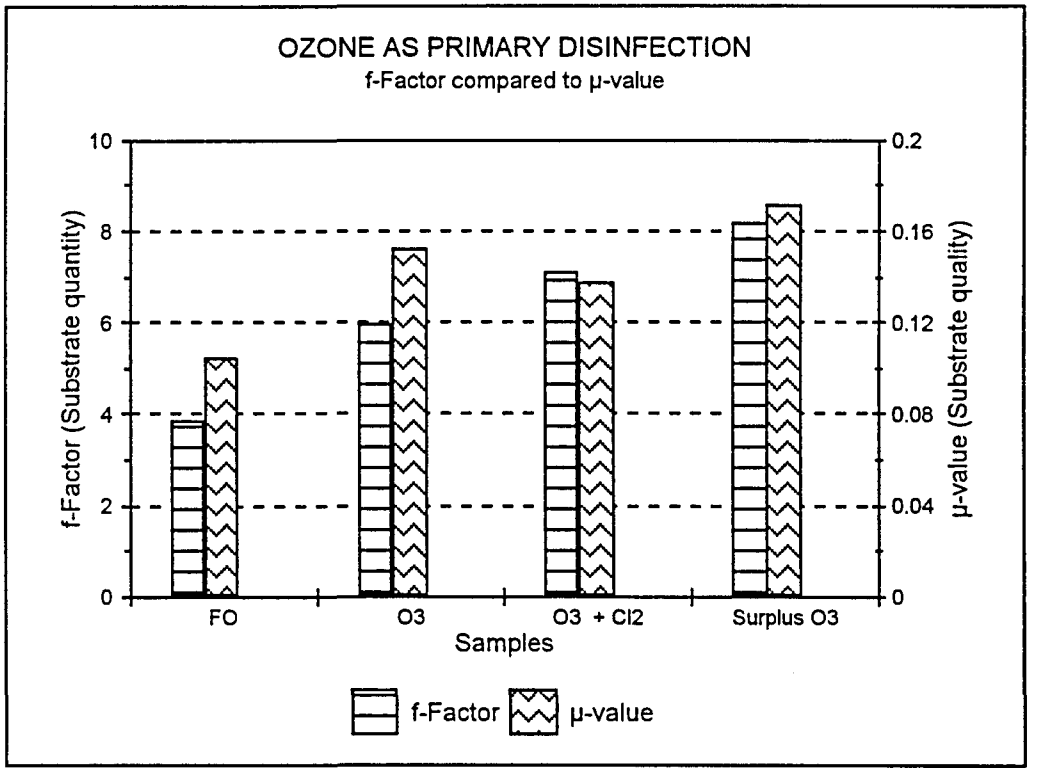
Sample		Parameter					
		AOC-P17 (μg acetate C eq/ℓ)	AOC-NOX (μg acetate C eq/ℓ)	Total AOC (μg acetate C eq/ℓ)	f-Factor	μ-value	Initial DOC (mg/ℓ)
FO	Min	11.3	13.2	30.2	0.87	0.0068	1.4
	Max	43.8	43.4	77.8	5.13	0.1363	1.8
	Avg	30.02	22.02	52.03	3.84	0.1045	1.60
O_3	Min	18.8	40.6	75	3.91	0.1026	1.5
	Max	78.1	122.6	188.2	8.35	0.2039	2.4
	Avg	50.78	76.55	127.33	5.93	0.1517	1.86
O_3 + Cl_2	Min	14.1	59.4	83.9	3.84	0.106	1.4
	Max	70.3	122.6	164.8	10.76	0.1892	2.1
	Avg	37.25	79.38	116.63	7.10	0.1371	1.80
Surplus O_3	Min	21.9	39.6	61.5	4.05	0.1066	1.4
	Max	54.7	169.8	221.4	16.69	0.2548	3.6
	Avg	37.77	96.23	134.0	8.20	0.1713	2.14

Table 5.2b: Percentage change in AOC concentration, f-Factor, μ-value and Initial DOC caused by different treatments with ozone.

Source	Treat-Ment	Parameter					
		AOC-P17 (μg acetate C eq/ℓ)	AOC-NOX (μg acetate C eq/ℓ)	Total AOC (μg acetate C eq/ℓ)	f-Factor	μ-value	Initial DOC (mg/ℓ)
FO	O_3	69.2%	247.6%	144.7%	54.4%	45.2%	16.3%
	O_3 + Cl_2	24.1%	260.5%	124.2%	84.9%	31.2%	12.5%
	Surplus O_3	25.8%	337.0%	157.5%	113.5%	63.9%	33.8%
O_3	O_3 + Cl_2	-26.6%	3.7%	-8.4%	19.7%	-9.6%	-3.2%



(a)



(b)

Figure 5.2: Ozonation as primary disinfection on (a) AOC concentration compared to f-Factor, and (b) f-Factor compared to μ-value (Test period: 20-06-95 to 02-08-95).

5.2.4 Discussion

When ozonation is used for primary disinfection, a definite increase in concentration as well as the availability of biodegradable organic carbon (Figure 5.2a and b and Table 5.2a and b). The initial DOC concentration of the samples showed an increase after ozonation. However a small decrease (-3.2%) after chlorination of the ozonated sample, was observed (Table 5.2b).

According to Van der Kooij and Hijnen (1984), chlorination usually caused an increase in the AOC concentration, but in most cases the average relative biodegradability was reduced. This can be seen between O_3 and $O_3 + Cl_2$, where the f-Factor increased by 19.7%, while the μ-value decreased by 9.6% (Table 5.2b). This was not clear in the AOC concentration or initial DOC values. The DOC value actually decreased from 1.86 to 1.80 and AOC-P17 decreased from 50.78 to 37.25, while the AOC-NOX increased from 76.55 to 79.38 (Table 5.2a). Huck *et al.* (1991) found that ozonation increased and decreased the AOC concentration - AOC-NOX always increased whereas AOC-P17 could increase or decrease.

5.2.5 Conclusions

Ozonationation as primary disinfection causes a definite increase in both the concentration and availability of biodegradable organic carbon. As these determinations were done in a bench test, simulating the treatment process (contact time not exactly the same), more work on an actual plant and distribution network will be necessary to confirm these findings in practice. At this stage, ozonation is not a very common practice in water treatment in South Africa. Only experimental plant systems are usually available, except for the plant at Western Transvaal Regional Water Company and the Wiggins Water Works of Umgeni Water, where pre-ozonation is used.

5.3 CHLORAMINATION AS SECONDARY DISINFECTION

Bench tests were performed to evaluate the effect of chloramination as secondary disinfection on the possible formation of biodegradable organic carbon directly after treatment. The possible effect of the order in which chlorine and ammonia were added, was also investigated.

5.3.1 Water samples

A water sample was taken directly after chlorination (AC). The sample was split into four sub-samples (Diagram 5.3) of which three were treated to give the following:

AC	-	sample directly after chlorination (average 1.26 mg Cl_2/ℓ prechlorination)
Cl + N	-	AC treated with an average 1.1 mg Cl_2/ℓ and 4.6 mg ammonia/ℓ - in that order.
N + Cl	-	AC treated with an average 4.6 mg ammonia/ℓ and 1.1 mg Cl_2/ℓ - in that order.
Cl + h-N	-	AC treated with an average 1.1 mg Cl_2/ℓ and 6.9 mg ammonia /ℓ - in that order with a higher dosage of ammonia.

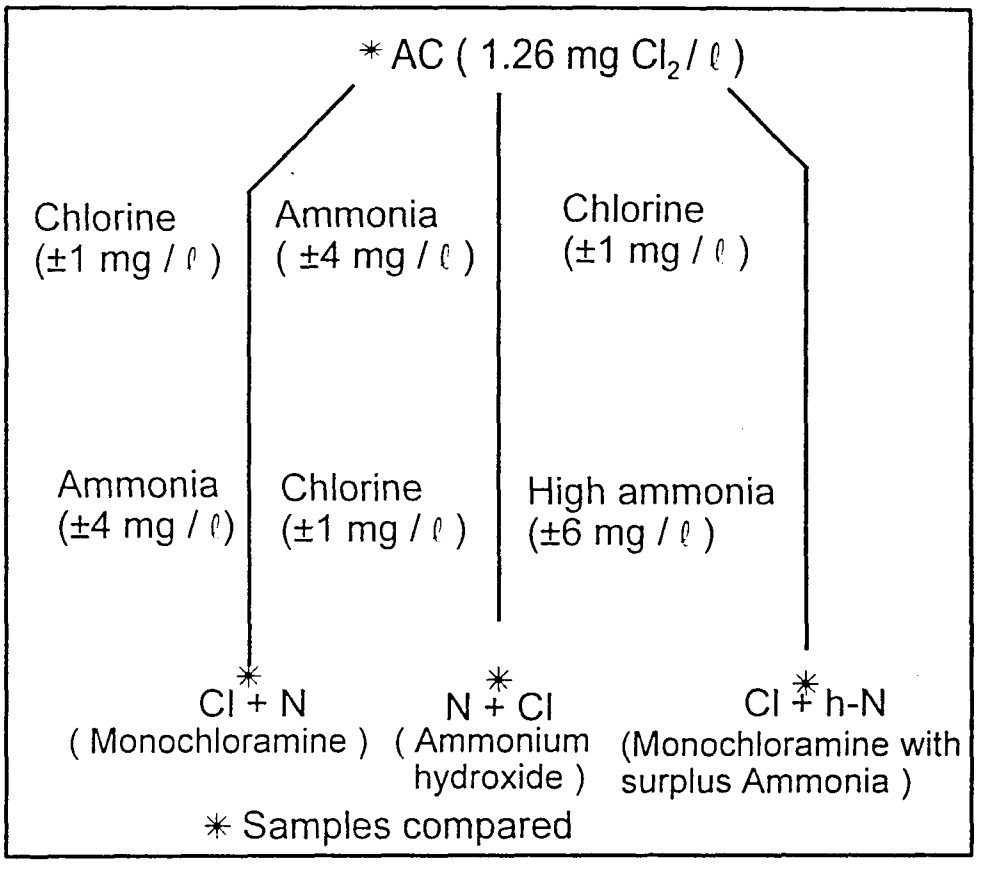


Diagram 5.3 Flow diagram simulating the sample treatment with chloramination as secondary disinfection.

5.3.2 Experimental work

All the water samples were treated with sodium thiosulphate to neutralise the effect of the chlorination and chloramination. The Van der Kooij and Werner methods were used to do the determinations for the biodegradable organic carbon. Both *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX were used as inoculum for the Van der Kooij method. Chemical and DOC analysis were performed on the samples.

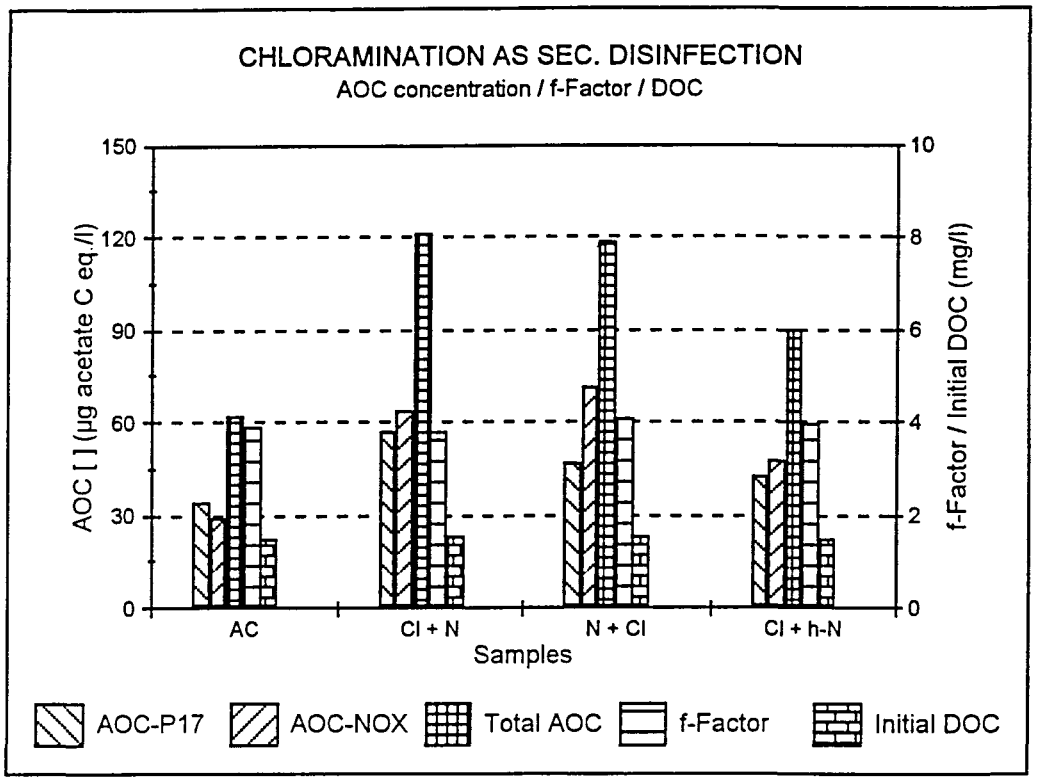
5.3.3 Results

Table 5.3a: Range for results obtained with chloramination as secondary disinfection.

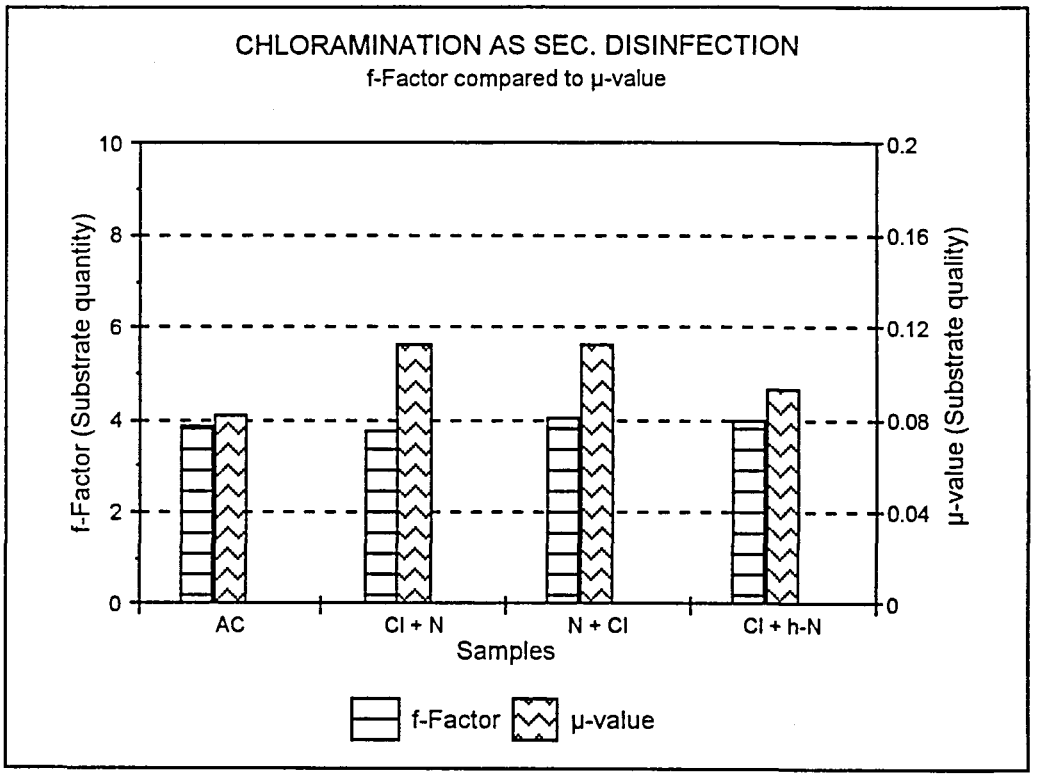
Sample		Parameter					
		AOC-P17 (μg acetate C eq/ℓ)	AOC-NOX (μg acetate C eq/ℓ)	Total AOC (μg acetate C eq/ℓ)	f-Factor	μ-value	Initial DOC (mg/ℓ)
AC	Min	25.0	21.7	53.9	1.25	0.0271	1.0
	Max	42.3	37.7	67.7	6.08	0.1168	1.7
	Avg	33.46	28.48	61.94	3.89	0.0816	1.44
Cl + N	Min	45.3	52.8	98.1	1.71	0.0757	1.2
	Max	82.8	83.0	165.8	4.93	0.1589	2.0
	Avg	56.86	63.78	120.64	3.76	0.1130	1.54
N + Cl	Min	31.3	69.8	97.2	1.84	0.0799	1.1
	Max	73.4	82.1	143.2	6.61	0.1492	2.0
	Avg	46.56	71.44	118.08	4.06	0.1125	1.52
Cl + h-N	Min	29.7	32.1	61.8	1.63	0.0496	1.2
	Max	59.4	57.5	116.9	6.55	0.1366	1.8
	Avg	42.52	47.54	90.06	3.97	0.0928	1.44

Table 5.3b: Percentage change in AOC concentration, f-Factor, μ-value and Initial DOC caused by chloramination.

Source	Treat-Ment	Parameter					
		AOC-P17 (μg acetate C eq/ℓ)	AOC-NOX (μg acetate C eq/ℓ)	Total AOC (μg acetate C eq/ℓ)	f-Factor	μ-value	Initial DOC (mg/ℓ)
AC	Cl + N	69.9%	123.9%	94.8%	-3.3%	38.5%	6.9%
	N + Cl	39.2%	150.8%	90.6%	4.4%	37.9%	5.6%
	Cl +h-N	27.1%	66.9%	45.4%	2.1%	13.7%	0%



(a)



(b)

Figure 5.3: **Chloramination as secondary disinfection on (a) AOC concentration compared to f-Factor and DOC and (b) f-Factor compared to μ-value (Test period: 30-08-95 to 25-10-95).**

5.3.4 Discussion

The total AOC concentration increased, no matter in which order the chlorine and ammonia were added (Table 5.3a and b and Figure 5.3a). The same trend was observed with the μ-value (Table 5.3a and b and Figure 5.3b), indicating that a more biodegradable substrate was formed. Although the Cl + h-N treatment increased both concentration and availability of the biodegradable organic carbon, the percentage increase was less than that obtained with the Cl + N and N + Cl treatments (Table 5.3b). The initial DOC concentration also increased 6.9% and 5.6% respectively for the Cl + N and N + Cl treatment, but no increase took place with the Cl + h-N treatment (Table 5.3b). The f-Factor stayed almost constant with a 3.3% decrease after the Cl + N treatment. This difference could probably be due to the type of organisms present in the indigenous inoculum used in the Werner method.

5.3.5 Conclusions

Chloramination caused an increase in the biodegradable organic carbon directly after treatment. More detailed work on the treatment plant will be necessary to confirm this observation and to compare the concentration directly after treatment, to that at a distribution end point.

5.4 GENERAL CONCLUSIONS

Both ozonation and chloramination caused an increase in the concentration of biodegradable organic carbon in water, directly after the treatment process (Sections 5.2 and 5.3). Although ozonation as primary disinfection increased the concentration and availability of the biodegradable organic carbon, chlorination as secondary disinfection has a decreasing effect on the biodegradability of the carbon source. Although chloramination caused an increase directly after treatment (Section 5.3), it seemed to be reduced as water moved through the distribution system (Section 5.1).

CHAPTER 6

ACTIVATED CARBON FOR THE REMOVAL OF BIODEGRADABLE ORGANIC CARBON

In this part of the project, an investigation on activated carbon as part of the treatment process, was done. The effect of activated carbon on the possible removal, and/or change in availability of biodegradable organic carbon in the water, were determined.

Samples were taken from a small water purification plant which included granular activated carbon (GAC) treatment after sand filtration, before chlorination. The three GAC contactors were arranged in series and cross connected in such a way that the column with freshly reactivated carbon, was at the end of the series. Lime and alum were used in the coagulation/flocculation process (Diagram 6). Information about the carbon columns is given in Appendix I.

6.1 WATER SAMPLES

The following water samples were taken:

Before - after sand Filtration, before the GAC columns
GAC_1 - sample point between columns 1 and 2
GAC_2 - sample point between columns 2 and 3
GAC_3 - sample point after column 3, before chlorination

Samples were taken from various points in a small water purification plant which included granular activated carbon (GAC) treatment in three contactors arranged in series.

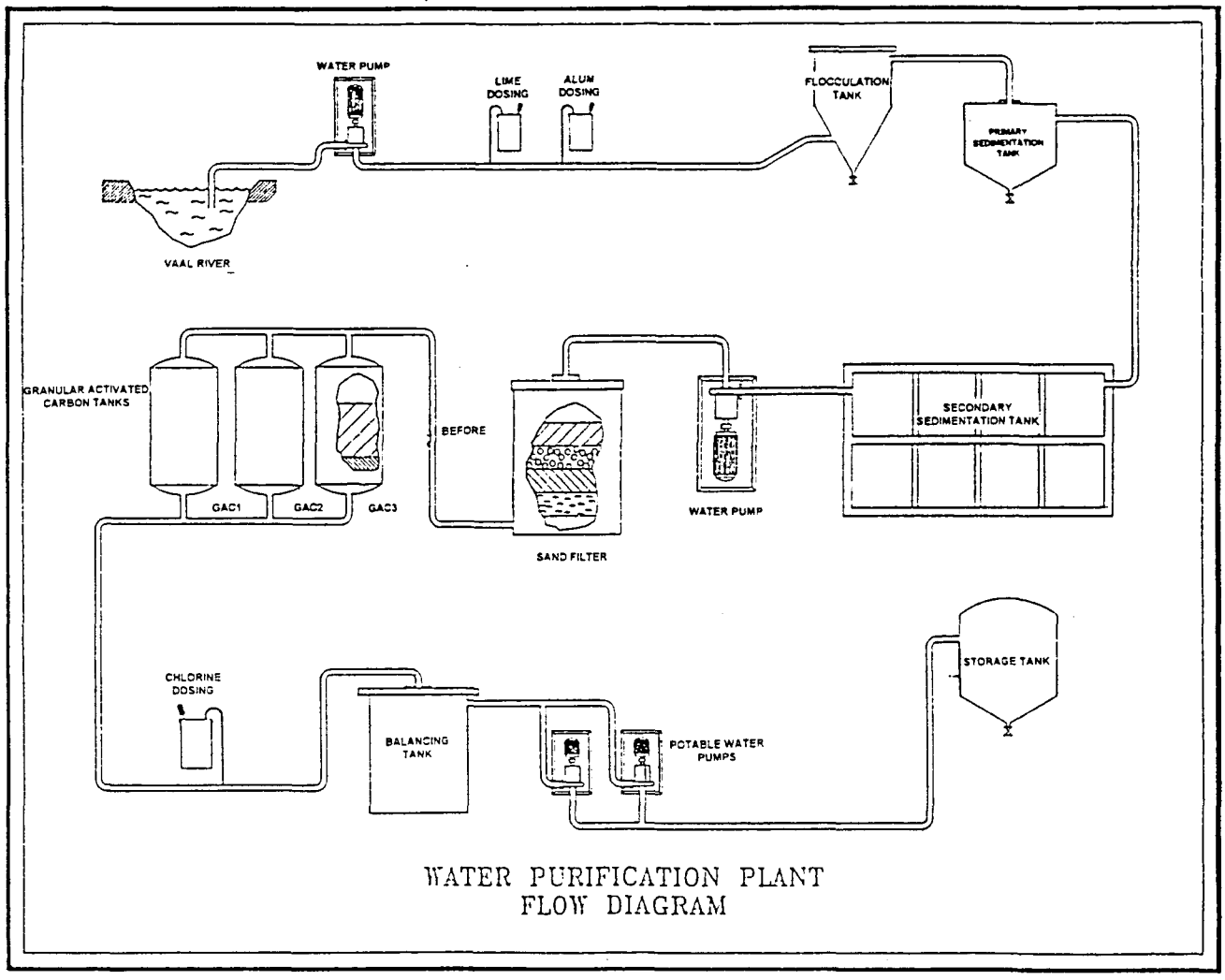


Diagram 6: Flow diagram of treatment plant.

6.2 EXPERIMENTAL WORK

Analysis on these water samples were done with the Werner and Van der Kooij-methods. Both *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX were used in the Van der Kooij method. Chemical analysis and DOC analysis were done.

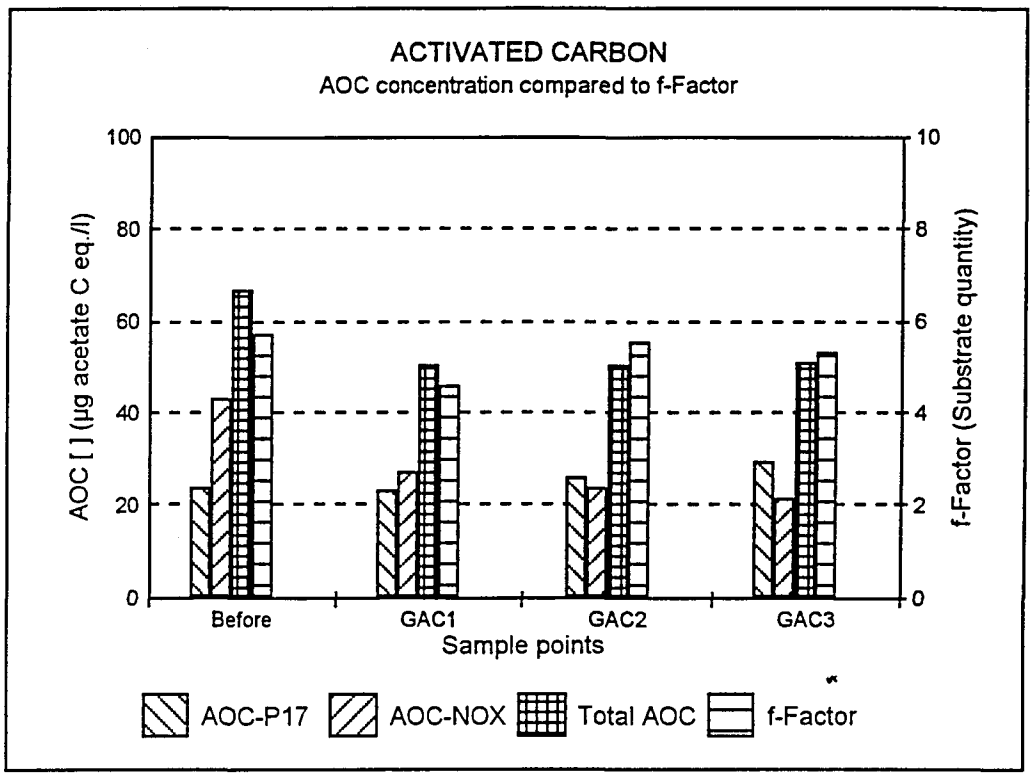
6.3 RESULTS

Table 6a : Range for results obtained with activated carbon in the removal of biodegradable organic carbon

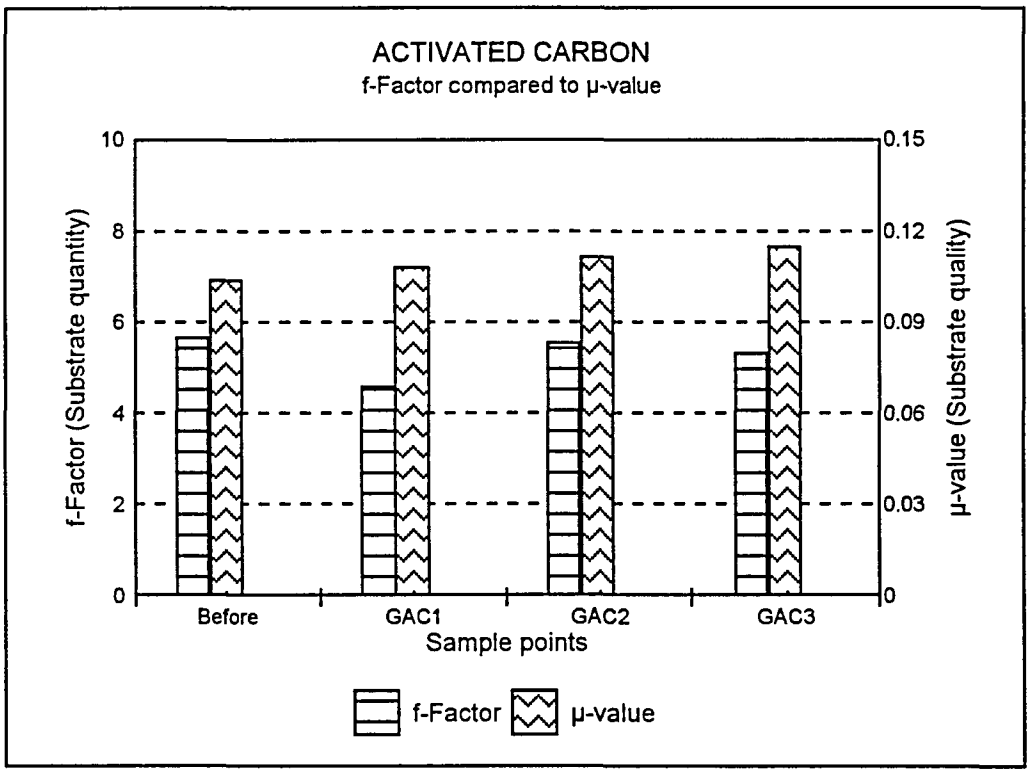
Sample		Parameter					
		AOC-P17 (μg acetate C eq/ℓ)	AOC-NOX (μg acetate C eq/ℓ)	Total AOC (μg acetate C eq/ℓ)	f-Factor	μ-value	Initial DOC (mg/ℓ)
Before	Min	18.8	27.4	47.7	3.73	0.0748	2.0
	Max	37.5	53.8	83.7	7.84	0.1425	4.7
	Avg	23.7	42.9	66.65	5.68	0.1038	3.12
GAC_1	Min	17.2	23.6	43.6	3.29	0.0851	0.99
	Max	39.1	30.2	62.7	6.29	0.1354	4.2
	Avg	23.14	26.8	49.94	4.59	0.1081	2.17
GAC_2	Min	15.6	19.8	40.1	3.17	0.0683	0.99
	Max	32.8	28.3	59.2	7.11	0.1596	1.7
	Avg	26.03	23.9	49.93	5.52	0.1113	1.38
GAC_3	Min	13.3	15.1	28.4	2.93	0.0757	0.99
	Max	53.1	30.2	74.8	6.82	0.1698	1.7
	Avg	29.57	21.38	50.95	5.29	0.1144	1.18

Table 6b: Percentage change in AOC concentration, f-Factor, μ-value and Initial DOC caused by activated carbon in the treatment process.

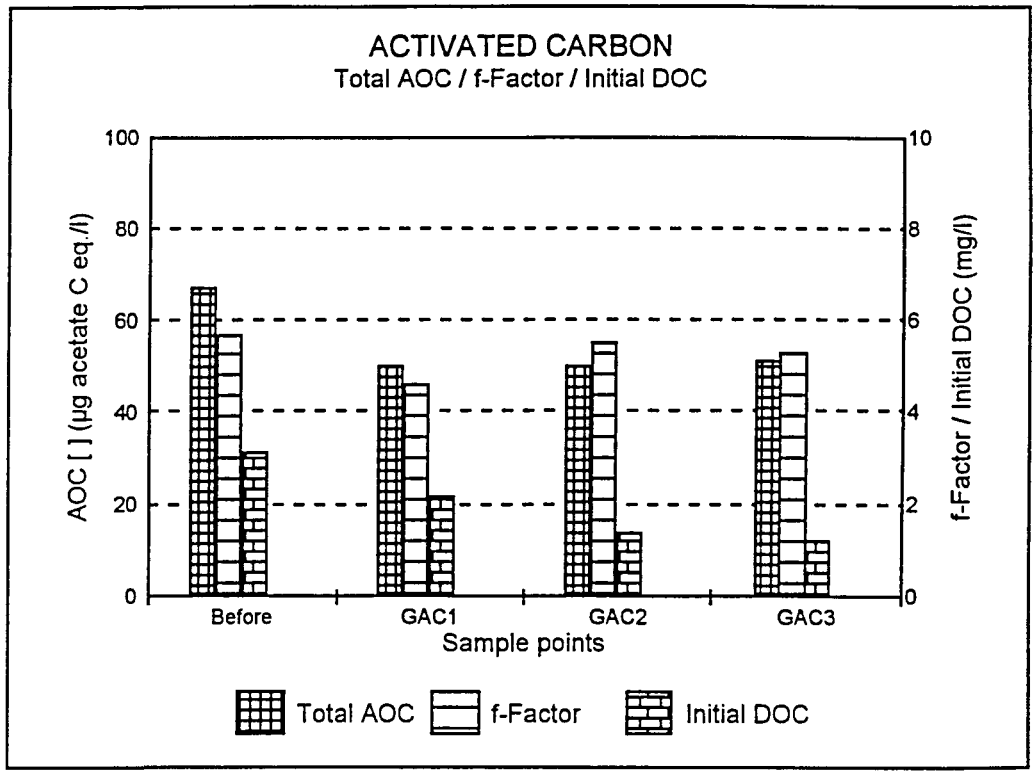
Source		Parameter					
		AOC-P17 (μg acetate C eq/ℓ)	AOC-NOX (μg acetate C eq/ℓ)	Total AOC (μg acetate C eq/ℓ)	f-Factor	μ-value	Initial DOC (mg/ℓ)
Before	GAC_1	-2.36%	-37.53%	-25.07%	-19.10%	4.14%	-30.45%
	GAC_2	9.83%	-44.29%	-25.09%	-2.82%	7.23%	-55.77%
	GAC_3	24.77%	-50.16%	-23.56%	-6.87%	10.21%	-62.18%
GAC_1	GAC_2	12.49%	-10.82%	-0.02%	20.26%	2.96%	-36.41%
	GAC_3	27.79%	-20.22%	2.02%	15.25%	5.83%	-45.62%
GAC_2	GAC_3	13.6%	-10.54%	2.04%	-4.17%	2.79%	-14.49%


ACTIVATED CARBON
AOC concentration compared to f-Factor
AOC [ ] (µg acetate C eq./l)
f-Factor (Substrate quantity)
100
80
60
40
20
0
10
8
6
4
2
0
Before
GAC1
GAC2
GAC3
Sample points
AOC-P17
AOC-NOX
Total AOC
f-Factor

(a)


ACTIVATED CARBON
f-Factor compared to µ-value
f-Factor (Substrate quantity)
µ-value (Substrate quality)
10
8
6
4
2
0
0.15
0.12
0.09
0.06
0.03
0
Before
GAC1
GAC2
GAC3
Sample points
f-Factor
µ-value

(b)



(c)

Figure 6: Activated carbon for the removal of biodegradable organic carbon during treatment, as reflected in (a) AOC concentration compared to f-Factor, (b) f-Factor compared to μ-value and (c) total AOC/f-Factor and Initial DOC (Test period: 26-06-95 to 07-08-95).

6.4 DISCUSSION

The GAC decreased the concentration of biodegradable organic carbon, but increased the availability of these carbon compounds (Table 6a and b and Figure 6a to c). From the inlet ("Before") to the GAC columns, towards the outlet of each column (GAC_1, GAC_2, and GAC_3) a definite decrease in concentration of biodegradable organic carbon was observed for AOC-NOX, Total AOC, f-Factor and Initial DOC. With AOC-P17 a decrease of 2.36% was observed for the first column (GAC_1) but increases of 9.83% and 24.77% respectively for GAC_2 and GAC_3. The percentage change between columns varied. For AOC-P17, increases took place, while the AOC-NOX constantly decreased. The accumulative effect on the Total AOC was an insignificant decrease of 0.02% between GAC_1 and GAC_2, with a slight increase of 2.04% between GAC_2 and GAC_3.

Almost the same trend was observed with the f-Factor with a 19.19% decrease from "Before" to GAC_1, but with an increase of 20.26% between GAC_1 and GAC_2 with another decrease of 4.17% between GAC_2 and GAC_3. Although a definite decrease took place in the DOC concentration (Table 6a and b), some change took place in the biodegradability of those carbon compounds as the μ-value constantly increased

towards the end of column 3 (Table 6b and Figure 6b). Although filters 1 and 2 were already in operation for 12 and 10 months respectively, while filter 3 was only in operation for 3 months, it seemed that most of the biodegradable organic carbon was removed by the first filter, irrespective of the operational age, in this case 12 months.

6.5 CONCLUSIONS

The concentration of biodegradable organic carbon in the treated water (after flocculation, sedimentation and sand filtration) decreased with the use of GAC in the treatment process. In contrary to this initial decrease in concentration, the availability of the biodegradable organic carbon compounds increased after each GAC column (μ-value ≈ 4.14% - GAC_1; 7.23% - GAC_2 and 10.21% - GAC_3). An increase in the OAC-P17 (12.49%) and f-Factor (20.26%) occurred after the second GAC cp:i,m, with another increase in the AOC-P17 (13.6%) after the third GAC column.This observation may be the result of changes that have taken place in the GAC columns due to biological activity. As most of the biodegradable organic carbon was removed by the first GAC column, irrespective of its operational age, a logical conclusion will be to use GAC most recently reactivated in this first column. These aspects should be investigated in more detail to be able to make any significant recommendations for the use of GAC in a treatment plant with the main aim of removal of biodegradable organic carbon from the treated water.

CHAPTER 7

A POSSIBLE MINIMUM AOC VALUE AT WHICH NO REGROWTH WOULD BE EXPECTED.

In this part of the project it was attempted to determine a possible minimum concentration of biodegradable organic carbon at which no growth of heterotrophic or coliform bacteria, especially *Aeromonas* and *Pseudomonas* species, would be expected.

It was decided to isolate *Aeromonas* and *Pseudomonas* species from a raw water source (RW), as these organisms were generally isolated and identified in the routine laboratory, especially from mEndo agar when a high number of atipical background growth occurred some times without any typical total coliforms. *Pseudomonas* species are also known to be utilisers of almost any type of carbon source. Therefore, if a possible minimum concentration of biodegradable organic carbon can be determined at which *Pseudomonas* species do not grow, the assumption can be made that little or no growth of other bacteria could be expected.

7.1 ISOLATION AND IDENTIFICATION OF *AEROMONAS* AND *PSEUDOMONAS* SPECIES FROM DRINKING WATER.

7.1.1 Isolation

Isolation was done by means of membrane filtration. Pseudomonas Agar Base with CN supplement for the isolation of *Pseudomonas* spp and Ryan medium for the isolation of *Aeromonas* spp were used as isolation media. Duplicate sets of different aliquotes or volumes, ranging from 0.1 to 50 mℓ of raw water were filtered through 0.45 μm cellulose nitrate membranes, which were placed on the agar plates. The agar plates were incubated at 35 $\pm$ 2 °C for 24 to 48 h.

7.1.2 Verification and identification

Presumptive *Pseudomonas* and *Aeromonas* colonies were identified, using the following tests:

* **Gram stains**: To separate Gram positive from Gram negative bacteria. *Aeromonas* and *Pseudomonas* spp are Gram negative.

* **Oxidase test**: To determine the presence of the oxidase enzyme, It is used to separate the Pseudomonadaceae from the oxidase - negative members of the Enterobacteriaceae (MacFaddin, 1980). *Aeromonas* and *Pseudomonas* spp are oxidase positive.

* **Catalase test**: To test for the presence of the enzyme, catalase. Primarily used to differentiate between genera and to aid in species differentiation (MacFaddin, 1880). *Aeromonas* and *Pseudomonas* spp are catalase positive.

* **Indole**: To determine the ability of an organism to split indole from the tryptophane molecule. To aid in differentiation between genera and between species (MacFaddin, 1980). *Aeromonas* is indole positive and *Pseudomonas* is negative.

* **β-galactosidase (ONPG)**: To demonstrate the presence or absence of the enzyme β-galactosidase by utilizing the organic compound o-nitrophenyl β-D-galactophyranoside (ONPG). To differentiate lactose-delayed organisms from lactose-negative organisms. To aid in species differentiation: *Pseudomonas cepacia* (positive) and *Pseudomonas maltophila* (positive) from other *Pseudomonas* species (negative) (MacFaddin, 1980). *Aeromonas* is ONPG positive.

* **OF (oxidation/fermentation test):** To determine the oxidative or fermentative metabolism of a carbohydrate or its non utilization. Primarily to differentiate nonenteric, gram negative, intestinal genera from the Enterobacteriaceae (MacFaddin, 1980). *Aeromonas* is fermentative and *Pseudomonas* is oxidative.

* *Aeromonas* test on AH medium: For the further indentification of the genus *Aeromonas* (Kaper *et al.*, 1979).

* **API 20NE system:** For further identification. It is a standardized micromethod combining eight conventional and twelfth assimilation tests for the identification of non-fastidious Gram-negative rods not belonging to the Enterobacteriaceae for example *Pseudomonas* and *Aeromonas.*

7.1.3 Results

Aeromonas sobria and *Pseudomonas fluorescens* were isolated and identified. These two isolates were used as inoculum in both the methods of Van der Kooij and Werner.

7.2 EXPERIMENTAL TESTS OF PURIFIED ISOLATES.

Two groups of samples were used in conducting the tests. The first group of samples consisted of a laboratory tap water sample(treated water of the same quality as the AC-after chlorination sample) that was "diluted" with freshly distilled water (7.2.1). The second group of samples consisted of a laboratory tap water sample to which three different concentrations of acetate were added (7.2.2).

7.2.1 Treated water diluted with freshly distilled water.

7.2.1.1 Water Samples

A water sample was collected at the laboratory tap and dechlorinated by addition of sodium thiosulphate. Four different samples were prepared:

kH_2O = sample without any dilution.
1:1 = 500 mℓ sample + 500 mℓ distilled water.
1:10 = 100 mℓ sample + 900 mℓ distilled water.
1:100 = 10 mℓ sample + 990 mℓ distilled water.

7.2.1.2 Experiment

The methods of Van der Kooij and Werner were used. One set of samples were inoculated with the *Pseudomonas fluorescens* isolates and another set of samples were inoculated with the *Aeromonas sobria* isolates, instead of the P17/NOX culture for the Van der Kooij and an indigenous inoculum for the Werner method. The samples in the Van der Kooij method were plated in triplicate. The cultures were prepared in the same way as was done with the P17 and NOX cultures. TOC analysis were done on samples using the Werner method - at the beginning and end of each experiment.

7.2.1.3 Results

The yield factor of *Pseudomonas* - P17 (6.4×10^6) was used to calculate the AOC concentration for results obtained with the Van der Kooij method. Average AOC concentration values are given in Table 7.1

Table 7.1: Results obtained for *Aeromonas sobria* and *Pseudomonas fluorescens* in diluted treated water.

Test Organism	Sample	Parameters					
		AOC [ ] (μg acetate C eq/ℓ)	f-Factor	μ-value	TOC-Begin (mg/ℓ)	TOC-End (mg/ℓ)	Δ TOC (mg/ℓ)
Aeromonas sobria	kH_2O	8.44	0.92	0.0143	5.45	4.77	0.68 (12.48%)
	1:1	12.97	1.31	0.0336	3.17	2.94	0.23 (7.26%)
	1:10	NG	1.01	0.0059	1.41	1.12	0.29 (20.27%)
	1:100	NG	0.58	-0.0007	1.11	0.86	0.24 (21.62%)
Pseudomonas fluorescens	kH_2O	62.5	4.9	0.2929	5.45	4.81	0.64 (11.74%)
	1:1	95.31	4.82	0.2809	3.17	2.72	0.45 (14.2%)
	1:10	32.81	2.53	0.2042	1.31	0.93	0.48 (34.04%)
	1:100	NG	2.08	0.1364	1.11	0.47	0.64 (57.66%)

NG = No growth. ΔTOC = TOC-Begin - TOC-End

7.2.1.4 **Discussion and conclusions**

Low AOC and f-Factor values were obtained for *Aeromonas sobria*. With the two higher dilutions no growth occurred. The very low μ-values indicated that the present substrate was not biodegradable. The TOC values at the beginning of the experiment, showed a decreasing trend with an increasing dilution, while an increasing ΔTOC was observed. It means that although there was less substrate available, a bigger portion of it was available for growth.

Much higher AOC values were obtained with *Pseudomonas fluorescens* than with *Aeromonas sobria*. This trend was confirmed by the f-Factor values and much higher μ-values. The same trend was observed for the TOC analysis with *Aeromonas sobria*. The fact that the *Pseudomonas* culture produced higher values than the *Aeromonas* culture, confirmed the findings that *Pseudomonas* species can utilise a wider variety of carbon sources at a lower concentration than some of the other organisms.

It was concluded that the carbon source for *Aeromonas* in water can possibly be diluted to such an extent, that no growth will occur, while for *Pseudomonas* a much higher dilution point will be required. Thus while

regrowth problems could be experienced with *Aeromonas*, problems could still occur where *Pseudomonas* are present.

7.2.2 Low concentrations acetate added to treated water.

7.2.2.1 Water samples

A water sample was collected at the laboratory tap and dechlorinated by addition of sodium thiosulphate.Four different samples were prepared:

0 μg = sample without any added acetate carbon
3 μg = sample plus 3 μg acetate C/ℓ
6 μg = sample plus 6 μg acetate C/ℓ
12 μg = sample plus 12 μg acetate C/ℓ

7.2.2.2 Experiment

The same procedure was used as in 7.2.1.2.

7.2.2.3 Results

The yield factor of *Pseudomonas* P17 (6.4 x 10^6) was also used in calculations done on the results for the AOC concentrations. Average AOC values were given in Table 7.2

Table 7.2: Results obtained for *Aeromonas sobria* and *Pseudomonas fluorescens* in different concentrations acetate carbon added to treated water

Test Organism	Sample	Parameters					
		AOC [ ] (μg acetate C eq/ℓ)	f-Factor	μ-value	TOC-Begin (mg/ℓ)	TOC-End (mg/ℓ)	Δ TOC (mg/ℓ)
Aeromonas sobria	0μg	7.03	9.01	0.0771	5.45	4.71	0.74 (13.58%)
	3μg	7.99	5.35	0.0794	4.94	5.33	-0.39 -
	6μg	8.28	6.72	0.0892	5.18	5.14	0.04 (0.77%)
	12μg	12.19	5.91	0.0711	5.055	4.91	0.145 (2.87%)
Pseudomonas fluorescens	0μg	73.44	7.55	0.3285	5.45	4.73	0.72 (13.21%)
	3 μg	110.94	4.74	0.2971	4.94	4.91	0.03 (0.61%)
	6 μg	112.5	4.6	0.2965	5.18	4.91	0.27 (5.21%)
	12 μg	123.44	5.64	0.3224	5.055	5.52	-0.465 -

NG = No growth. ΔTOC = TOC-Begin - TOC-End

7.2.2.4 Discussion and conclusions

Both test organisms gave increasing higher AOC values as the acetate content increased. The *Pseudomonas* gave a much higher AOC concentration than the *Aeromonas*, indicating that the substrate was more biodegradable for the first organism than for the second. There was no difference in the f-Factor of both organisms, not even an increasing value with the increasing acetate added. The samples with added acetate gave lower F-Factor values than the samples without any acetate. The reason for this is unknown at this stage. The μ-value confirmed the results found with the AOC concentrations - the substrate was more biodegradable for *Pseudomonas* than for *Aeromonas*. The TOC values were also confusing. It may be, that the added concentrations of acetate were too low to be detected by the TOC method.

Acetate carbon was more biodegradable for *Pseudomonas* than for *Aeromonas*. Thus any addition of carbon to a water source, no matter how little, will contribute to bacterial regrowth.

7.3 GENERAL CONCLUSIONS

It will be difficult to determine a possible minimum concentration of biodegradable organic carbon at which no bacterial growth can be expected, because the indigenous bacteria present in water consist of a wide variety of species, each with its own nutritional requirements. Depending on what kind of species present, a specific carbon source will have different effects. Regarding the two organisms tested, it is clear that with both being present in the water, more regrowth problems can be expected with *Pseudomonas* than with *Aeromonas* under the same nutritional conditions. Organic carbon concentrations may vary to such a small extend that differences may not even be detectable by TOC or DOC analysis commonly used.

CHAPTER 8

WATER TREATMENT METHODS FOR THE REMOVAL OF BIODEGRADABLE ORGANIC CARBON.

The type of source water determines what kind of methods will be suitable for treatment. In this section, water samples were received from a treatment plant where pre-chlorination formed an essential part of the treatment process due to high algal concentrations in the raw water. Chemical dosing with rapid mixing, consists of polyelectrolyte /ferric chloride /lime as main coagulants. After flocculation and sedimentation, carbonation took place. Following rapid sand filtration, the water was chlorinated for disinfection (See Appendix J).

8.1 WATER SAMPLES

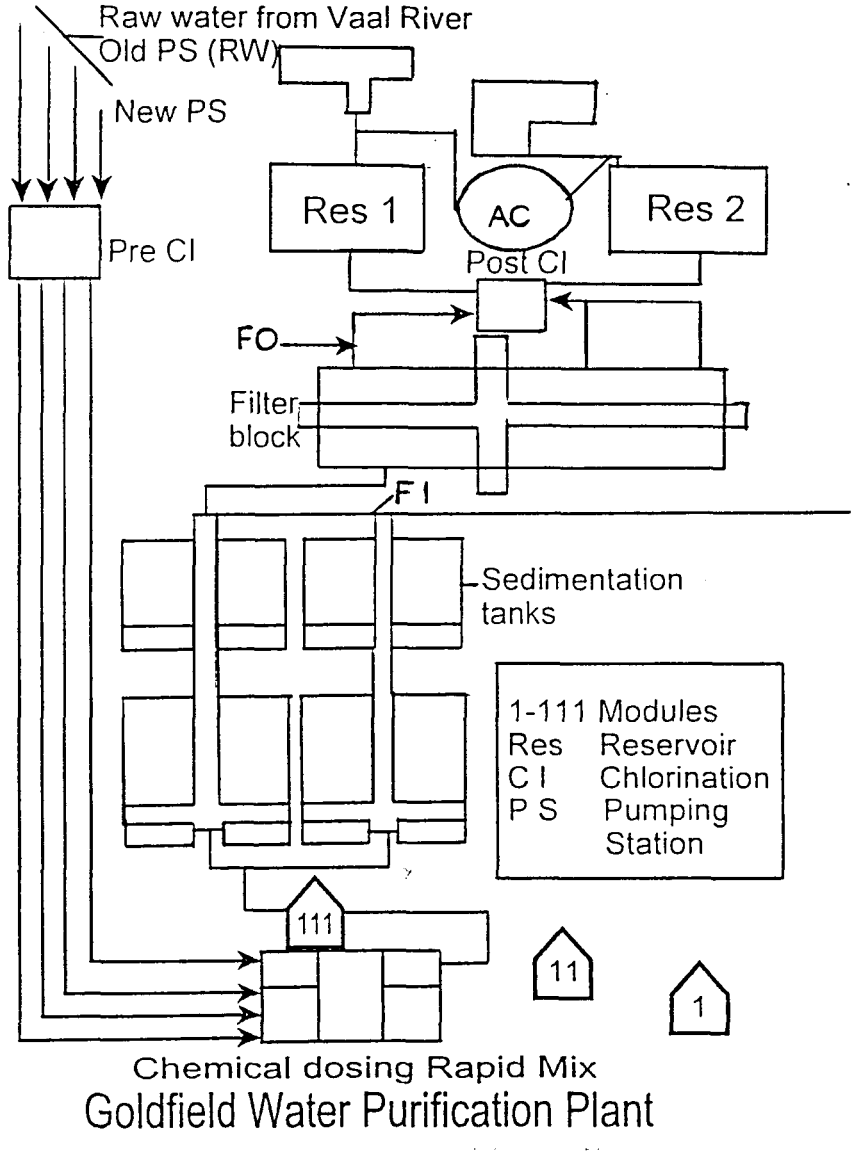


Diagram 8: Diagram of the treatment plant and sample points (with pre-chlorination)

The following water samples were received:

RW - raw water from intake before pre-chlorination
FI - after flocculation/sedimentation - before sand filtration
FO - after rapid sand filtration
AC - after post chlorination.

8.2 EXPERIMENTAL WORK

The Van der Kooij and Werner methods were used to evaluate the treatment process. Chemical analysis and DOC determinations were done on all the samples Analysis were done on 14-02-96; 29-02-96; 13-03-96 and 28-03-96.

8.3 RESULTS

Results for the treatment process without pre-chlorination, were obtained from the evaluation of the seasonal effect on removal of biodegradable organic carbon from water during the conventional treatment process used at Rand Water during the period of 17-01-94 to 13-12-94.

Table 8.1a: Range for results obtained with samples from a water treatment plant with pre-chlorination.

Sample		Parameter						
		AOC-P17 (μg acetate C eq/ℓ)	AOC-NOX (μg acetate C eq/ℓ)	Total AOC (μg acetate C eq/ℓ)	f-Factor	μ-value	Initial DOC (mg/ℓ)	Turbidity (NTU)
RW	Min	93.8	84.1	177.9	2.48	0.0610	6.8	160
	Max	134.4	138.1	272.5	8.96	0.1928	9.4	540
	Avg	109.8	115.1	224.9	5.50	0.1291	8.1	275
FI	Min	18.8	33.3	70.8	2.52	0.0520	4.2	0.39
	Max	37.5	107.9	143.8	5.57	0.1354	4.7	12
	Avg	32.4	69.8	102.3	3.79	0.1010	4.4	5.02
FO	Min	10.5	92.1	115.5	2.73	0.0530	4.1	0.25
	Max	56.3	150.8	180.1	4.74	0.1777	4.2	1.60
	Avg	28.8	125.4	154.2	3.82	0.1138	4.2	0.63
AC	Min	28.1	138.1	205.3	4.02	0.0970	3.6	0.24
	Max	93.8	269.8	316.0	7.71	0.1237	5.3	1.8
	Avg	60.6	213.1	273.6	5.52	0.1106	4.4	0.995

Table 8.1b: Percentage change in AOC concentration, f-Factor, μ-value, DOC and turbidity caused by pre-chlorination in the water treatment process.

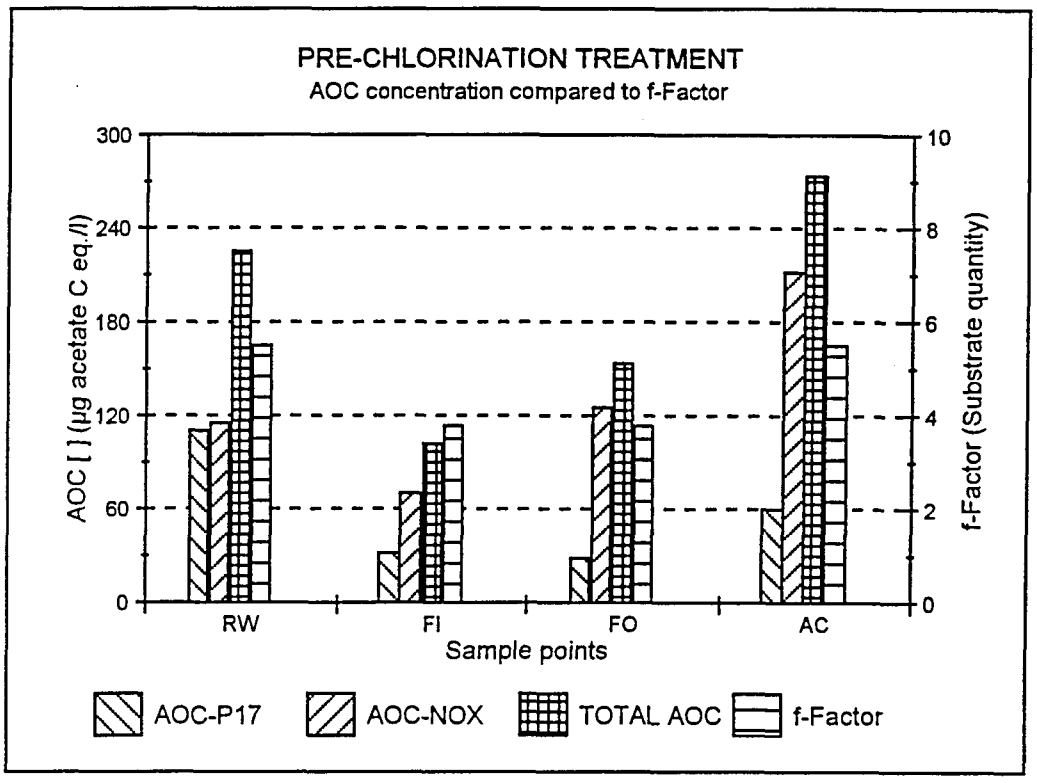
Sample		Parameter						
		AOC-P17 (μg acetate C eq/ℓ)	AOC-NOX (μg acetate C eq/ℓ)	Total AOC (μg acetate C eq/ℓ)	f-Factor	μ-value	Initial DOC (mg/ℓ)	Turbidity (NTU)
RW	FI	-70.5%	-39.4%	-54.5%	-31.1%	-14.8%	-45.7%	-98.2%
	FO	-73.8%	8.9%	-31.4%	-30.5%	-11.9%	-48.1%	-99.8%
	AC	-44.8%	85.1%	21.7%	0.4%	-14.3%	-45.7%	-99.6%
FI	FO	-11.1%	50.7	50.7%	0.8%	12.7%	-4.5%	-87.5%
	AC	87.0%	167.4%	167.4%	45.6%	9.5%	0	-79.8%
FO	AC	110.4%	77.4%	77.4%	44.5%	-2.8%	4.8%	57.9%

Table 8.2a: Range for results obtained with samples from a water treatment plant without pre-chlorination.

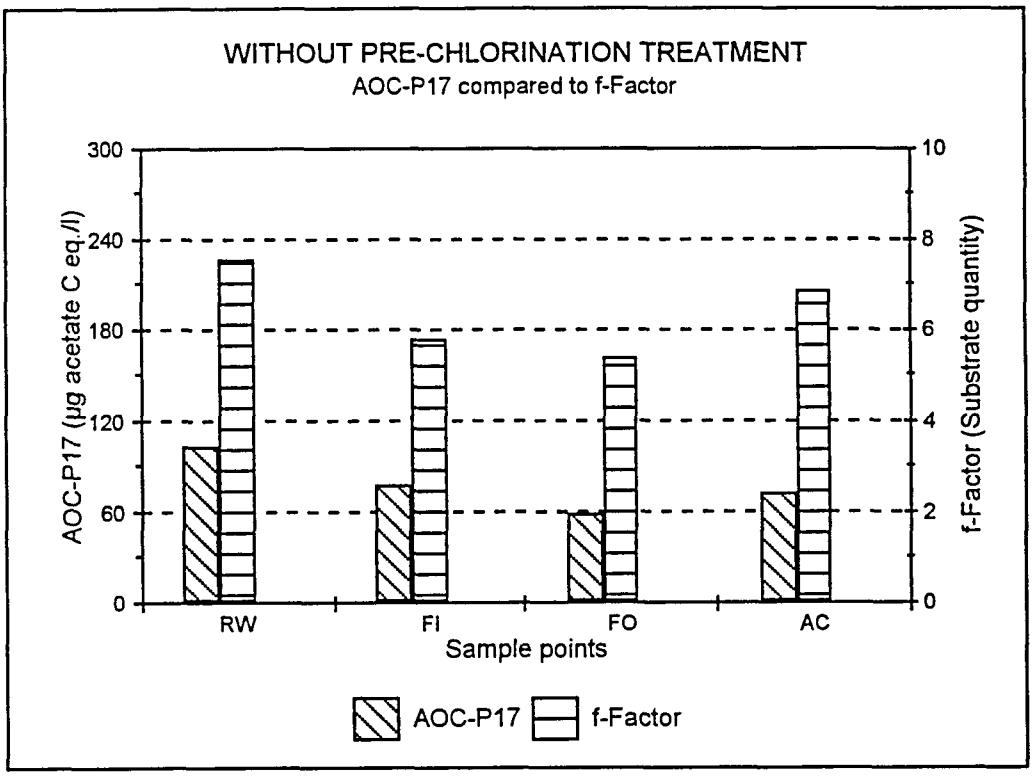
Sample		Parameter				
		AOC-P17 (μg acetate C eq/ℓ)	f-Factor	μ-value	DOC-Begin (mg/ℓ)	Turbidity (NTU)
RW	Min	46.4	2.47	0.0592	2.70	105
	Max	362.3	27.66	0.5777	4.70	185
	Avg	103.4	7.53	0.1777	3.57	138.38
FI	Min	33.3	2.33	0.557	1.80	0.64
	Max	202.9	23.27	0.5718	4.40	11.0
	Avg	77.9	5.78	0.1429	3.21	3.13
FO	Min	29.0	1.00	0.0493	1.50	0.12
	Max	120.3	29.46	0.5621	4.40	1.70
	Avg	57.3	5.34	0.1420	3.23	0.43
AC	Min	37.7	2.26	0.0445	1.40	0.21
	Max	150.7	29.06	0.5690	4.50	4.20
	Avg	72.1	6.84	0.1403	3.20	1.59

Table 8.2b: Percentage change in AOC concentration, f-Factor, μ-value, DOC and turbidity for samples from a water treatment plant without pre-chlorination

Sample		Parameter				
		AOC-P17 (μg acetate C eq/ℓ)	f-Factor	μ-value	DOC-Begin (mg/ℓ)	Turbidity (NTU)
RW	FI	-24.7%	-23.2%	-19.6%	-10.1%	-97.7%
	FO	-44.6%	-29.1%	-20.1%	-9.5%	-99.7%
	AC	-30.3%	-9.2%	-21.0%	-10.4%	-98.9%
FI	FO	-26.4%	-7.6%	-0.6%	0.6%	-86.3%
	AC	-7.4%	18.3%	-1.8%	-0.3%	-49.2%
FO	AC	25.8%	28.1%	-1.2%	-0.9%	269.8%

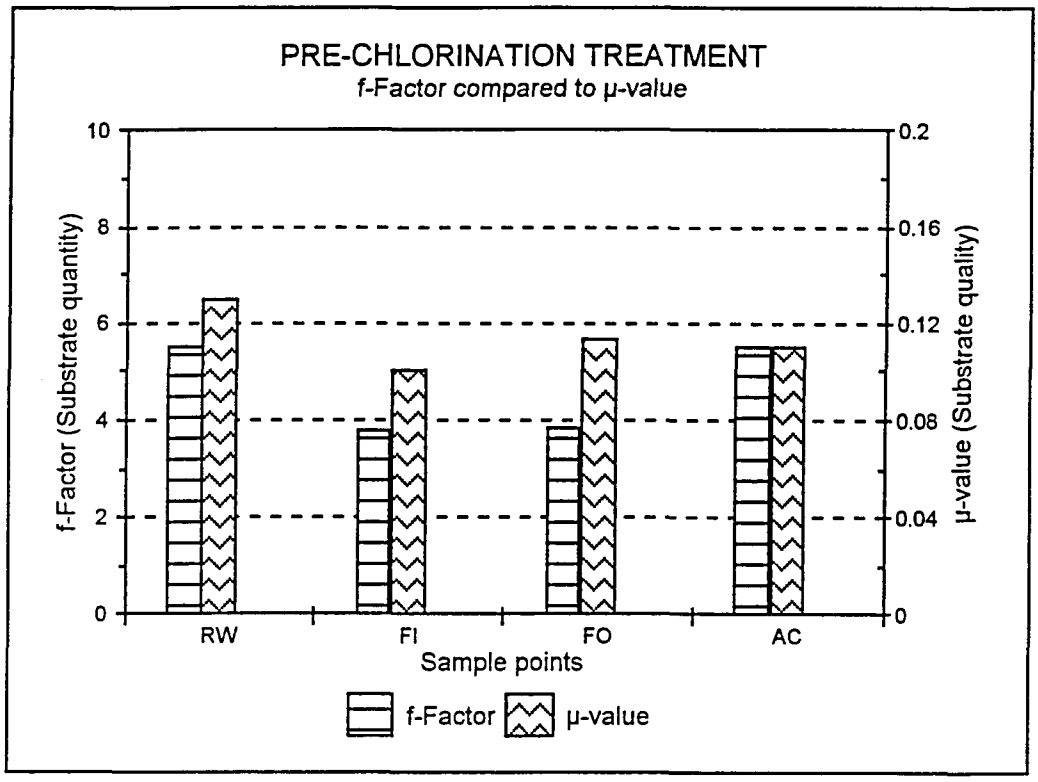


(a)

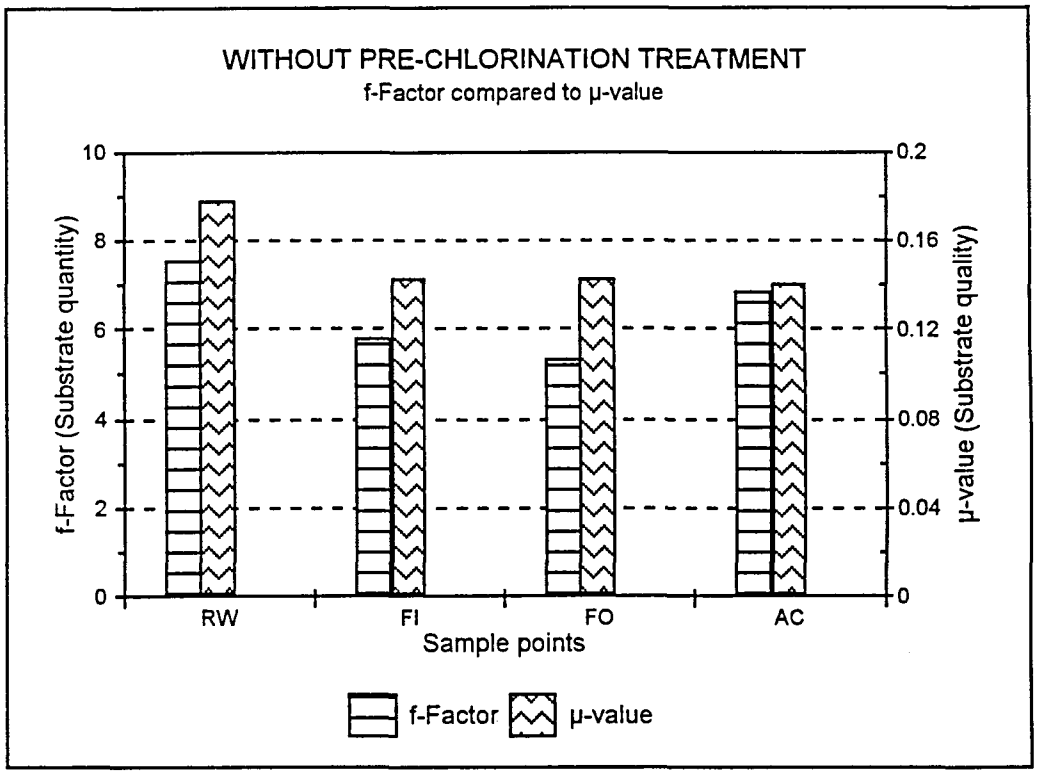


(b)

Figure 8.1 **Comparison of two different water treatment methods as reflected in the AOC concentration and f-Factor (a) with pre-chlorination and (b) without pre-chlorination.**

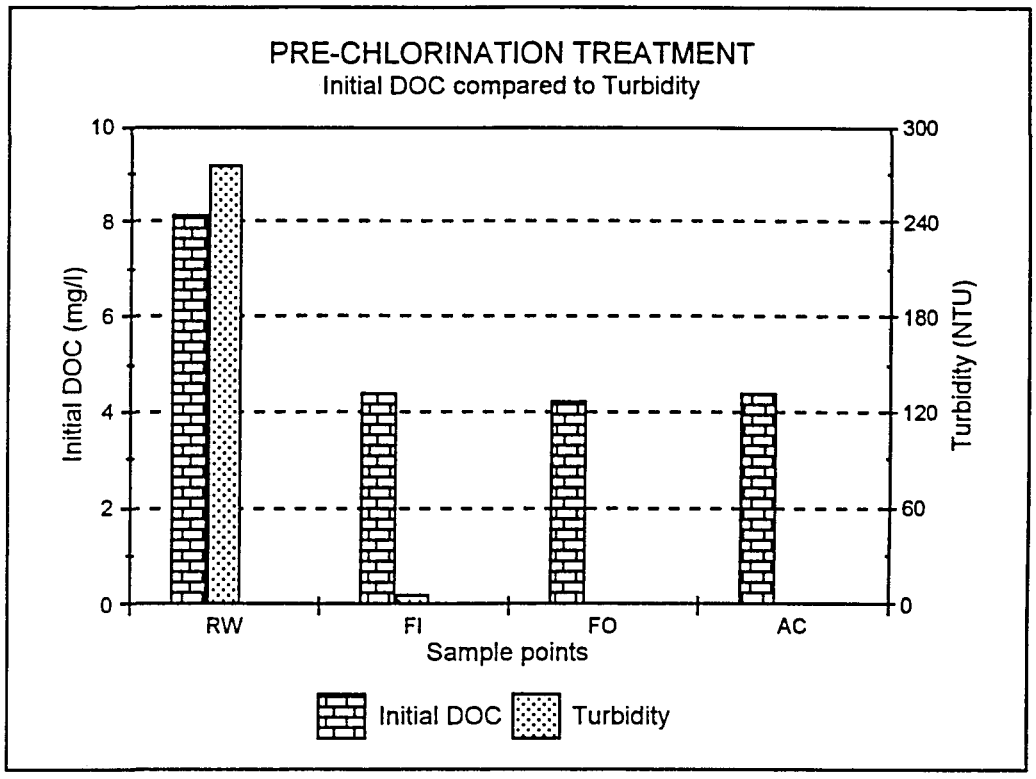


(a)

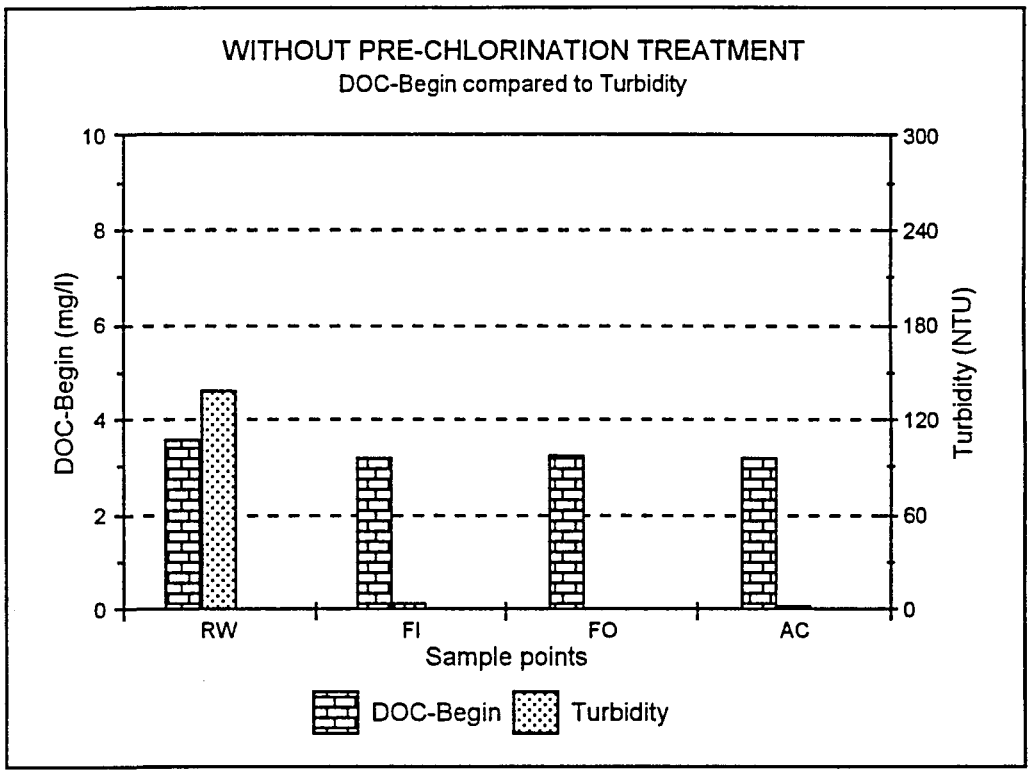


(b)

Figure 8.2: **Comparison of two different water treatment methods as reflected in the f-Factor compared to the μ-value (a) with pre-chlorination and (b) without pre-chlorination.**



(a)



(b)

Figure 8.3: Comparison of two different water treatment methods as reflected in the Initial DOC and Turbidity values (a) with pre-chlorination and (b) without pre-chlorination

8.4 DISCUSSION

Both treatment processes showed the same trend of a decrease in the concentration (AOC concentration, f-Factor and DOC) of biodegradable organic carbon from RW towards FO, with an increase from FO towards AC except for the DOC-Begin in the treatment process without pre-chlorination.(Tables 8.1a and b and 8.2a and b and Figures 8.1, 8.2 and 8.3).

Although an increase in the concentration (AOC concentration and f-Factor) of biodegradable organic carbon was caused by the chlorination process, the biodegradability (μ-value) of these carbon compounds decreased by 2.8% and 1.2% respectively for the with pre-chlorination and without pre-chlorination treatment plants. A higher percentage change in the test parameters was observed for the treatment plant with pre-chlorination than for the treatment plant without pre-chlorination.

AOC-P17 and AOC-NOX contributed almost equally (average 109.8 and 115.1 μg acetate C eq/ℓ respectively) to the Total AOC of RW in the treatment plant with pre-chlorination (Table 8.1a and Figure 8.1a). After treatment, AOC-NOX increased much more than AOC-P17 (Table 8.1a and Figure 8.1a). This point to a definite change in the kind and quantity of biodegradable organic carbon available after each treatment step.

The turbidity of the raw water was decreased by the complete treatment process with 99.6% and 98.7% respectively for the with and without pre-chlorination treatment plants (Tables 8.1b and 8.2b). The cause of the increase in turbidity after the chlorination process, is unknown.

8.5 CONCLUSIONS

- The same trend of a decrease in the concentration of biodegradable organic carbon from RW towards FO, with an increase at AC was observed with both treatment plants under investigation.

- Although an increase in the concentration of biodegradable organic carbon was observed at AC, the biodegradability (μ-value) decreased. Therefore less bacterial regrowth should take place.

- AOC-NOX was increased by rapid sand filtration and chlorination, while AOC-P17 was increased only by chlorination.

- Higher average values were obtained for AOC-P17, f-Factor and μ-value in the treatment plant without pre-chlorination than in the treatment plant with pre-chlorination, although AOC-P17 for RW was almost the same (103.4 and 109.8 μg acetate C eq/ℓ respectively).

- An experiment should be run where a sample is taken directly after the pre-chlorination process and where both *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX should be used to compare the effect of the treatment processes, with and without pre-chlorination.

- Depending on the changes following each treatment process, modifications on that process may be done to improve the removal or decrease the formation of biodegradable organic compounds at that point of treatment.

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

9.1 GENERAL CONCLUSIONS

9.1.1 Suitable methods to determine the biodegradable organic carbon in water:

- The methods of Van der Kooij, (using both *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX) and Werner are both suitable for use with different kinds of water, like raw water with a high concentration of biodegradable organic carbon (AOC-P17 ≈ 362 μg acetate C eq/ℓ; AOC-NOX ≈ 138.1 μg acetate C eq/ℓ; f-Factor ≈ 27.66) to low concentrations as obtained after sedimentation (AOC-NOX ≈ 33.3 μg acetate C eq/ℓ) and sand filtration (AOC-P17 ≈ 10.5 μg acetate C eq/ℓ; f-Factor ≈ 1).

- The Van der Kooij method is cheaper to perform but more labour intensive and results are only available after two to four weeks. *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX must both be used during experiments to ensure utilisation of most of the carbon sources available. If only one strain is used, valuable information can be lost and a false impression obtained about the growth potential of that specific type of water or treatment process.

- The AOC analyser used for the Werner method is expensive, but analyses are less labour intensive and results are available within three to five days. The inoculum obtained from the water sample is a mixed culture and has therefore already been adapted to utilise most of the available carbon sources in that water.

- The BDOC methods of Joret-Lévi and Billen-Servais are easy to perform. Care should however be taken to work in a "DOC-free" environment to prevent DOC contamination via the air. The instrument used for the DOC analyses should be sensitive enough to record changes in μg/ℓ units when work is done at very low DOC concentrations (BDOC ≈ 0.2 mg/ℓ).

- The method that relies on ATP measurements proposed by Jago-Stanfield was found to be unreliable and is not recommended for use.

- The methods of Van der Kooij and Werner were found to be reliable and were used for the remaining part of the project.

9.1.2 Possible seasonal effects on biodegradable organic carbon in water during the conventional treatment process at Rand Water:

- Increases in the concentration of biodegradable organic carbon, measured as

AOC-P17, f-Factor and DOC-Begin, occurred around the beginning of autumn (April) and during spring (October/November) in the raw water through the treatment process until after chlorination.

- The difference in trend between the AOC-P17 concentration and the f-Factor, is possibly due to the fact that only *Pseudomonas fluorescens* strain P17 was used and not *Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX.

9.1.3 Effect of different treatment processes on the concentration and availability of biodegradable organic carbon:

Silica/lime vs lime/ferric chloride, high ferric chloride/low lime vs low ferric chloride/high lime and pre-chlorination vs pre-ozonation were evaluated.

- Both the concentration and availability of the biodegradable organic carbon in the raw water, were increased by each of these different treatment processes under evaluation. Although the silica/lime treatment resulted in a higher percentage (549%) increase in AOC-P17 in comparison with the other treatment processes, it was of low quality, only 1.15% increase in the μ-value of the raw water after treatment.

- Pre-ozonation resulted in an increase of up to 243% for the availability (μ-value) of the biodegradable organic carbon.

9.1.4 Biodegradable organic carbon present directly after treatment vs the possible formation in the distribution network:

Primary vs secondary disinfection, ozonation as primary disinfection and chloramination as secondary disinfection were investigated.

- Both ozonation and chloramination caused an increase in the concentration of biodegradable organic carbon in water directly after the treatment process (Total AOC $\approx$ 144.7% and 94.8%; f-Factor $\approx$ 54.4% and -3.3% respectively).

- Although ozonation as primary disinfection increased the concentration (Total AOC $\approx$ 144.7%; f-Factor 54.4%) and availability (μ-value $\approx$ 45.2%) of the biodegradable organic carbon, chlorination as secondary disinfection reduced the biodegradability of the carbon source (μ-value $\approx$ -9.6%).

- The increase caused by chloramination directly after treatment (Total AOC $\approx$ 94.8% and 90.6%; f-Factor $\approx$ -3.3% and 4.4%; μ-value $\approx$ 38.5% and 37.9% respectively for chlorine followed by ammonia vs ammonia followed by chlorine) seemed to be reduced as water moved through the distribution system (AOC-P17 $\approx$ -1.21%; f-Factor $\approx$ 3.3%; μ-value $\approx$ -4.47% after secondary disinfection to the distribution endpoint).

9.1.5 Activated carbon for the removal of biodegradable organic carbon:

- Although the concentration of biodegradable organic carbon decreased during the treatment process with GAC, the availability increased after each GAC column (μ-value $\approx$ 4.14% - GAC_1, 7.23% - GAC_2 and 10.21% - GAC_3). This observation may be the result of changes that took place in the GAC columns due to biological activity.

- As most of the biodegradable organic carbon was removed by the first GAC column, irrespective of its operational age (12 months in this case), a logical conclusion may be to use fresh GAC, in the first column.

9.1.6 A possible minimum AOC value at which no regrowth would be expected:

- It will be difficult to determine a possible minimum concentration of biodegradable organic carbon at which no bacterial growth can be expected, because the indigenous bacteria present in water consist of a wide variety of species, each with its own nutritional requirements.

- Depending on what kind of species present, a specific carbon source will have different effects.

- Regarding the two organisms tested, it is clear that with both being present in the water, more regrowth problems can be expected with *Pseudomonas* than with *Aeromonas* under the same nutritional conditions.

- Organic carbon concentrations may vary by such a small margin that differences may not be detectable using standard TOC or DOC methods of analysis.

9.1.7 Water treatment methods for the removal of biodegradable organic carbon:

- Whether pre-chlorination was practised or not, the same trend was observed in treatment plants - a decrease in the concentration of biodegradable organic carbon up till sand filtration, with an increase after chlorination.

- Although chlorination resulted in an increase of the concentration, a decrease in the availability (μ-value) of the biodegradable organic carbon took place. Therefore, less bacterial regrowth should be expected.

9.2 REVIEW OF PROJECT IN TERMS OF OBJECTIVES

9.2.1 Evaluation of some of the available methods to choose the most suitable method(s) to determine biodegradable organic carbon present in water:

The methods of Van der Kooij and Werner are suitable to determine the

concentration and availability of the biodegradable organic carbon compounds present in water. Changes in the concentration and availability of the carbon compounds may be detected with these methods, while it is not always the case with DOC analyses.

9.2.2 **Determination of the extent to which results, obtained with these methods, are comparable:**

The results obtained with both methods follow the same trend during the evaluation of treatment processes in practice , but not necessarily when bench tests were performed. It is also important that both cultures (*Pseudomonas fluorescens* strain P17 and *Spirillum* sp strain NOX) should be used in the method of Van der Kooij, to obtain a total true AOC value.

9.2.3 **Evaluation of different treatment processes with respect to the removal of biodegradable organic carbon by using the most suitable method(s):**

The effect of the different treatment processes on the removal and availability of biodegradable organic carbon, can be determined and evaluated by using either or both of these methods (Methods of Van der Kooij and Werner).

9.2.4 **Determination of a possible minimum concentration of biodegradable organic carbon at which no growth of heterotrophic or coliform bacteria, especially *Aeromonas* and *Pseudomonas* species would be expected:**

It will be difficult to determine a minimum concentration of biodegradable organic carbon that will not support bacterial growth, because the indigenous bacterial population present in water consists of a wide variety of species, each with its own nutritional requirements.

9.2.5 **Investigation into the relationship between the presence of biodegradable organic carbon and the concentration of easily measurable determinands:**

No firm relationship between biodegradable organic carbon and an easily measurable determinand could be found.

9.3 RECOMMENDATIONS FOR FUTURE RESEARCH

9.3.1 Both the BDOC methods should be evaluated, with the assurance of continuous DOC analysis being done on a sensitive instrument capable of detecting low concentrations DOC (BDOC ≈ 0.2 mg/ℓ).

9.3.2 A continuous monitoring programme should be initiated to evaluate the concentration and availability of biodegradable organic carbon in the water from the raw water intake through the treatment process at different stages, taking into consideration parameters like rainfall, temperature and water source. The methods

of Van der Kooij and Werner should be used, making use of both cultures for the Van Der Kooij method.

- These results may give an answer to the problem of increasing bacterial counts during summer and after heavy rainfall.

- Depending on the changes following each unit treatment process, modifications to that process may be recommended to improve the removal of, or decrease the formation of biodegradable organic compounds at that point of treatment.

9.3.3 The effect of the different unit treatment processes and chemicals (silica/lime, lime/ferric chloride, pre-chlorination, pre-ozonation) was evaluated by means of bench tests, without being able to use the same retention time as in practise. Therefore analyses should be done on an operational plant where these treatment processes are in use to determine the actual effect of these treatment processes on the concentration and/or availability of biodegradable organic carbon present in the water.

9.3.4 The effect of pre-ozonation on the concentration and availability of biodegradable organic carbon should be investigated under operational conditions in a water treatment plant.

9.3.5 A long term investigation should be initiated to determine the relation between the potential of GAC in removing biodegradable organic carbon during the water treatment process and the frequency of reactivation required to obtain maximum removal.

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APPENDIX A : METHOD OF WERNER

Determination of bacterial growth dynamics - growth rate (μ) and growth factor (f).

The procedure described here allows the measurement of the biodegradability of organic compounds in water. This is achieved by means of growth experiments with mixed biocenoses (mixtures of different strains of bacteria) under standardized conditions.

This method applies to the analysis of drinking water, water after various steps of treatment, as well as ground and surface water. The growth curves provide quantitative information about the so-called regrowth potential of drinking water.

Principle of the method:

This microbiological test for bacterial growth, consists in recording a growth curve after sterile filtration of a water sample and its inoculation with a mixed biocenosis, which is normally obtained from the examined water sample. The increase in biomass is monitored semi-continuously by turbidity measurements (12° forward scattering). These measurements, as well as the evaluation at the end of an experiment, are automated.

Definition of the parameters:

The two parameters, μ (growth rate) and **f** (growth factor), are determined by recording a growth curve.

Growth rate is calculated from the slope of the (logarithmic) growth curve during the logarithmic growth phase of the bacteria. It is a parameter for the substrate quality.

The growth factor, **f**, is calculated from the ratio of biomass at the end of an experiment to that at the beginning and gives an indication of substrate quantity.

A1 EQUIPMENT

A.1.1 **Instruments and Materials**

Autoclave
Oven (0 °C to 550 °C)
Analytical balance
Automatic measuring system for bacterial growth (MONITEK MODEL 251-4 mAOC)
Complete glass filter unit, 47 mm diameter (Millipore, Cat. No. 1504700)
Vacuum pump
0.2 μm Nucleopore polycarbonate membranes, 47 mm diameter (Cat. No. 111106)
Pleated filters, 125 mm diameter (Schleicher & Schuell, Cat. No. 311644)
1 mℓ Glass pipettes
Aluminum foil to cover the cuvettes

100 mℓ and 400 mℓ Calibrated glass beakers
Fibre-glass pre-filters (Millipore, Cat. No. AP 400 705)

A.1.2 **Chemicals**

- Phosphate-free Extran (Merck, Cat. No. 7550.5000) to clean all glassware.
- Physiological sodium chloride solution (8.5 g/ℓ distilled water). Dispense in 30 mℓ volumes, autoclave for 15 min at 121 to 124 °C. The solution is used to suspend the bacteria for the inoculum.
- Nutrient medium contains all salts necessary for bacterial growth and is added to the prepared samples in a ratio 1 + 10 to guarantee that none of the salts will be a minimum factor during the growth experiment.

100 mg NH_4Cl (Merck)
100 mg $Ca(NO_3)_2.4H_2O$ (Merck)
100 mg $CaCl_2.2H_2O$ (Merck)
500 mg $MgSO_4.7H_2O$ (Merck)
100 mg KH_2PO_4 (Merck)
50 mg $Na_2SiO_3.9H_2O$ (Sigma)
10 mg $Al_2(SO_4)_3.18H_2O$)
0.1 mℓ Hoagland A-Z solution
Dissolve in 1000 mℓ distilled water (pH 6.8)

Hoagland A - Z solution:
Solution of trace elements which completes the above mentioned medium

1.0 g $Al_2(SO_4)_3$ (Sigma)
0.5 g KJ (Merck)
0.5 g KBr (Sigma)
1.0 g TiO_2 (Sigma)
0.5 g $SnCl_2.2H_2O$ (Merck)
0.5 g LiCl (Sigma)
7.0 g $MnCl_2.4H_2O$ (Sigma)
11.0 g H_3BO_3 (Merck)
1.0 g $ZnSO_4$ (Merck)
1.0 g $CuSO_4.5H_2O$ (Merck)
1.0 g $NiSO_4.6H_2O$ (Sigma)
1.0 g $Co(NO_3)_2.6H_2O$ (Sigma)
Dissolve in 1800 mℓ distilled water

A2 PREPARATION OF. THE SAMPLES

A.2.1 **Preparing of glassware**

The most important issue is to make sure that no traces of organic carbon are left in the glassware used in the experiment for this might lead to false results in this highly sensitive test.

- Cuvettes:
  After usage, first soak the cuvettes for 6 to 8 hours in phosphate-free Extran solution. Rinse thoroughly with hot water and then with distilled water. Finally dry it at 105 °C in an oven. After cooling down, cover with aluminum foil and store in a cupboard, ready for use.

- Stirring magnet:
  After soaking as described above, it is sufficient to rinse the magnet with very hot water and let it dry.

A.2.2 **Filtration of samples and nutrient medium**

Filtration has to be carried out very carefully to avoid any contamination with organic substances from outside (*e.g.* from the glass vessels) which could serve as nutrients for the bacteria and thus produce false results.

First of all, clean the working table and assemble the filtration apparatus (vacuum pump, suction bottle, filter frit and filter top). Then insert a polycarbonate filter with a pore size of 0.2 µm. Before filtering the sample, filter and suction bottle should be flushed by pumping through ± 1 ℓ of distilled water in several smaller portions.

Now filtration of the sample can begin.

- First, let 100 mℓ of the sample through the membrane to rinse the suction bottle and discard it.
- Then pump through 150 mℓ and rinse the prepared cuvettes with the stirring magnets and the 400 mℓ beaker.
- Now add 40 mℓ glass-fibre-filtered nutrient medium to 400 mℓ of the sample.
- Filter 300 mℓ into the 400 mℓ beaker and fill the rinsed cuvette.
- Cover it with aluminum foil.
- Keep a sub sample of this filtrate for DOC determination.

Repeat this procedure with each sample. If the different DOC concentrations are already known, the samples with the lowest DOC content should be filtered first. Pump through 1 ℓ of distilled water between two samples.

Change the filter only when necessary and keep it for the preparation of the inoculum. Except for the addition of the inoculum, the water samples are now ready for the experiment.

A.2.3 **Preparation of the inoculum**

The inoculum for the test is obtained from the mixed biocenosis of the respective sample water, of which 3 to 5 ℓ are filtered through polycarbonate filters (Nucleopore, 0.2 µm pore size). The filters with the filter residue (mainly bacteria) are then put into ± 30 mℓ sterilized physiological sodium chloride solution and stirred for half an hour with a magnetic stirrer. After

that, the bacteria are suspended in the solution. In case larger non-bacterial particles remain in the solution, it should be removed by filtering the inoculum through a pleated filter, previously rinsed with hot water.

The inoculum is now ready for use. Later, after calibration of the instrument, the inoculum is pipetted into the cuvettes with the prepared water sample, until a turbidity reading of between 0.2 and 0.4 is obtained.

A.2.4 Sampling from the cuvettes

To control and compare the measuring data, determinations of cell number and DOC should be carried out. The samples should not be taken with pipettes but simply poured out to reduce risk of introducing organic substances. The following samples are recommended:

DOC

- original sample
- after sterile filtration of the sample (including nutrient medium), *i.e.* before starting the experiment
- after the experiment

Cell number

- inoculum
- after the experiment

A.2.5 Determination of cell number

The recommended method of fluorescent microscopy for the determination of a total cell number - both dead and living cells - was not used.

The spread plate method with Lab-Lemco agar was used to determine the cell number of the inoculum as well as at the end of the experiment.

This evaluation is to control whether the increase in turbidity is actually caused by bacterial growth and not, for example, by inorganic precipitation.

A.2.6 DOC determinations

DOC determinations were done by the Organic Chemistry Section, according to their prescribed method.

A3 CONDUCTING AN EXPERIMENT

The instructions as set out in the "Manual and Operating Instructions" for the mAOC-Analyser, as from section "5.2 Starting Procedure" were followed.

REFERENCE:

LINK/HARTMANN/EBENHAGEN/HAMBSCH (1992). *mAOC - Analyser : Manual and Operating Instructions* - Automatic Analysis of Bacterial Growth Dynamics for the Characterization of Substances Dissolved in Water and the Determination of the Regrowth Potential.

APPENDIX B : METHOD OF VAN DER KOOIJ

The original method of Van der Kooij determined AOC in a water sample by measuring the growth of *Pseudomonas fluorescens* strain P17. This strain can utilize a variety of compounds at relatively high and low (a few micrograms per litre) concentrations. It does not need specific growth factors and can use nitrate and ammonia as nitrogen sources. This strain was isolated from drinking water and represented biotype 7.2, which occurs commonly in drinking water, surface water and ground water (Huck, 1990)

Pseudomonas P17 can utilize easily biodegradable compounds, like amino acids, carboxylic acids, hydrocarboxylic acids, alcohols and carbohydrates (polysaccharides excluded), with the exception of oxalic acid, a compound frequently produced during ozonation. *Spirillum*, strain NOX, capable of using oxalate, has been identified and incorporated in the test procedure.

Principle of the method:

The vegetative cells in the water sample are destroyed by heat treatment. After cooling, the sample is inoculated with the precultured cells of P17 and/or NOX. The samples are incubated at approximately 15 °C without shaking, until a maximum number (Nmax) of colony forming units per mℓ (cfu/mℓ) is reached. Growth is determined by periodic colony counts. Yield factors for P17 and NOX are determined, using known concentrations of acetate. The yield factor is expressed as colony forming units (cfu) per μg acetate C/ℓ for each strain. The AOC results are expressed as μg acetate C eq/ℓ.

B1 EQUIPMENT

B.1.1 Glassware

10 mℓ pipettes
1 mℓ pipettes
1000 mℓ glass stoppered Pyrex Erlenmeyer flasks/1000 mℓ Schott bottles with polypropylene screw caps.

B1.2 Media and chemicals

Lab-Lemco agar (Oxoid Cat.no. CM17)
Physiological sodium chloride solution (8.5 g/ℓ distilled H_2O) - sterilise at 121 to 124 °C for 15 min.
1 ℓ H_2SO_4
60 g $K_2Cr_2O_7$

Chemicals for the mineral medium

1. Basal medium A

5.0 g	$CaCl_2.2H_2O$
0.005 g	$CoCl_2.6H_2O$
0.01 g	H_3BO_3
1000 mℓ	distilled H_2O

Dissolve the chemicals in distilled water and make up to 1000 mℓ. The solution can be stored for up to 5 years in a dark place at room temperature

2. Basal medium B

5.00 g	$MgSO_4.7H_2O$
0.01 g	$CaSO_4.5H_2O$
0.5 g	$MnSO_4.7H_2O$
0.01 g	$ZnSO_4.7H_2O$
0.30 g	$FeSO_4.7H_2O$
1000 mℓ	distilled H_2O

Dissolve in distilled water and make up to 1000 mℓ. The solution can be stored for up to 5 years in a dark place at room temperature.

3. Basal medium C

0.27 g	KH_2PO_4
0.53 g	$K_2HPO_4.3H_2O$
0.80 g	$Na_2HPO_4.12H_2O$
1000 mℓ	distilled H_2O

Dissolve in distilled water and make up to 1000 mℓ. The solution can be stored for up to 5 years in a dark place at room temperature.

4. Ammonium chloride solution

0.0077 g	NH_4Cl
100 mℓ	distilled H_2O

Dissolve in distilled water and make up to 1000 mℓ. The solution can be stored for up to 5 years in a dark place at room temperature.

B1.3 **Pure culture**

Pseudomonas fluorescens strain P17 (Obtained from D.van der Kooij)
Spirillum species, strain NOX (Obtained from D.van der Kooij)

B2 PREPARATION OF THE SAMPLES

B2.1 **Preparation of glassware**

Preparation of chromic acid:

Dissolve 60 g $K_2Cr_2O_7$ in 40 mℓ distilled water. Fill up to a 1000 mℓ with concentrated sulphuric acid. Follow safe work procedures.

Cleaning procedure:

Place the pipettes into a 1ℓ measuring cylinder and cover pipettes with chromic acid. Leave the pipette in the acid for 30 minutes. Pour the acid back into the acid storage bottle and rinse the pipettes thoroughly with warm tap water. Let the pipettes dry and heat overnight at ±250 °C in stainless steel pipette canisters. Rinse all other glassware with chromic acid and then with tap water, let them dry. Cover the openings of the bottles and wrap the glass stoppers with aluminum foil. Heat overnight at 250 °C.

B2.2 **Preparation of the inoculum**

The inoculum was prepared according to one of two methods, namely (i) in autoclaved tap water containing 1 mg of acetate C/ℓ, (Van der Kooij *et al*, 1982a) or (ii) in a prepared mineral medium (Huisvoorschrift: LMB-004, Datum: 93-05-25 - Copy received from Dr D van der Kooij).

(i) *Pseudomonas fluorescens* strain P17 and *Spirillum* strain NOX were inoculated each on a Lab-Lemco agar slant and incubated for approximately 24 h at 25 to 28 °C. Prepare a solution of tap water containing 1 mg sodium acetate C/ℓ. Dispense 50 mℓ volumes into 250 mℓ screw cap Schott bottles and autoclave for 15 minutes at 121 to 124 °C. Cool down. Prepare a turbid suspension of each 24 h - slant culture and inoculate into the prepared water acetate solution. Incubate at 25 to 28 °C until Nmax (± 4 x 10^6 cfu/mℓ) is reached (±7 days). Store these precultures refrigerated at ± 6 °C or below. Always predetermine the cell number (cfu/mℓ) before samples are inoculated.

(ii) *Pseudomonas fluorescens* (P17) and *Spirillum* NOX were each inoculated onto a Lab-Lemco slant. Incubate for approximately 24 h at 25 to 28 °C. Prepare a slight turbid suspension from each slant. Inoculate 0.2 mℓ of this suspension into 50 mℓ mineral medium to obtain a cell concentration of about 4 x 10^4/mℓ. Incubate at 15 °C. Determine the cell number (cfu/mℓ) periodically (± 3 times per week) until a maximum of about 3 x 10^6 cfu/mℓ has been obtained. This preculture is now ready to be used as an inoculum. Keep refrigerated at ± 4 °C. Always determine the cell number (cfu/mℓ) before the sample bottles are inoculated.

B2.3 Preparation of the mineral medium as described by Van der Kooij

3.42 mg	CH_3COONa
10 mℓ	Basal medium C
10 mℓ	Basal medium A
10 mℓ	Basal medium B
970 mℓ	Distilled water

The final pH should be 6.8. Adjustment of the pH is not necessary, even undesired as a precipitate may develop. Dispense in 50 mℓ quantities and sterilise at 121 °C ±1 °C for 15 minutes. Let it cool down. Add aseptically 0.5 mℓ ammonium chloride solution. Can be kept at 0 to 6 °C for one year.

B2.4 Treatment of water samples

Samples were taken from the sample points. Sodium thiosulphate was added to the samples to neutralize the chlorine (approximately 5 drops of a 10 % solution/1 ℓ water sample). Pour 600 mℓ water sample into each 1 ℓ glass stoppered Erlenmeyer flask. Pre-heat a waterbath to 60 to 70 °C. An extra flask with a thermometer is also put into the pre-heated waterbath with the other bottles. This dummy flask contains 600 mℓ tap water. When the thermometer in the dummy flask reads 70 °C then the flasks are left in the waterbath for an hour. After the hour the flasks are taken out and put into a waterbath with cold water. The water in the waterbath are constantly replaced by cold water so that the water samples can cool down quickly. Inoculate each flask with the pre-calculated volume of the inoculum to obtain 50 to 500 cfu/mℓ in the sample. Incubate the flasks at 15 °C. The water was not autoclaved but heat treated for an hour at 60 °C to kill the vegetative cells of micro-organisms in the water. This treatment was used to preserve the organic content of the water as much as possible in its original state (Van der Kooij *et al.*, 1982b).

B2.5 Determination of the number of organisms

Standard plate counts are done periodically by means of the spread-plate procedure. Lab-Lemco agar plates are poured before hand and left in a laminar flow cabinet to dry before it is used. Use precleaned 1 mℓ pipettes to withdraw 1 mℓ of the sample to be added to 9 mℓ physiological sodium chloride solution. A triplicate set of decimal dilutions are done for every sample bottle. A 0.1 mℓ of the chosen decimal dilutions are pipetted onto the Lab-Lemco agar plates and spread out. Counting is performed after 40 to 48 h of incubation at 25 to 28 °C. This was done until a maximum count (Nmax) was obtained and a reduction or no change in counts of two successive plate counts were observed.

B2.6 Calculation of the concentration of available AOC

Calculate the available concentration AOC used by the organism (P17 and/or NOX) during an experiment by means of the maximum colony count (Nmax) and the yield factor (γ).

Yield factor for that organism in a known acetate solution.

The acetate C concentrations used for determining the yield factor were 0,12,24,48,96 and 192μg acetate C/ℓ tap water. This was done in the same way as in the above mentioned procedure for the water samples. The following calculations was used to determine the yield factor for P17 or NOX at a given concentration of μg acetate C/ℓ:

$$\gamma = \frac{x-x_0}{c} \times 1000 \text{ m}\ell/\ell$$

γ = yield factor
x = maximum standard plate count (cfu/mℓ at a specific acetate concentration)
x_0 = maximum standard plate count at zero acetate concentration (in blank)
c = concentration (μg C / 1000 mℓ) (same concentration as used by x)

Determine the mean γ value at all the different concentrations acetate C used.

To determine the available AOC concentration as μg acetate C equivalents/ℓ in a test sample:

$$\text{Available AOC concentration} = \frac{\text{Nmax} \times 1000}{\gamma}$$

γ = yield factor for the specific test organism used - P17 and/or NOX

According to literature:

γP17 = 3.3×10^6 cfu/μg acetate C (Huck *et al.*, 1991)
4.1×10^6 cfu/μg acetate C (Van der Kooij *et al.*, 1989)
γNOX = 1.4×10^7 cfu/μg acetate C (Huck *et al.*, 1991)
1.2×10^7 cfu/μg acetate C (Van der Kooij *et al.*, 1989).

Yield factors obtained during project:

γP17 = 1993 - 4.31×10^6 cfu/μg acetate C
1994 - 6.9×10^6 cfu/μg acetate C
1995 - 5.8×10^6 cfu/μg acetate C
1996 - 6.4×10^6 cfu/μg acetate C
γNOX = 1995 - 6.4×10^6 cfu/μg acetate C
1996 - 6.3×10^6 cfu/μg acetate C

REFERENCES:

1. VAN DER KOOIJ, D., A. VISSER and W.A.M. HIJNEN, 1982a. Determining the concentration of easily assimilable organic carbon in drinking water. *J. Am. Water Works Assoc.*, 74 : 540 - 545.

2. KIWA, 1993. Huisvoorschrift : LMB - 004. *Internal publication.*

3. VAN DER KOOIJ, D., A. VISSER and J.P. ORANJE, 1982b. Multiplication of fluorescent Pseudomonads at low substrate concentration in tap water. *Antonie van Leeuwenhoek*, 48 : 229 - 243.

APPENDIX C : METHOD OF JAGO-STANFIELD

ATP determination is used as an indication of the concentration of assimilable organic carbon in water.

Micro-organisms are capable of utilizing or assimilating only a small fraction of the total organic carbon (TOC) fraction which is composed of biodegradable compounds. This portion of the TOC, termed assimilable organic carbon (AOC), cannot be quantified by simple chemical means since it is composed of a wide variety of compounds each of which is difficult to measure at low concentrations and, therefore, microbiological methods (bioassays) have been developed to measure the AOC content of water (Stanfield and Jago, 1989).

Principle of the method

The test involves monitoring the growth response of a natural population of bacteria added to a sterilised sample of the water being studied. The ATP concentration is used as a measure of the growth response and this is monitored daily until a maximum yield is obtained. The ATP yield is related to the quantity of AOC in the sample.

C1 EQUIPMENT

C1.1 Instruments and Materials

Oven (0 °C to 550 °C)
250 mℓ Erlenmeyer flasks
Complete glass filter unit, 47 mm diameter (Millipore, Cat.no.1504700)
Vacuum pump
0.2 µm Nucleopore polycarbonate membranes, 47 mm diameter (Cat. No. 111106)
1 mℓ Glass pipettes
Aluminum foil to cover the glassware
MacCartney bottles
Lumac Biocounter M 1500 P
Lumac Cuvettes (Cat.no. 9200-0)

C1.2 Chemicals

Lumac Water Microbial kit (Cat.no. 9288-5) with the following contents:
- Lumit PM
- Lumit buffer
- NRB (Nucleotide releasing reagent for microbial cells)

Lumac ATP stock solution
Phosphate-free Extran (Merck, Cat. No. 7550.5000) to clean glassware.

C2 PREPARATION OF SAMPLES

C2.1 Preparation of glassware

Soak glassware for 6 to 8 hours in phosphate free Extran. After that, rinse thoroughly with hot water and then with distilled water. Sterilize for 5 hours at 550 °C.

C2.2 Sample preparation

The water sample (±500 mℓ) and inoculum (raw water source) (±100 mℓ) was collected in sterile glass bottles. The inoculum is the indigenous bacterial population of the sample. Filter the water sample through a 0.2 µm polycarbonate membrane using a complete glass filter unit. Filter sterilization of the water sample is necessary to remove the indigenous bacterial population. Discard the first 50 mℓ of the filtrate because the first 25 mℓ of the filtrate could contain soluble organic carbon eluted from the polycarbonate membrane (Stanfield and Jago, 1989). Dispense 100 mℓ volumes of the filtrate into 250 mℓ Erlenmeyer flasks covered with aluminum foil. Add an inoculum of 1 mℓ into the flask and incubate at 22 °C on a shaker. Take a 3 mℓ sample for ATP measurements on a daily basis.

C2.3 ATP Measurement

ATP measurements were carried out using a LUMAC luminometer and Lumac ATP reagents (NRB and Lumit PM). Pipette 0.1 mℓ of the sample into a disposable Lumac cuvette, add 0.1 mℓ of the nucleotide releasing reagent (NRB) and place it into the Luminometer. The addition of NRB extracts ATP from the bacteria in the sample. Furthermore, the NRB initiates light measurement by the instrument and this reading is treated as the instrument background of the sample. After the initial measurement, 0.1 mℓ of the luciferin-luciferase reagent (Lumit PM) must be added to the cuvette and another light reading must be taken. The light generated as a result of the ATP is the difference between the two readings. Perform triplicate determinations on each sample.

C2.4 Calculation of ATP concentration (according to LUMAC Water Microbial Kit instructions)

To calculate the ATP concentration of a sample from its light reading (RLU = Relative Light Units) it is necessary to produce a calibration curve of the light emissions obtained from standard ATP solutions.

Internal standardization technique -

a. Sample measurement: measure the RLU value of the sample. The result is **Xa**.

b. Immediately add 20 µℓ of a known amount of ATP to the sample cuvette and measure again. The result is **Xb**

Note: For accurate internal standardization, the amount of ATP added should give a RLU value which is 2 to 5 times the value obtained with the sample.

c. Calculate the amount of ATP per RLU (the k-factor) as follows:

$$k = \frac{\text{ATP added}}{Xb - Xa}$$

d. Calculate the amount of ATP in the actual samples as follows:

ATP in the sample = k . (Xa - blank)

C2.5 **Conversion of the ATP value to an AOC value expressed as µg acetate C/ℓ**

A calibration curve of ATP yield from known concentrations of supplemented acetate was compiled according to Jago and Stanfield (1984). ATP values obtained from samples were read on the graph to obtain a derived AOC value.

REFERENCE:

STANFIELD, G. and P.H. JAGO. 1989. Application of ATP determinations to measure the concentration of assimilable organic carbon in water. In: ATP Luminescence - Rapid methods in Microbiology (Editors : P.E. Stanley, B.J. McCarthy and R. Smither). *Soc. Appl. Bacterial.. Tech. Series.* 26 : 99 - 108. Blackwell Scientific Publications, Oxford.

APPENDIX D : METHOD OF JORET-LÉVI: BDOC

In an attempt to provide a more rapid assay for BDOC, Joret and Lévi (Internal Publication) make use of pre-washed, biologically active sand as inoculum. The change in DOC of the sample is monitored daily until no further change in the DOC value occurs.

Principle of method:

An inoculum of biologically active sand, coming from a plant without pre-chlorination, is pre-washed until no further release of DOC. Fixed volume/mass of sample and inoculum are placed together into a pre-cleaned Erlenmeyer flask, aerated and incubated at room temperature (± 20 °C). Daily DOC measurements are done until no further change takes place. BDOC is calculated as the difference between the initial DOC value and the minimum DOC value reached.

D1 EQUIPMENT

D1.1 Instruments and Materials

500 mℓ Erlenmeyer flasks
1000 mℓ Glass measuring cylinder
Glass pipettes
Borosilicate Dreschel bottles (Cat.no. MF 29/3/500)
Borosilicate Dreschel bottle heads (Cat .no. MF 28/3/500)
Aquarium air pump

D1.2 Chemicals

Phosphate-free Extran (Merck, Cat. No. 7550.5000) to clean glassware

D2 PREPARATION OF THE SAMPLES

D2.1 Preparation of glassware

Soak the glassware for 6 to 8 hours in phosphate free Extran. Rinse thoroughly with hot water, and then with distilled water. After the glassware is dry, cover with aluminum foil and sterilize at 550 °C for 4 hours.

D2.2 Preparation of BAS (Biological Active Sand)

BAS is taken from a water treatment plant without any pre-chlorination. BAS is washed 10 times with ± 500 mℓ dechlorinated tap water (thiosulfate was added to the tap water to neutralize the chlorine), then 3 times with ± 500 mℓ distilled water, until no detectable DOC is released by the sand in the last washing water sample in comparison with distilled water.

The BAS is now considered ready for use as an inoculum. If released DOC is detected, additional washing must be performed.

D2.3 Storage of the sand

Store BAS fully emerged in dechlorinated tap water at ambient temperature. Before use, rinse the sand 3 times with freshly dechlorinated tap water (2 mℓ of a 10% thiosulfate solution in 5000 mℓ water).

D2.4 Methodology of the bioassay

Weigh 100 g of BAS into a previously heat treated (550 °C/ 4 h) Erlenmeyer flask (500 mℓ). Rinse sand with 100 mℓ of the water sample to be analyzed. Gently pour 300 mℓ of water into the flask containing BAS. Avoid too vigorous mixing. Measure the DOC of the water with BAS ($DOC_{t=0}$). Begin the test by aerating the water sample (flow rate 4 ℓ/h) with a normal aquarium air pump. Check the DOC daily until minimum DOC occurs. The flask with the sample must be kept open.

D2.5 Measurement of DOC

All DOC analysis are done in duplicate with a Technicon Auto-Analyzer 2 using the Persulfate - Ultraviolet Oxidation Method. DOC analysis were done by the Organic Chemistry Section according to a prescribed method.

D2.6 Calculation of BDOC

The BDOC of the water sample is calculated by taking the difference between mean values of initial and minimum remaining DOC reached after a few days incubation period (usually 5 days) of the sample in contact with BAS.

$$BDOC\ (mg/l) = DOC_{t=0} - DOC_{min(t=n)}$$

REFERENCE:

COMPAGNIE GéNéRALE DES EAUX. Protocol for BDOC determination in water using a fixed inoculum (BAS) - *Internal Publication.*

APPENDIX E : METHOD OF BILLEN-SERVAIS: BDOC(ΔDOC)

The change in the DOC value of the water sample is monitored over a time period.

Principle of the method:

The water sample is filter sterilised, re-inoculated with an inoculum from the same origin as the sample and incubated in the dark at approximately 20 °C for a period of four weeks. The DOC level is measured at the beginning and end of the incubation period and the difference is taken as the amount of BDOC.

E1 EQUIPMENT

E1.1 Instruments and materials

Oven (0 °C to 550 °C)
1000 mℓ Erlenmeyer flasks
Complete glass filter unit, 47 mm diameter (Millipore, Cat.no.1504700)
Vacuum pump
0.2 µm Nucleopore polycarbonate membranes, 47 mm diameter (Cat. No. 111106) (Cellulose acetate membranes were used by Servais *et al.*, 1987)
2.0 µm Nucleopore polycarbonate membranes, 47 mm diameter (Cat. No. 111111)
1 mℓ Glass pipettes
1000 mℓ Glass measuring cylinder
Aluminum foil to cover the glassware

E1.2 Chemicals

Phosphate-free Extran (Merck, Cat. No. 7550.5000) to clean glassware

E2 PREPARATION OF THE SAMPLES

E2.1 Preparation of glassware

Soak the glassware (except the filter frit) for 6 to 8 hours in phosphate free Extran. Rinse thoroughly with hot water, and then with distilled water. After the glassware is dry, cover with aluminum foil and sterilize at 550 °C for 4 h .

E2.2 Filtration , inoculation and incubation of the samples

A 500 mℓ sample is sterilized by filtration through a 0.2 µm Nucleopore polycarbonate membrane. The first 100 to 200 mℓ is discarded to avoid possible contamination by the filter. From the filter sterilised sample, a 300 mℓ volume is measured into a pre-cleaned Erlenmyer flask. A 3 mℓ (1 %) inoculum from the same natural environment from which the sample was

taken, is filtered through a 2 µm Nucleopore polycarbonate membrane, in order to eliminate big particles and protozoans, and is added to the sample. Incubate in the dark at ± 20 °C for a period of 4 weeks.

E2.3 SAMPLING FOR DOC ANALYSIS

DOC is measured at the beginning (t = o) and at the end of the incubation period. A Technicon Auto-Analyzer 2, using a Persulfate-Ultraviolet Oxidation Method was used. DOC analysis were done by the Organic Chemistry Section according to a prescribed method.

E2.4 CALCULATION OF BDOC (ΔDOC)

BDOC is calculated as the difference between the DOC at the beginning and the DOC at the end of the incubation period.

REFERENCE:

SERVAIS, P., G. BILLEN and M.C. HASCOËT. 1987. Determination of the biodegradable fraction of dissolved organic matter in waters. *Wat. Res.*, 21 (4) : 445 to 450.

APPENDIX F : PURIFICATION PROCESS AS USED BY RAND WATER

Rand Water abstracts almost all its raw water requirements from the Vaal River system via a canal and a gravity pipeline from the Vaal Dam, and by pumping from the Vaal River at Lethabo and from the Barrage reservoir.

Water from the Vaal Dam and the Barrage contains highly dispersed, suspended particles which must be forced to coagulate and settle during purification. The purification process consists of six stages:

1. **Coagulation**:
   Activated sodium silicate (dosage rate between 1 and 3 mg/ℓ as silicon dioxide) is added to the raw water to promote flocculation. Slaked lime (dosage rate varies between 55 and 70 mg/ℓ as calcined lime) is then added to the water as the main coagulant. The high pH of between 10.0 and 11.0 obtained during lime coagulation limits algal growth and is very effective towards the removal of heavy metals, some organic material, bacteria and viruses.

2. **Flocculation**:
   The destabilised particles form heavier visible particles called floc. The floc remains in suspension as water flows at high velocity through either spiral flocculators or conditioning bays.

3. **Sedimentation:**
   The water enters the sedimentation tanks where the floc settles to the bottom of the tanks in the form of sludge. Depending on the turbidity of the incoming raw water, between 75% and 97% of the suspended particles are removed during this process.

4. **Carbonation:**
   The use of lime as a coagulant raises the pH of the water to about 10.5. After sedimentation, the water flows into the carbonation bays where it is stabilised by treating it with carbon dioxide gas. This reduces the pH to levels of around 8.2.

5. **Filtration:**
   Following carbonation, the water flows through rapid gravity sand filter beds of finely graded silica sand and pebbles. The remaining suspended particles are removed at this stage.

6. **Chlorination and chloramination:**
   At the main pumping station reservoir, chlorine is added to disinfect the water. Depending on the raw water quality, the chlorine dosage may vary between 1.5 and 4.0 mg/ℓ. At the booster pumping stations, ammonia and chlorine are added to the water to control bacterial growth. Ammonia is used to prolong the effectiveness of the disinfectant.

TABLE F1 : WATER QUALITY DURING THE PERIOD OF JULY TO NOVEMBER 1993 (LIMS SYSTEM - routine analyses)

Parameter	Sample point		
	Raw Water (Vaal Dam)	After Chlorination	After Chloramination
Turbidity (NTU)	150	0.43	0.43
pH	7.7	8.1	8.2
Conductivity (mS/m)	12	21	18
Hardness (as $CaCO_3$ in mg/ℓ)	40	91	69
DOC (mg/ℓ)	3.2	< 2.0	5.1
Chlorophyll	7.28	0.12	-

Mean values

TABLE F2 : WATER QUALITY DURING THE PERIOD OF JANUARY TO DECEMBER 1994 (LIMS SYSTEM - routine analyses).

Source	Season	Turbidity (NTU)	pH	Conductivity (mS/m)	Hardness (as $CaCO_3$ in mg/ℓ)	Alkalinity (as $CaCO_3$ in mg/ℓ)	DOC mg/ℓ	Chloro phyll a
Raw Water (Vaal Dam)	Summer	160	7.9	15	50	48	3.9	10.72
	Autumn	160	7.9	18	59	56	5.0	11.02
	Winter	130	8.0	18	60	57	5.1	6.93
	Spring	110	7.8	19	64	60	5.7	6.97
	Summer	115	7.9	20	58	63	5.7	5.92
After chlorination	Summer	0.27	8.1	23	86	83	<2.0	0.19
	Autumn	0.47	8.3	28	110	100	3.4	0.22
	Winter	0.38	8.3	30	115	120	3.7	0.24
	Spring	0.30	8.1	24	91	81	4.0	0.28
	Summer	0.68	7.9	21	63	63	4.1	0.19
After chloramin-ation	Summer	0.39	8.3	22	83	77	2.4	N/A
	Autumn	0.55	8.2	26	99	95	3.0	N/A
	Winter	0.50	8.2	29	125	115	3.3	N/A
	Spring	0.62	7.9	24	84	85	3.8	N/A
	Summer	0.52	8.0	22	67	73	3.7	N/A

Mean values for each test period
N/A ≡ not applicable

TABLE F3 : WATER QUALITY THROUGH THE PURIFICATION PROCESS AS USED BY RAND WATER (Scientific Services Graphical Data January to December 1996).

	Turbidity (NTU)	**Alkalinity as $CaCO_3$ in mg/ℓ**	**pH**	**Conductivity (mS/m)**
Vaal Dam Water	75 - 240	42 - 65	7,5 - 8,2	12 - 17
After Sedimentation	5 - 10	7 - 120	10.6 - 11.1	18 - 28
After Carbonation	5 - 10	70 - 120	8.0 - 8.4	18 - 28
After Filtration	0.41 - 0.84	70 - 115	8.0 - 8.4	18 - 28
After Chlorination/Chlora-mination Consumer	0.41 - 0.84	69 - 115	7.9 - 8.3	18 - 28

APPENDIX G : COMPARISON BETWEEN LAB-LEMCO AND R_2A AGAR WITH THE POUR AND SPREAD PLATE METHODS

A comparison between the two types of agar and the two standard plate count methods were done to find the best combination to use for the Van der Kooij method. Four different concentrations of acetate in dechlorinated tap water, were used as samples. From each sample, one set of pour plates and one set of spread plates were done on each of the two types of media.

For the pour plate method, 1 mℓ from each dilution was pipetted into a sterile, disposable petri dish (90 mm diameter), mix with the specific media and left to solidify.

For the spread plate method, 0.1 mℓ from each dilution was pipetted onto a prepared agar plate from each type of agar and spread with a bent glass rod, sterilised with alcohol and a gas bunsen burner.

All the plates were inverted and incubated for 42 to 48 h at 28 $\pm$ 1.0 °C.

All results were processed with the STAT-GRAPHICS computer packages.

PROCESSED RESULTS OBTAINED (STAT-GRAPHICS)

Variable	LLLPOUR[1]	LLLSPREAD[2]	LR2APOUR[3]	LR2ASPREAD[4]
Sample size	51	51	51	51
Average	5,91276	6,00639	5,874	5,94023
Median	6	6,04139	6	6
Arithmetical mean	5,90253	6,00096	5,8602	5,93208
Variance	0,119865	0,0651261	0,16031	0,0968385
Standard deviation	0,346216	0,255198	0,400388	0,311189
Standard error	0,0484799	0,0357349	0,0560655	0,0435751
Range	1,44284	1,19584	1,59908	1,30103

[1]LLLPOUR Log Lab-Lemco agar pour plate
[2]LLLSPREAD Log Lab-Lemco agar spread plate
[3]LR2APOUR Log R2A agar pour plate
[4]LR2ASPREAD Log R2A agar spread plate

The average and median values of all the combinations do not differ much from each other. The Lab-Lemco agar/spread plate combination gave the highest average, median and arithmetic mean with the smallest variance and standard deviation in the counts. According to these results, the Lab-Lemco agar/ spread plate combination was chosen to use in the Van der Kooij method.

APPENDIX H : CHEMICAL ANALYSIS ON RW SAMPLES DURING SEASONAL INVESTIGATIONS

Results were discussed in Chapter 3.

Figure [i] - [iv] : Results from chemical analysis

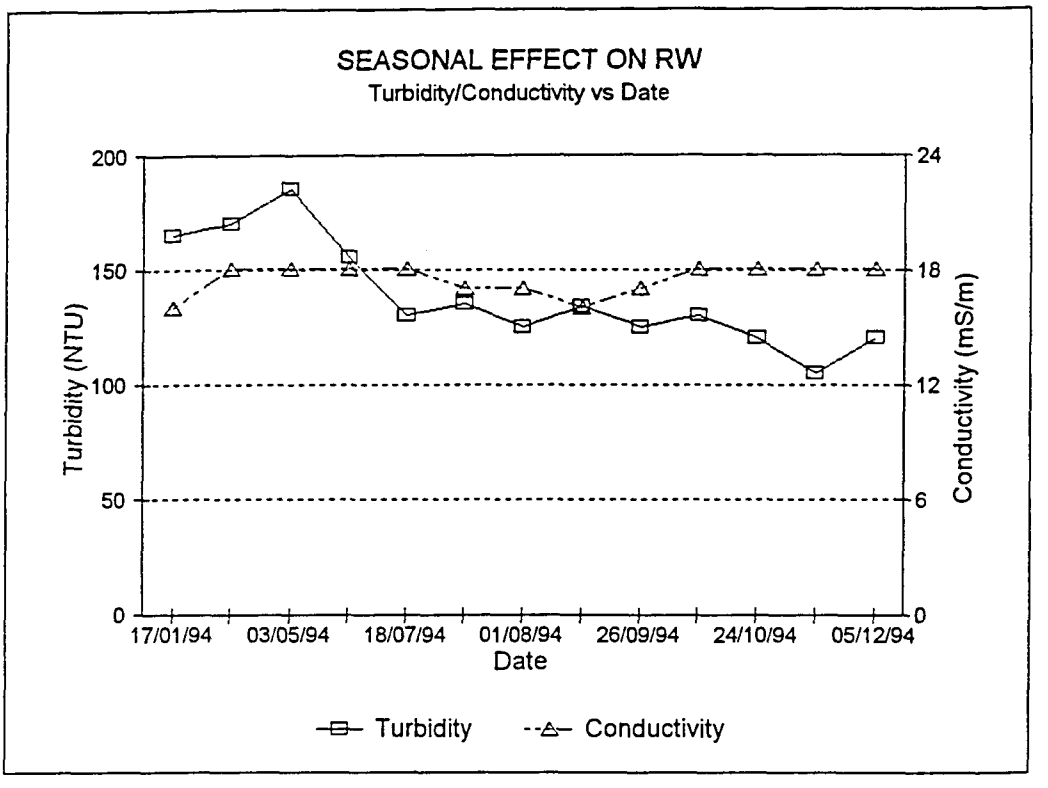

Figure [i] : Seasonal effect on turbidity and conductivity for RW.

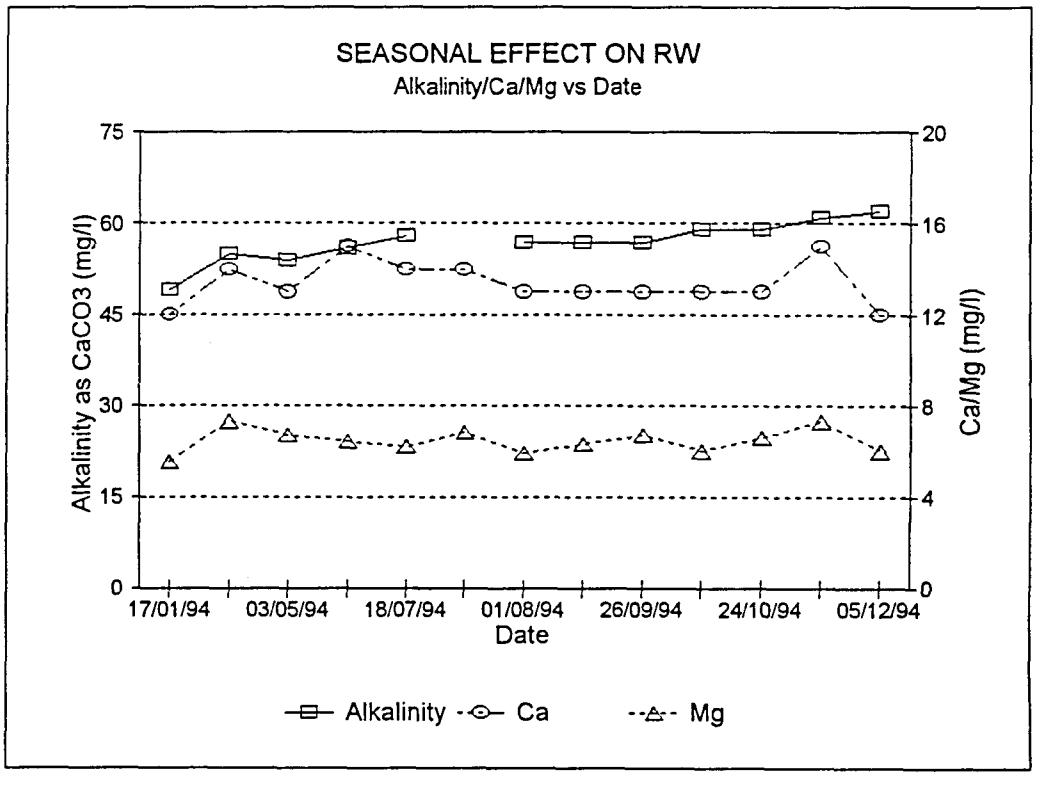

Figure [ii] : Seasonal effect on Alkalinity, Ca, and Mg for RW.

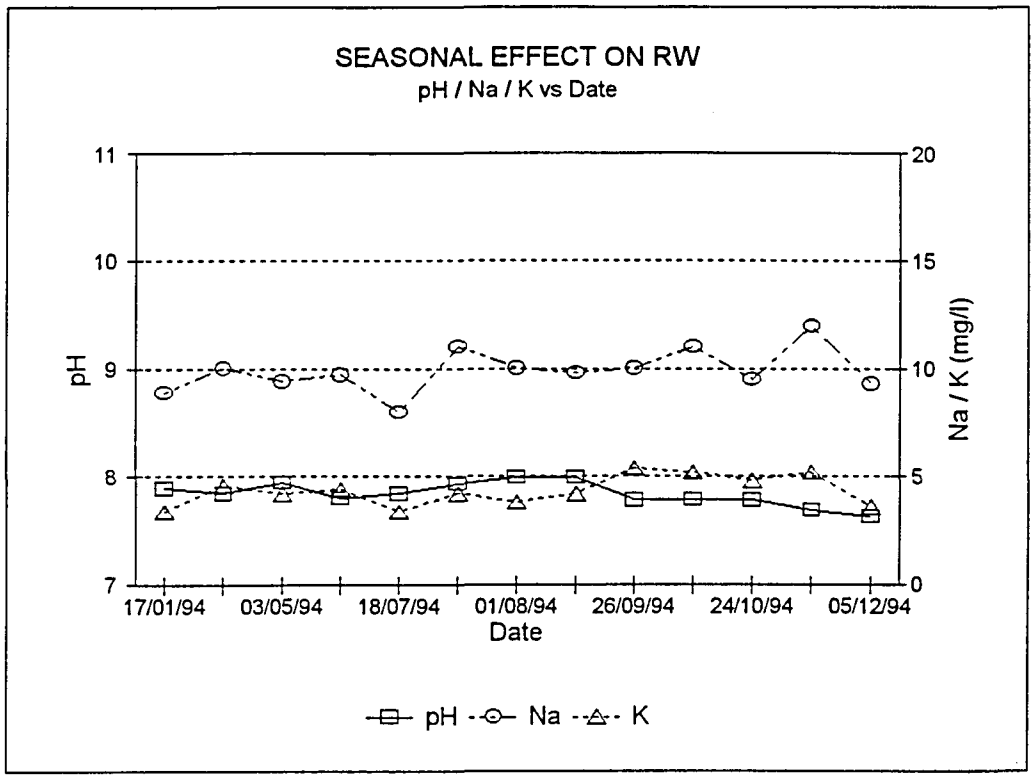

Figure [iii] : Seasonal effect on pH, Na and K for RW.

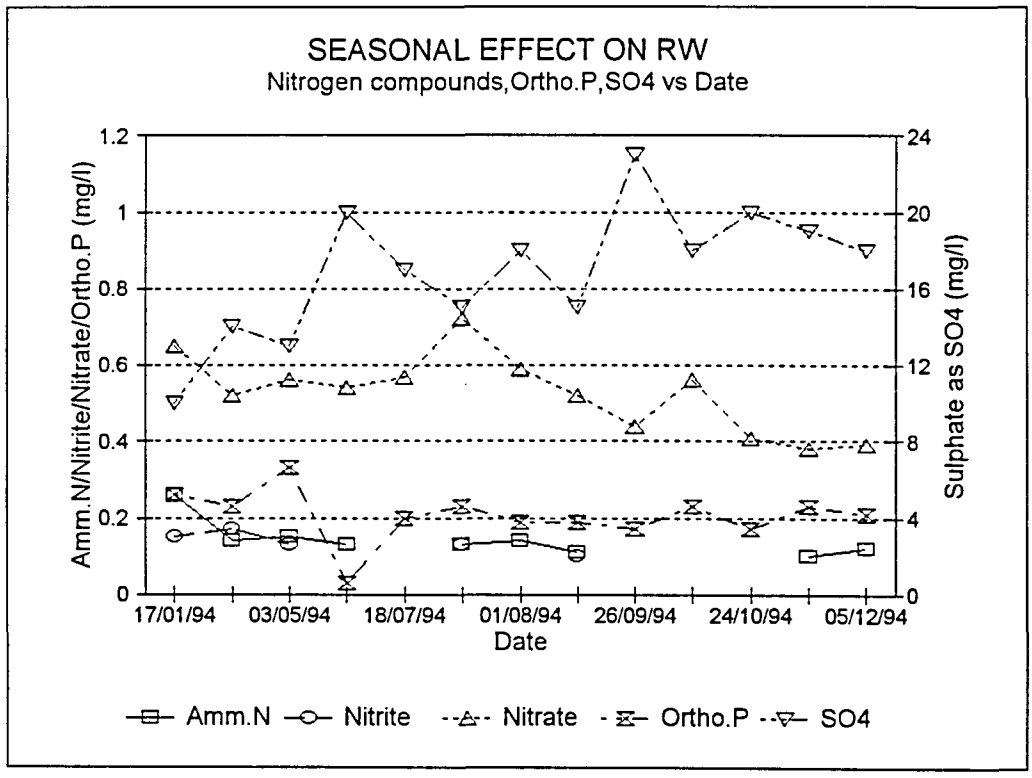

Figure [iv] : Seasonal effect on nitrogen, phosphate and sulphate for RW.

APPENDIX I : ACTIVATED CARBON FOR THE REMOVAL OF BIODEGRADABLE ORGANIC CARBON

Information on the granular activated carbon used in determining the effect of activated carbon on the removal of biodegradable organic carbon.

Type: WCM 006 granulated activated carbon from bitumen coal.

Bed depth	:	1.2 m		
Flow rate	:	7 m^3/h		
Time in use	:	Column 1	-	12 months
		Column 2	-	10 months
		Column 3	-	3 months

Period of evaluation : 26 June 1995 to 7 August 1995.

APPENDIX J : CHEMICAL DOSAGE DURING TREATMENT OF WATER WHERE PRE-CHLORINATION IS NEEDED DUE TO ALGAL PROBLEMS IN THE SOURCE WATER.

Sampling Date	14-02-96	29-02-96	13-03-96	28-03-96
Flow (Mℓ)	73.39	72.19	66.4	66.4
Pre-chlorination (mg/ℓ)	3.3	3.9	4.1	3.8
Lime (mg/ℓ)	52.2	76.2	38.4	48.5
Ferric chloride product (mg/ℓ)	38.8	19.7	21.4	30.5
Polymer (mg/ℓ)	1.3	9.2	8.5	1
CO_2	23.3	41.2	64	26.3
Post-chlorination (mg/ℓ)	5	5.1	5.3	4.3
Post-lime (mg/ℓ) for pH correction	3.7	3.6	1.3	6.5

Water samples were received from Goudveld Water treatment plant at Balkfontein. Water abstraction took place from the Vaal River. Due to a high algal concentration in the water, pre-chlorination was used as part of the standard treatment process. Chemical dosing with rapid mixing consists of polyelectrolyte/ferric chloride/lime. Depending on the water quality, lime/polyelectrolyte could be added before or after the ferric chloride. The ferric chloride and polyelectrolyte are the main coagulants. After flocculation and sedimentation took place, CO_2 was added. Following rapid sand filtration, the water was chlorinated for disinfection. Post-lime treatment was applied for pH correction of the water

APPENDIX K : CHEMICAL DOSAGE AT PLANT 2 ('82 SCHEME) - VEREENIGING PUMPING STATION AND THE CHLORAMINATION DOSAGE AT EIKENHOF BOOSTER STATION

The chemical dosages used in the treatment processes during the project period when samples were taken at different stages of treatment.

CHEMICAL DOSAGE AT PLANT 2 ('82 SCHEME)
RAND WATER - VEREENIGING PUMPING STATION

DATE	CALCINED LIME mg/l	SODIUM SILICATE mg/l	FERRIFLOC 1010 mg/l	POLY ELECTROLYTE U5000 mg/l	FERRIC CHLORIDE mg/l	CHLORINE mg/l
50793	68.00	1.89			0.45	1.58
120793	64.51	2.18			0.56	1.36
190793	59.22	1.43			0.29	1.26
260793	64.03	1.89			0.73	1.47
20893	60.43	1.57			2.32	1.66
90893	61.50	1.79			0.55	1.58
160893	55.69	1.07			1.57	1.35
230893	54.31	1.06			0.82	1.31
300893	56.35	1.47			0.39	1.36
60993	55.90	1.37			1.12	1.35
130993	56.35	0.95				1.12
200993	55.15	1.13			1.70	1.12
270993	59.45	0.62			1.30	1.29
41093	55.46	1.36			1.72	1.27
111093	55.70	1.79			0.95	1.11
181093	86.61	1.74		7.08	2.45	1.26
251093	54.58	0.85			2.92	1.50
11193	56.83	0.32			1.23	1.52
81193	67.08	1.75			1.00	1.50
151193	63.92	1.59			0.51	1.42
170194	63.33	3.66			1.18	1.47
140194	63.35	1.38			0.94	1.36
310194	69.81	1.93			1.36	1.69
70294	80.12	1.20			0.69	1.96
140294	64.63	2.06			1.18	1.90
210294	66.38	2.74			1.93	1.83
110494	62.23	2.24			1.29	1.57
250494	60.07	2.75			1.00	1.76
20594	62.24	1.51			1.37	1.81
90594	56.81	1.67			1.60	1.66
160594	58.70	1.85			0.88	1.77
230594	54.34	2.53			0.44	1.84
110794	61.96	2.89			3.38	2.13
180794	54.48	2.45			0.23	2.05
200794	63.51	2.77			1.39	2.34
250794	53.73	3.83			1.90	2.05
10894	52.71	2.80				2.01
80894	52.85	2.77			1.25	1.97
150894	45.64	2.44			0.76	1.68
220894	53.40	2.39			0.91	1.78
31094	56.73	2.08			1.57	1.90
131094	53.43	2.00			1.08	1.80
171094	54.05	2.14			1.22	1.87
241094	53.13	1.08			1.08	1.83
311094	53.28	2.01			1.85	1.85
71194	54.91	2.40			1.48	1.87
141194	55.41	2.43	9.57		1.04	1.78
51294	50.85	1.64			1.73	1.84
121294	46.91	1.14			0.70	1.91
('94-avg.)	57.90	2.23			1.27	1.84

CHEMICAL DOSAGE AT PLANT 2 ('82 SCHEME)
RAND WATER - VEREENIGING PUMPING STATION

DATE	CALCINED LIME mg/l	SODIUM SILICATE mg/l	FERRIFLOC 1010 mg/l	POLY ELECTROLYTE U5000 mg/l	FERRIC CHLORIDE mg/l	CHLORINE mg/l
160195	56.15	2.37			1.06	1.77
230195	56.12	1.44			1.08	1.93
300195	56.47	1.63				1.92
130295	66.51	3.09			1.17	2.13
200295	58.41	2.23			1.97	2.00
270295	66.13	1.48				1.94
60395	66.27	1.47			1.62	1.91
130395	66.47	2.76	10.04	4.15	1.54	1.91
270395	76.22	2.11			0.86	1.82
30495	79.05	1.56			0.43	2.10
190695	55.74	1.43			0.94	1.44
270695	54.81	1.52			1.27	1.31
40795	58.55	1.27			2.31	1.34
110795	51.53	1.11			1.66	1.27
250795	54.63	1.28			1.43	1.26
10895	47.09	1.25			0.70	1.21
290895	52.17	1.49			0.63	1.31
50995				3.78	0.93	1.22
120995	50.73	1.63			0.95	1.21
190995	51.22	1.31			0.76	1.21
101095	56.51	2.06			1.42	1.18
241095				4.10	1.84	1.49

CHLORAMINATION DOSAGE
EIKENHOF BOOSTER STATION

DATE	CHLORINE mg/l	AMMONIA mg/l
30893	0.49	0.28
70993	0.85	0.51
121093	0.47	0.54
161193	0.61	0.38
250194	0.96	0.3
80294	1.13	0.28
220294	1.19	0.31
30594	1	0.23
170594	1.08	0.23
310594	0.69	0.23
120794	0.24	0.13
260794	0.82	0.31
90894	0.77	0.26
230894	0.71	0.26
41094	0.88	0.23
181094	0.79	0.23
11194	0.87	0.25
151194	0.71	0.25
131294	0.87	0.24
160195	1.09	0.27
230195	0.85	0.27
300195	0.85	0.27
130295	0.64	0.21
200295	1.03	0.21
270295	1.1	0.21
60395	0.75	0.41
130395	0.78	0.28
270395	0.75	0.22
30495	0.76	0.21