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DEVELOPMENT OF FLOTATION AND NANOFILTRATION TECHNOLOGIES TO REMOVE CYANOBACTERIA AND CYANOTOXINS IN DRINKING WATER TREATMENT

(Tese para a obtenção do grau de doutor no ramo Ciências e Tecnologias do Ambiente, especialidade de Tecnologias do Ambiente)

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To my Parents Manuela e Manuel

Development of flotation and nanofiltration technologies to remove cyanobacteria and cyanotoxins in drinking water treatment

ABSTRACT

Dissolved air flotation (DAF) and nanofiltration were optimised and integrated to remove cyanobacteria and cyanotoxins from drinking water. The removal mechanisms of the most commonly occurring cyanobacteria (cultured cells and aggregates of *Microcystis aeruginosa* and *Plankthotrix rubescens* filaments) and cyanotoxins (hepatotoxic microcystins, and neurotoxic anatoxin-a) were investigated, as well as the impact of the water background organic (NOM) and inorganic matrixes, using both model and natural waters.

Results showed that coagulation/flocculation/DAF is the best process for clarifying cyanobacterial-rich waters (93-99% chlorophyll *a* removal), without toxin release to water and with lower NOM effect, using low recycle (8%), and lower coagulant doses, slower coagulation, stronger but shorter flocculation than the conventional coagulation/flocculation/settling.

Studies with a negatively charged tight nanofiltration membrane demonstrated that while nanofiltration fluxes of low NOM and moderately hard water are largely influenced by the background pH and calcium hardness, rather than by the type of NOM, anatoxin-a and microcystins are efficiently removed (anatoxin-a by electrostatic interactions and steric hindrance, microcystins mainly by steric hindrance), producing a final water of superior quality, also in terms of NOM content, regardless of the variations in feed water quality (pH, calcium hardness, NOM, toxins) and water recovery rate (up to 90%).

Key-words: Dissolved air flotation, nanofiltration, cyanobacteria, cyanotoxins, natural organic matter, drinking water

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TÍTULO DA TESE:

Desenvolvimento das tecnologias de flutuação e nanofiltração para remoção de cianobactérias e cianotoxinas no tratamento de água para consumo humano.

RESUMO

A flutuação por ar dissolvido (DAF) e nanofiltração foram optimizadas e integradas para remover cianobactérias e cianotoxinas de água para consumo humano. Investigaram-se os mecanismos de remoção de formas celulares, coloniais (*Microcystis aeruginosa)* e filamentosas (*Planktothrix rubescens)*, de microcistinas (hepatotóxicas) e anatoxina-a (neurotóxica), e a influência das matrizes orgânica (NOM) e inorgânica da água usando soluções modelo e águas naturais.

Demonstrou-se que a coagulação/floculação/DAF é o melhor processo para tratamento de águas ricas em cianobactérias (93-99% remoção de clorofila *a*), sem libertação de toxinas na água – requer baixa recirculação, menor dose de coagulante, menor agitação na coagulação, floculação mais intensa mas mais curta e tem menor efeito da NOM relativamente à coagulação/floculação/sedimentação convencional.

Os estudos de nanofiltração (com membrana apertada, negativa) demonstraram que o pH e dureza cálcica influenciam mais os fluxos de água moderadamente dura com baixo teor em NOM do que o tipo de NOM, mas que a anatoxina-a e as microcistinas são eficientemente removidas (anatoxina-a por interacções electrostáticas e impedimentos estereoquímicos, microcistinas principalmente por impedimentos estereoquímicos) independentemente das variações da qualidade da água bruta (pH, dureza cálcica, NOM, toxinas) e da taxa de recuperação (até 90%), produzindo-se uma água de qualidade superior, também em termos de NOM.

Palavras-chave: Flutuação por ar dissolvido, nanofiltração, cianobactérias, cianotoxinas, matéria orgânica natural, água para consumo humano.

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SYMBOLS AND ABBREVIATIONS

AHA	=	Aldrich humic acid
ATX-a	=	Anatoxin-a
C	=	Coagulation
C_b	=	Bulk concentration
C_f	=	Final concentration
C_i	=	Initial concentration
C_m	=	Concentration at the membrane surface
C_p	=	Permeate concentration
Chl_a	=	Chlorophyll *a*
DAF	=	Dissolved air flotation
DBPs	=	Disinfection by-products
DCO_2F	=	Dissolved CO_2/air flotation
DI	=	Deionised water
DOC	=	Dissolved organic carbon
DW	=	Decanted water
Extra MC	=	Extracellular microcystins
f	=	Rejection
F	=	Flocculation
G	=	Velocity gradient
Intra MC	=	Intracellular microcystins
i.e.p.	=	Isoelectric point
MC	=	Microcystin
MC-LF	=	Microcystin-LF
MC-LR	=	Microcystin-LR
MC-LW	=	Microcystin-LW
MC-LY	=	Microcystin-LY
MF	=	Microfiltration
NF	=	Nanofiltration
NOM	=	Natural organic matter
OW	=	Ozonated water
R/Q	=	Pressurised recycle

RO	=	Reverse osmosis
RW	=	Raw water
S	=	Sedimentation
SA	=	Salicylic acid
SUVA	=	Specific UV absorbance
THM	=	Trihalomethanes
THMFP	=	Trihalomethanes formation potential
TOC	=	Total organic carbon
TW	=	Tap water
UF	=	Ultrafiltration
WHO	=	World Health Organization
WTP	=	Water Treatment Plant

ΔE	=	Streaming potential
ΔP	=	Pressure
ξ	=	Apparent zeta potential
η	=	Viscosity of the permeate
κ	=	Conductivity of the solution
ε_o	=	Permittivity of vacuum
ε_r	=	Dielectric constant of the medium

CHAPTER 1
INTRODUCTION

ABSTRACT

This chapter introduces the cyanobacteria and cyanotoxins problem, especially when they are found in drinking water reservoirs. Special attention is given to the two technologies studied for the removal of those contaminants from drinking water, namely flotation and nanofiltration, including the parameters and operating conditions to optimise. Finally, the approach used to study flotation and nanofiltration, the scope, and the outline of this thesis are described.

Part of this chapter was presented in "11º Encontro Nacional de Saneamento Básico", APESB, University of Algarve, Faro, 11-15 of October 2004 as "Flotação e nanofiltração na remoção de cianobactérias e cianotoxinas de água para consumo humano".

1 INTRODUCTION

1.1 GENERAL

The presence of toxic cyanobacteria and cyanotoxins in a water body represents a potential risk for human health because cyanotoxins are responsible for hepatic and neuromuscular lesions and tumours. Cyanobacteria release to the water, not only cyanotoxins but also other compounds that may cause odour and taste decreasing the water's organoleptic and chemical quality. Hence, the growth of cyanobacteria in a water body used for human supply is a problem faced by water managements and Water Treatment Plants (WTP) engineering, which may result in the need to upgrade the water treatment system.

Therefore, the management and control of water bodies used for human water supply with episodes of cyanobacteria and cyanotoxins must be tackled at different levels in the hierarchy of the total water supply system. The first level is the assessment of the water body regarding the potential impact of blooms and cyanotoxins on water quality and public health. In this level the preference for control is the prevention of eutrophication. The second level of the hierarchy is related with engineering techniques at the water body or reservoirs to change its hydrophysical conditions and reduce the cyanobacterial growth. These techniques include the positioning of offtakes, the selection of intake depth and the use of barriers to restrict scum movements. Another intervention is the chemical treatment with algicides. This last technique has created controversy in the scientific community because of the environmental impacts, which include the release of cyanotoxins in the water body. The last level for controlling cyanobacteria and cyanotoxins in water supplies is related with the treatment system. At this level, the priority should be the use of water treatment technologies that remove intact cells and then remove the toxins present in the water (present in the raw water or released during the treatment).

This thesis concentrates on the last level. It focuses on the study of new methods for water treatment to face this growing problem, endowing the WTP engineering with alternative technical options. These methods could lead to an actualisation of the water treatment system, if the prevention management options did not succeed. The methods include flotation and nanofiltration technologies.

In recent years, surveys have been carried out in a number of countries in Europe, Africa, Asia, Australia and South America. The conclusions from these surveys are that toxic cyanobacteria are worldwide, and that as further surveys are carried out more toxic cyanobacterial blooms and new toxic species will be discovered (Sivonnen and Jones (1999)). In Portugal, the situation is similar and cyanotoxins (microcystins) have already been found from north (Vasconcelos *et al.* (1996)) to south (Rosa *et al.* (2004a)).

Funcho dam reservoir is one of the most important drinking water sources in western Algarve (southern of Portugal) and has a record of toxic cyanobacteria occurrences in this region. Funcho dam reservoir (*ca*. 2 km^2 of surface area and a volume capacity of 43.4 hm^3) has been used for water abstraction to Alcantarilha WTP since January 2000. Therefore, this water body was chosen as the case-study of the present work.

Alcantarilha WTP, run by Águas do Algarve, SA (an affiliate of Águas de Portugal, SGPE, SA) is responsible for providing a reliable supply of safe drinking water to *ca.* half million people in southern Algarve. This WTP was designed to treat up to 3 m^3/s by a conventional treatment of pre-ozonation, coagulation (C) /flocculation (F) /sedimentation (S), rapid sand filtration and chlorination. The coagulant used is aluminium polyhydroxichlorosulphate of high basicity. Alcantarilha WTP has to face a strong seasonal variation in raw water quality

together with a seasonal water demand (in 2002, it supplied *ca.* 180,000 of people during winter and 650,000 people in summer), so it has three treatment lines in parallel (1 m^3/s each).

Continued monitoring of these waters showed that seasonal variations correspond to two major types of raw water quality: clear waters (1 – 6 NTU) and turbid waters (25 – 40 NTU) (Ribau Teixeira *et al.* (2002)). Increases in turbidity usually occur after intense rainfall periods and give rise to higher organic carbon contents (Ribau Teixeira *et al.* (2002)); Ribau Teixeira and Rosa (2002) and Rosa *et al.* (2004b)). Microcystin results obtained in a monitoring program during September 2002 until October 2003 indicate the presence of extracellular microcystins (microcystin-LR and, probably microcystin-LY) throughout most of the year (September 2002 to January 2003, March, August and October 2003) (Rosa *et al.* (2004a)).

1.2 BACKGROUND

1.2.1 CYANOBACTERIA

Cyanobacteria, also known as blue-green algae, are a group of organisms that occur both in freshwater and marine environments. They are uni- and multicellular prokaryotes that possess chlorophyll *a* and use photosynthesis as their principal mode of energy metabolism. Therefore, their life processes require only water, carbon dioxide, inorganic substances and light.

The basic morphology comprises unicellular, colonial and multicellular filamentous forms. Unicellular forms have spherical, ovoid or cylindrical cells. The cells may aggregate into irregular colonies, being held together by the slimy matrix secreted during the growth of the colony (*Microcystis sp*). The multicellular structure consisting of a chain of cells is called

trichome, which may be straight or coiled. Cell size and shape show great variability among the filamentous cyanobacteria. Some species (order Oscillatoriales) are composed of essentially identical cells, unseriated and unbranched trichomes. Other filamentous species (orders Nostocales and Stigonematales) are characterised with trichomes having a heterogeneous cellular composition, with or without branches (Mur *et al.* (1999)).

Cyanobacteria have several important adaptations that help them to survive in environments where no other microalgae can exist, like the ability to store essential nutrients and metabolites within their cytoplasm and to fix nitrogen in the heterocyst cells. Some of them possess gas vesicles as another adaptation. These gas vesicles enable the buoyancy regulation, allow them to regulate their position in the water column and give them a distinct ecologic advantage over other planktonic species. A gas vesicle has a density of about one tenth that of water and thus gas vesicles can give cyanobacterial cells a lower density than water. Gas vesicles become more abundant when light is reduced and its growth rate slows down. Increases in the turgor pressure of cells, as a result of the accumulation of photosynthate, cause a decrease in existing gas vesicles and therefore a reduction in buoyancy. Cyanobacteria can, by such buoyancy regulation, poise themselves within vertical gradients of physical and chemical factors (Walsby *et al.* (1992)).

Cyanobacterial cells are microscopic, typically less than 10 μm in length or diameter, but the presence of very small cells of cyanobacteria (in the size range from 0.2 – 2 μm) has been recognised as a potentially significant source of primary production in various freshwater and marine environments (Mur *et al.* (1999)). Cyanobacteria can form blooms with millions of colonies in nutrient-rich water bodies. Factors such as nitrogen, phosphorus, temperature, light, micronutrients and buoyancy, have all been referred as factors affecting bloom

formation. These blooms are usually found in lakes and reservoirs, but more recently very slow flowing rivers have also been affected (Lawton and Robertson (1999)).

1.2.2 CYANOTOXIN

Cyanobacteria produce a variety of metabolites either toxic (cyanotoxins) or non-toxic, which natural function is unclear. Cyantoxins are produced and retained within healthy and actively growing cyanobacterial cells (*i.e.* these cyanotoxins are intracellular or in the particulate form) when the growing conditions are favourable. The amount of cyanotoxin increases in a culture during the logarithmic growth phase, being highest in the late logarithmic phase. They are only released into the surrounding water when cells senesce, die and lise (extracellular or dissolved toxins). Normally in healthy logarithmic phase cultures less than 10-20% of the total toxin is extracellular. As cells enter stationary phase the increased rate of cell death may lead to an increase in the extracellular dissolved fraction (Sivonnen and Jones (1999)). The range of measured concentration for dissolved cyanotoxins is 0.1 – 10 µg/L, except if a major bloom is breaking down (Jones and Orr (1994), Lahti *et al.* (1997)).

The effects of several environmental factors on growth and toxin production by cyanobacteria have been studied. Culture age, temperature, light, nutrients, salinity, pH and micronutrients concentration are the most frequently examined parameters. Environmental factors affect toxin content of cyanobacteria, but within a range of less than an order of magnitude (Sivonnen and Jones (1999)).

Cyanotoxins are a diverse group, both from the toxicological and the chemical points of view. They are classified toxicologically into hepatotoxic, neurotoxic and dermatotoxic. Classified

by their chemical structure, they are included into three groups: cyclic peptides, alkaloids and lipopolysaccharides.

1.2.2.1 CYCLIC PEPTIDE HEPATOTOXINS: MICROCYSTINS

Hepatotoxic cyclic peptides of the microcystin (MC) family are the most frequently found cyanobacterial toxins in blooms from fresh and brackish waters. Microcystins have been described from the genera *Microcystis*, *Anabaena*, *Planktothrix*, *Nostoc* and *Anabeanopsis* (Carmichael (1997)). At least 76 different microcystins found in natural blooms and laboratory cultures of cyanobacteria are reported in scientific literature (Sivonnen and Jones (1999), Spoof (2004)).

The cyclic peptides are rather small molecules with molecular weight ranging from 800 – 1100 g/mol. Microcystins contain seven amino acids, with the two terminal amino acids of the linear peptide being condensed (joined) to form a cyclic compound. The general structure of microcystins is cyclo(-D-Ala^1-L-X^2-D-*erythro*-β-methylisoAsp^3-L-Z^4-Adda^5-D-Glu^6-*N*-methyledydroAla^7), where Adda is (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Figure 1.1). The main structural variation in microcystins is in the L-amino acids residues 2 (designated as X) and 4 (Z). For the commonly occurring microcystin-LR (MC-LR), leusine is in position X and arginine is in position Z.

The cyclic peptides microcystins are water soluble, extremely stable and resistant to chemical hydrolysis or oxidation at near neutral pH. They are reported to withstand many hours of boiling and persist for many months or years in the dark, in natural waters (Sivonnen and Jones (1999), Lawton and Robertson (1999)). Slow hydrolysis has been observed at high temperatures (40 ºC) and high or low pH, with the times to achieve greater than 90%

breakdown being approximately 10 weeks at pH 1 and greater than 12 weeks at pH 9. Microcystins can be oxidized by ozone and other strong chemical oxidants agents, and by intense ultra violet light. However, all these conditions are unlikely to contribute to degradation occurring in the natural environment (Sivonnen and Jones (1999)).

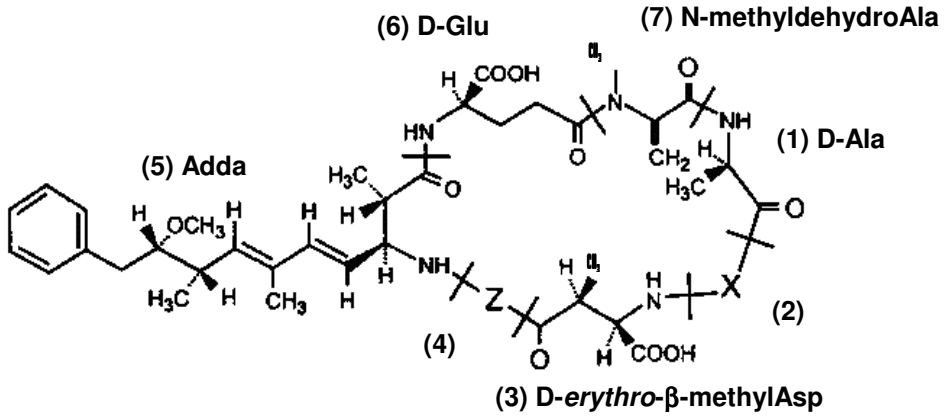

Figure 1.1 General structure of microcystins (Meriluoto (1997)).

Microcystins do not cross cell membranes and hence do not enter most tissues. They enter hepatocytes through the bile acid transport mechanism. The molecular basis of microcystins toxicity is by the inhibition of protein phosphatases 1 and 2A, which are crucial in cell regulation. This inhibition leads to a higher overall level of protein phosphorylation in hepatocytes, leading to cytoskeletal damage in the hepatocytes and haemorrhaging in liver (Carmichael (1997), Hitzfeld *et al.* (2000)). Therefore they cause severe liver damage and are tumour promoters (Matsushima *et al.* (1992)), so its presence in water, even at low concentrations, has particular interest to the water managers due to the acute toxicity and sublethal toxicity of these toxins. Most of the structural variants of microcystins are highly toxic within a comparatively narrow range. The LD_{50} (lethal dose resulting in 50% deaths) by the intra-peritoneal is in the range 25-150 μg/kg body weight in mice (a value of 50 or 60 μg/kg body weight is commonly accepted) (Kuiper-Godman *et al.* (1999)).

1.2.2.2 ALKALOID TOXINS

The alkaloid toxins are diverse, both in their chemical structures and in their mammalian toxicities. Alkaloids, in general, are a broad group of heterocyclic nitrogenous compounds (*i.e.* they contain ring structures with, at least, one carbon-nitrogen bond) usually of low to moderate molecular weight (< 1000 g/mol). Alkaloids have varying chemical stabilities, often undergoing spontaneous transformations to by-products which may have higher or, lower potencies than the parent toxin (Sivonnen and Jones (1999)).

Anatoxin-a (ATX-a) is one of the three families of neurotoxic alkaloids toxins known and has been found in *Anabeana*, *Oscillatoria Planktothrix spp*, *Aphanizomenon* and *Cylindrospermum*. Anatoxin-a is a postsynaptic neuromuscular blocking agent, *i.e.* nicotinic agonists because it mimics the effect of acetylcholine. It can induce muscle twitching and cramping, followed by fatigue and paralysis and death by respiratory arrest (Carmichael (1994)). The LD_{50} for this toxin is 200 μg/kg body weight (intra-peritoneal mouse) (Spoof (2004)).

Anatoxin-a is a low molecular weight alkaloid, 166 g/mol, a secondary amine, 2-acetyl-9-azabicyclo(4-2-1)non-2-ene (Figure 1.2). Anatoxin-a is relatively stable in dark, but in pure solution and in the absence of pigments it undergoes rapid photochemical degradation in sunlight. Breakdown is further accelerated by alkaline conditions (Sivonnen and Jones (1999)).

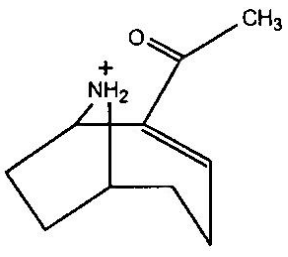

Figure 1.2 The chemical structure of anatoxin-a (Sivonnen and Jones (1999)).

1.2.3 DISSOLVED AIR FLOTATION

1.2.3.1 GENERAL

Dissolved air flotation (DAF) is a unit operation used to separate solid particles from a liquid phase. Separation is brought about by introducing fine gas (usually air) bubbles into the liquid phase. Bubbles are generated by the release of pressurised water which has first been air saturated at higher pressure than the atmospheric pressure. The bubbles attach to the particulate matter and the buoyant force of the combined particle and gas bubbles is great enough to cause the particle to rise to the surface.

If the particles have a flocculant character and/or can be destroyed (as is the case of the cyanobacterial flocs), they should not be subjected to shearing stresses associated with pressurisation. In this case, pressurised recycle should be used. The recycled flow is mixed with the unpressurised main stream just before entering the flotation tank, so the air comes out of solution in contact with the particulate matter at the entrance of the tank (Figure 1.3). Coagulation / flocculation (C/F) are usually required prior to DAF because, by the addition of reagents that act at the water-particle-air interfaces, these operations significantly increase the solid-liquid separation efficiency by DAF (Figure 1.3).

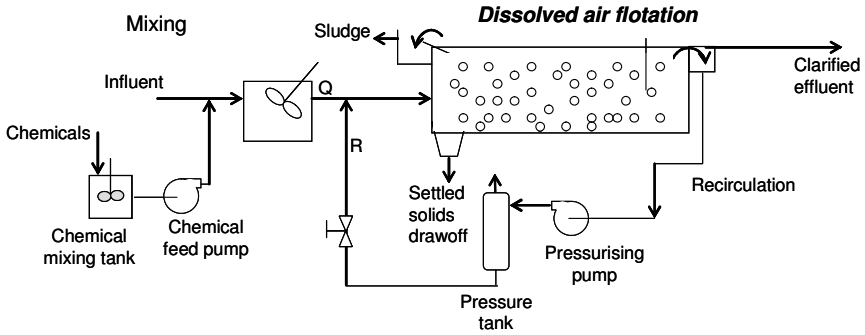

Figure 1.3 Schematic representation of a dissolved air flotation system with pressurised recycle and chemical addition (C/F) (adapted from Metcalf and Eddy (2003)).

Flotation is a technology used in some WTP to remove very small or light particles that settle slowly like algae, natural colour or clay. With flotation technology, this type of particles can be removed more completely in a shorter time. Once the particles have been floated to the surface, they can be skimmed off.

According to Edzwald *et al.* (1992), Mouchet and Bonnélye (1998) and Schofield (2001) the advantages of the DAF process over the conventional water clarification by C/F/ sedimentation (C/F/S) and/or filtration are: i) smaller sedimentation and flocculation tanks compared with those for C/F/S, lowering the capital costs; ii) lower coagulant and flocculant doses than for settling; iii) better removal of low density particles and algae that can cause short filter runs in conventional or direct filtration plants; and iv) higher solids content of sludge. In turn, DAF disadvantages are: i) higher power costs from pumping the recycled water (but these operation costs may be offset by the reduced costs for coagulants and flocculants aids) and ii) the need for qualified personnel. Mouchet and Bonnélye (1998) referred in some WTP, when the sludge treatment was also taken into account, flotation reduced the operation costs by 10-15% compared with sedimentation (the floated sludge was almost 10 times more concentrated than the settled sludge). The coagulant dose was also reduced by 20-40% in the flotation processes.

While it is not more effective than the conventional sedimentation process for removing extracellular toxins, DAF with pressurised recycle is generally more effective than sedimentation processes for treating algal-rich waters (Hrudey *et al.* (1999)). However, the type and dose of coagulant, as well as the C/F/DAF operating conditions are key parameters for removing intact cyanobacterial cells.

1.2.3.2 OPTIMISATION OF THE OPERATING CONDITIONS

There are many DAF studies on the laboratorial optimisation of the operating conditions of the process and comparison with the conventional sedimentation, as well as studies with flotation systems already working in a WTP. Generally, these studies are consonant about the importance of the C/F pre-treatment to the DAF process efficiency. This is attributed to the DAF dependency of the particle and bubble charges, effective flotation requiring destabilised particles of low or no charge and hydrophobic particles (Edzwald *et al.* (1992)), Fukushi *et al.* (1995), Han *et al.* (2001)).

In the C/F pre-treatment, the key operating conditions are the flocculation time, the size of the flocs, the velocity gradients (G) for coagulation and flocculation, and the coagulant type and dose. The parameter usually considered to optimise the DAF operating conditions is the bubble concentration, affected by the recycle ratio and the air saturation pressure. Other aspects like nozzle type have also been referred (Dupre *et al.* (1998), Schofield (2001), Ta *et al.* (2001)).

For instance, Edzwald *et al.* (1992) studied the effect of the flocculation time, the floc size and the air requirements on the DAF performance for treating three types of waters using both bench-scale and pilot plant data. They concluded that long flocculation times are not necessary for efficient DAF (5 – 8 minutes *vs.* the normal 20 min for flocculation prior to sedimentation), the floc size should be between 10 – 100 μm (ideally between 10 – 30 μm) and a bubble concentration of 1000 – 10,000 mg/L guarantees good collision opportunities between the particles and the air bubbles. To produce these microbubbles, a saturation pressure of 400 – 600 kPa is recommended. They found good removal efficiencies with 4600

mg/L of bubbles (8% recycle ratio) in waters with 2 – 15 mg/L of dissolved organic carbon (DOC), 20 – 100 mg/L of clay and $2x10^4 – 5x10^5$ of algae cells /mL.

In what concerns the reduction of the flocculation time, there has been some consensus among the authors: from 20 – 30 minutes in flocculation of the conventional sequence (C/F/S) to 5 – 10 minutes in the DAF sequence (C/F/DAF) (Malley and Edzwald (1991), Fukushi *et al.* (1995), Valade *et al.* (1996), Vlaski *et al.* (1996), Han *et al.* (2001)). However, the opinions diverge about the floc size. Valade *et al.* (1996), Jameson (1999) and Bache and Rasool (2001) referred that larger flocs are not necessary after flocculation, while Fukushi *et al.* (1995) demonstrated that larger flocs have higher collision efficiency.

For the velocity gradient, values between 30 and 80 s^{-1} have been proposed for flocculation. Valade *et al.* (1996) studied G values of 30 s^{-1} and 70 s^{-1}. The best results of turbidity and particle counts were obtained for the highest G and 5 minutes of flocculation. However, the G variation had a higher effect when $Fe_2(SO_4)_3$ was used as coagulant instead of alum ($Al_2(SO_4)_3$). Based on bench-scale experiments with G values of 10 s^{-1}, 23 s^{-1} and 70 s^{-1}, Vlaski *et al.* (1996) found that the algae removal efficiency was similar or better for G of 10 s^{-1} than 70 s^{-1} and they concluded that G values of 10 s^{-1} produced floc volume distributions which lead to a highest DAF removal efficiency, although the flocs were of a weaker structure. In another study, in pilot plant experiments, these authors obtained better turbidity removals with G values of 50 s^{-1}, because the increase of the flocculation energy input created better contact opportunities for the colloidal and particulate matter (Vlaski *et al.* (1997)). Zabel (1985), Hedberg *et al.* (1998) and Scriven *et al.* (1999) also presented G values between 70 – 80 s^{-1} associated with high algae and turbidity removals.

The recycle system effectiveness has been referred as crucial to the success and economy of the DAF process (Crossley *et al.* (2001)). In this line, Edzwald *et al.* (1992) studied different recycle ratios from 2 to 10% and verified that 8% was a good value for the recycle ratio in terms of clay, fulvic acids and algae removal efficiencies. They concluded that there is a minimum recycle or bubble volume concentration needed for effective DAF treatment which increases with increasing raw water concentration or flocculated water turbidity. Kempeneers *et al.* (2001) made experiments with 6% recycle ratio and Vlaski *et al.* (1996) with 8%. Schofield (2001) recommended values between 6 and 10%. Higher recycle ratios and saturation pressures resulted in an increase of the mean bubble values to sizes that lead to an insignificant increase of DAF efficiency (Vlaski *et al.* (1997)).

1.2.3.3 TREATMENT / DISPOSAL OF FLOATED SLUDGE

Treatment of sludge skimmed off from DAF is similar to sludge treatment from sedimentation, but DAF sludges are more concentrated. Their treatment depend on the composition of the raw water. If cyanobacteria are present in raw water, they will be concentrated in the sludge, therefore the potential release of toxins by cell lysis during treatment should be minimised by (Hall *et al.* (2005)):

- Avoiding recycling, if possible, when cyanobacteria concentrations are highest;
- Operating sludge thickeners to ensure good quality supernatants;
- Minimising sludge agitation to reduce the potential for cell lysis;
- Minimising sludge storage times prior to thickening and dewatering;
- Minimising sludge retention time in thickening, without compromising performance.

Sludge dewatering by centrifuge or filter press could result in significant cell lysis, and recycling of liquors from these processes should be avoided at times of highest cyantoxin risk.

Extended storage of sludge for several days prior to disposal (but following sludge treatment) during periods of cyanotoxins risk would be beneficial, since significant toxin biodegradation can occur (Hall *et al.* (2005)).

Disposal of sludge containing cyanotoxins must follow the procedures establish in legislation. In Portugal, it is the responsibility of the waste producer to identify and quantify any hazardous wastes. The producer is also responsible for the adequate elimination of these wastes. Sludge containing cyanobacteria or/and cyanotoxins must be disposed or incinerated as hazardous waste.

1.2.4 NANOFILTRATION

1.2.4.1 GENERAL

Nanofiltration (NF) is a pressure driven membrane process since it uses the pressure difference between the feed and the permeate sides as the driving force for solvent transport through the membrane. NF separation lies between ultrafiltration (UF) and reverse osmosis (RO) and is used when low molecular weight solutes are to be removed. Compared with UF, NF membranes have a smaller pore size (usually below 2 nm), hence organic compounds of lower molecular weight are retained (usually above 200 g/mol). Compared with RO, the retention of monovalents ions is lower, therefore NF requires lower operating pressures since the osmotic pressure gradients are minimised (Mulder (1997)).

Common NF applications are drinking water production, as well as wastewater treatment and industrial (*e.g.* biotechnological, pharmacological, chemical and textile) processes.

Membranes should combine high permeability and high selectivity with sufficient mechanical resistance stability. To accomplish that, asymmetric membranes were developed, *i.e.* consisting of a thin active layer (0.1 to 1 μm) responsible for the separation efficiency supported by one or more thicker layers with larger pores of the same or different polymeric materials – composite membranes. The supporting layers do not contribute to the resistance against mass transfer, so the permeability of the membrane is only determined by the thin active layer. These asymmetric membranes constituted a milestone for industrial applications of membrane processes since they combine high flux with sufficient mechanical strength. Most membranes commercially available nowadays are thin film composite membranes. The materials commonly used in NF membranes are aromatic polyamide, polysulfone/poly(ether sulfone)/sulfonated polysulfone, cellulose acetate, or poly(piperazine amide).

The membrane is permeable to the solvent (water), but it is less permeable or impermeable to the solute (salt) (Figure 1.4a). Therefore, to make the water pass through the membrane from the concentrate solution to the dilute solution, the driving force applied (such as pressure) must be higher than the osmotic pressure. In the membrane processes the concentrate stream represents *ca.* 10-20% of the feed stream, with a concentration 5 to 10 times higher than the feed stream (Van der Bruggen *et al.* (2003)). As a result, the treated water flux (permeate) is much higher than the contaminated effluent flux (concentrate) (Figure 1.4b).

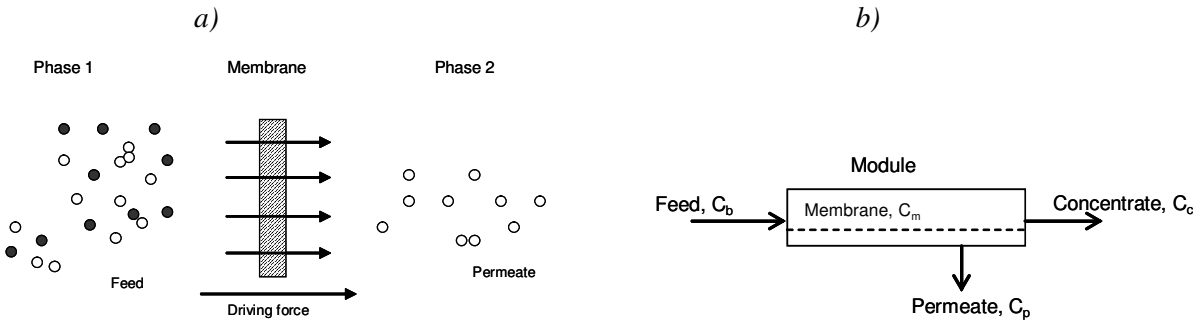

Figure 1.4 a) Schematic representation of a two-phase system separated by membranes (Adapted from (Mulder (1997)); **b)** Streams in the membrane process.

The separation mechanism is normally explained in terms of charge and/or size effects (Chaufer *et al.* (1996), Peeters *et al.* (1999)). Transport of uncharged solutes takes place by convection due to a pressure difference and by diffusion due to a concentration gradient across the membrane. The sieving mechanism (Figure 1.5a) is the motor responsible for the retention of uncharged solutes which will accumulate in the membrane surface and constitute the concentration polarisation layer, if the hydrodynamic conditions cannot return to the bulk solution (by diffusion) the uncharged material pushed (by convection) to the membrane surface (Figure 1.5b). However, neutral molecules also interact with membrane charge (NF membranes are usually (negatively) charge), mainly through polarity effects as explained by Van der Bruggen *et al.* (1999). For charged solutes an electrostatic interaction takes place between the solute and the membrane since most NF membranes are (negatively) charged (Figure 1.5c). The effect of membrane charge on the transport of charged components has already been described by Donnan in the beginning of the 20th century. Equilibrium between the charged membrane and the bulk solution is established, characterised by the Donnan potential, and leads to the retention of ionic species. This mechanism (Donnan exclusion) allows the removal of ions with a size below the pore size of the membrane.

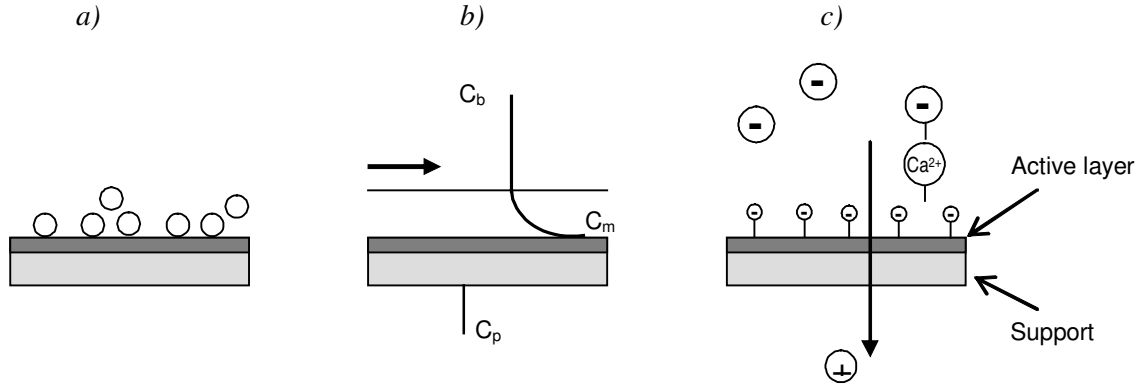

Figure 1.5 Transport in NF: **a)** sieving mechanism, **b)** concentration polarisation, and **c)** charge effects (Adapted from Schäfer (2001)).

There are a number of models to explain the transport phenomena through NF membrane. Much of them use the Extended-Nernst Planck equation that includes diffusion, convection

and electric field gradient. Solute transport by convection takes place due to an applied pressure gradient across the membrane. A concentration difference on both sides of the membrane causes the diffusive transport. The electric field gradient is fundamental in NF membranes, since they acquire a surface electrical charge. For uncharged solutes, electromigration may be omitted and the diffusive and convective flows govern the transport of solutes inside the membrane. Furthermore, the Nernst-Planck equations involve transport coefficients and parameters characteristic of the membrane-solute-solvent system of easy physical interpretation and capable of being related to the operating conditions.

The Nernst-Planck based model has proven to be successful for modelling the solute transport in simple electrolyte solutions, although its applicability in the presence of organics is questionable (Schäfer (2001)). Wang *et al.* (1997) developed a model to account for the transport phenomena of organic electrolytes, thus combining electrostatic and steric hindrance effects. Other models like the Steric Hindrance Pore Model has been used for the calculation of an effective pore radius and the ratio of membrane porosity to membrane thickness (Wang *et al.* (1995)). In fact, the majority of models are used more often to determine the effective pore size, because NF pores are too small to be measured directly (Schäfer (2001)).

1.2.4.2 REJECTION AND FOULING

NF membrane separation efficiency is usually related to membrane effective pore radius (average pore size and size distribution) or molecular cut-off defined as the molecular weight of the solutes that are more than 90% rejected by the membrane. The molecular cut-off of the NF membranes is between 100 and 1000 g/mol and the pore size is less than 2 nm (Mulder (1997)). Therefore, the rejection of organics should be high, while the salt rejection depends on the charge and the valence of the ions, and the membrane charge.

There are many studies regarding the determination of membrane charge and its relation with the salt rejection: the highest the charge, the highest the rejection (Berg *et al.* (1997), Peeters *et al.* (1999), Childress and Elimelech (2000)), being the membrane charge very dependent of the solution pH (Elimelech *et al.* (1994), Nyström *et al.* (1995), Childress and Elimelech (1996), Schaep and Vandecasteele (2001)). Both co- and counter-ions can absorb to charged membranes surface due to electrostatic and non-electrostatic interactions. As anions in the vicinity of non-polar surfaces are less hydrated than cations, the former can adsorb more closely to the surface, often resulting in an excess of negative charges in the layer nearest to the surface (Elimelech *et al.* (1994)). If a charged membrane is put in contact with an ionic solution, ions with the same charge sign of the membrane (the co-ions) are excluded and cannot pass the membrane, whereas the ions with opposite charge sign of the membrane (counter-ions) are able to pass the membrane (Mulder (1997)). For negatively charged membranes, the anions are therefore responsible for the rejections, while for positively charged membranes rejection is determined by the cations. For neutral membranes, the steric hindrance effects prevail. Due to the membrane charge, a concentration difference of the ions between the solution and the membrane is built-up, resulting in an osmotic pressure difference between the membrane and the solution. An additional potential across the membrane, the Donnan potential, will compensate this osmotic pressure, since equilibrium is assumed (Peeters et al. (1999)).

Some studies observed negative rejections due to the ion, usually H^+, increase in the permeate and its high permeation rate compared to the others ions (Childress and Elimelech (2000), Dey *et al.* (2000), Tanninen and Nyström (2002)).

Macromolecular or dissolved organic compounds have also been studied by some authors. Results showed that these compounds are responsible for the membrane fouling by accumulating on the membrane surface or inside the pores. As a result, the performance and the life of the membranes decrease. The dissolved organic substances have been referred as the main responsible for the membrane fouling in the filtration of natural waters (Hong and Elimelech (1997)). These substances include the humic and fulvic substances that represent the higher fraction of the natural organic matter (NOM), with low molecular weight.

Generally, factors influencing the membranes NOM fouling are often classified as: i) physical-chemical characteristics of NOM and membranes, ii) hydrodynamic conditions, and iii) chemical composition of the feed water (Hong and Elimelech (1997), Lee *et al.* (2004)).

Several studies demonstrated that the extent of NOM fouling is greatly influenced by the hydrophobicity of the membrane and NOM. Jucker and Clark (1994) concluded that humic substances adsorbed more favourably onto hydrophobic membranes. Nilson and DiGiano (1996) showed that hydrophobic fraction of NOM was mostly responsible for permeate flux decline, whereas the hydrophilic fraction caused much less fouling. They also showed that only the large molecular weight fraction of NOM contributed to the formation of a fouling layer.

Concerning the hydrodynamic conditions role on NOM fouling, Braghetta (1995) amongst others demonstrated that the permeate flux increases at higher crossflow velocities, due to the disruption of the NOM fouling layer. Chellam and Taylor (2001) evidenced that operating conditions such as feed water recovery had a significant negative impact on the rejection

(rejection decrease as the recovery increase) of total hardness and total triahlomethanes by NF membranes.

The chemical composition of the feed water, pH, ionic strength and the presence of multivalent cations have a great influence on NOM adsorption. Elimelech and co-workers (Childress and Elimelech (1996), Hong and Elimelech (1997), Faibish *et al.* (1998)) and Kilduff *et al.* (2004) observed a flux decline at low pH and a rejection increase at high pH. This behaviour was attributed to charge reduction of the membrane and the humic macromolecules at low pH. As the ionic strength increased, flux and rejection decreased due to the increase of the hydraulic resistance of the fouling layer (Hong and Elimelech (1997), Schäfer *et al.* (1998), Kilduff *et al.* (2004)). Hong and Elimelech (1997), Yoon *et al.* (1998) and Her *et al.* (2000) showed a water flux decrease with increasing calcium concentration. This was related with the reduction of the NOM charge due to effective charge screening and complex formation.

1.2.4.3 TREATMENT OF CONCENTRATE STREAMS

The treatment of the concentrate stream (that corresponds to a volume of 10-20% of the feed stream, Figure 1.4b) largely depends on the composition of the feed. The treatment of this stream is a cost that should be considered in the overall costs of this technology.

In the drinking water industry, the components to be removed are usually non-toxic (hardness, suspended solids) or toxic but in low concentrations (micropollutants, *e.g.* herbicides, pesticides or cyanotoxins). Methods for disposal of concentrate, when no toxic compounds are present, include discharge into saline water, irrigation in arid areas, and deep well

injection when a favourable injection zone is present. The latter technique is expensive and has a significant influence on the cost of the produced water (Van der Bruggen *et al.* (2003)).

If the concentrate stream contains an organic fraction, a biological treatment can be used in the case of biodegradable compounds or ozonation in case of recalcitrants. Another technique that has been studied is electro-oxidation, where recalcitrant organic compounds can be efficiently removed by anodic oxidation (Van Hege *et al.* (2004)). If the concentrate stream contains a large organic fraction and/or high toxic compounds (as cyanotoxins), the treatment can be by evaporation and deposition in a landfill, and incineration with energy recovery. As referred in section 1.2.3.3 and according to national waste legislation, it is the responsibility of the waste producer to identify and quantify any hazardous potential of wastes, and to guarantee an adequate elimination of these wastes.

1.3 GUIDELINES AND STANDARDS

Since cyanotoxins have been found in drinking waters, a provisional Guideline Value of 1.0 µg/L for MC-LR (one of the most commonly occurring cyanotoxins) in drinking water was proposed in 1998 by the World Health Organisation (WHO (1998)). In the European Union, these toxins are not clearly regulated. However, the European Water Framework Directive (2000/60/EC, European Union (2000)) characterises them as high priority water pollutants, and toxin producing cyanobacteria have been specifically highlighted as potential key hazardous pollutants (Table 1.1). Besides this, some European Countries, like Spain, France, Poland and Germany, have already created specific national legislation. Other guideline values for cyanobacterial toxins exist in several countries worldwide, most of these countries having a history of problems with cyanobacteria in drinking water reservoirs (Table 1.1).

Besides these guideline values, the WHO through the work of Bartram *et al.* (1999) established Alert Levels with the objective of providing the water treatment plant operators and managers with a graduated response to the onset and progress of cyanobacterial bloom. The Alert Levels Framework developed for the assessment of a potentially toxic cyanobacterial bloom provides appropriate actions and responses through three stages of progressing cyanobacterial numbers: Vigilance Level (initial detection), Alert Level 1 (moderate to high cyanobacterial number and possible detection of toxins above guideline values) and Alert Level 2 (very high cyanobacterial biomass level with potential health risks).

Table 1.1 Guideline values for cyanotoxins.

Country	Guideline value	Comments	Reference
Australia	1.3 µg/L MC	Guidelines are not mandatory standards, designed to provide guidance for a good quality drinking water.	NHMRZ/ARMCANZ (2001)
Brazil	1.0 µg/L MC	Guideline value adopted as mandatory.	Carmichael *et al.* (2001)
Canada	1.5 µg/L cyanobacterial toxins as MC	Canada uses guidelines as the standard of water quality.	Heath Canada (2002)
European Union	No specific values for cyanobacterial toxins	In European Water Framework Directive (2000/60/EC), toxin producing cyanobacteria have been specifically highlighted as potential key hazardous pollutants.	European Union (2000)
New Zealand	1.0 µg/L MC 3.0 µ/L ATX-a	Provisional maximum acceptable values.	Ministry of Health (2002)
USA	Not currently known	-	-
WHO	1.0 µg/L MC-LR	-	WHO (1998)

The indicative value for Vigilance Level is the detection of one colony or five filaments of a cyanobacterium in a 1 mL water sample, *i.e.* 200 cells per mL or 0.1 µg/L chlorophyll *a*. Taste and odours may become detectable but their absence does not indicate absence of toxic cyanobacteria. This level constitutes an early warning for potential bloom formation (Bartram *et al.* (1999)).

The Alert Level 1 derives both from the WHO guideline value for MC-LR in drinking water and from the highest recorded microcystin content for cyanobacterial cells. It is 2,000 cells per mL of cyanobacterial biomass or 0.2 mm^3/L biovolume or 1 μg/L chlorophyll *a*. This level requires consultation with health authorities for ongoing assessment of the status of the bloom and of the suitability of treated water for human supply (Bartram *et al.* (1999)).

The Alert Level 2, cyanobacterial biomass 100,000 cells per mL or 10 mm^3/L biovolume or 50 μg/L chlorophyll *a*, describes an established and toxic bloom with high biomass and possibly also localised scum (although scums may also form under Alert Level 1 conditions). Conditions in this level are indicative of increase in the risk of human health effects. The need for effective water treatment systems and on-going assessment of the performance of the system thus becomes of heightened importance (Bartram *et al.* (1999)).

In Algarve, vigilance is made by daily visual observations of the water bodies that may trigger specific physico-chemical and/or biological analysis and eventually the water treatment tuning.

1.4 OBJECTIVE AND STRUCTURE OF THE THESIS

From the previous sections, it is clear that cyanobacteria and cyanotoxins represent human heath risk potential especially for they appear in water bodies used for drinking water production and/or recreational activities. The study of technologies to remove intact cyanobacteria (*i.e.* without cell lysis) and to remove cyanotoxins from drinking water is a contribution that could lead to the minimisation (or even elimination) of their negative impact.

The aim of this thesis is the development of methodologies based on flotation and nanofiltration technologies to remove cyanobacteria and cyanotoxins from drinking water.

For it was impossible to study all cyanobacteria and associated toxins, this thesis addressed the most commonly occurring cyanotoxins: the hepatotoxic cyclic peptides microcystins, and particularly MC-LR, and the neurotoxic alkaloid anatoxin-a. Laboratory biomass culturing of *Microcystis aeruginosa* cells, cell aggregates (colonies) and *Planktothrix rubescens* filaments (the three cyanobacterial morphologies that produce mainly MC-LR and the two of most commoly occurring cyanobacteria, Hall *et al.* (2005)) are used.

The objective of flotation is to profit the flotation ability of cyanobacteria and remove them without cell lysis, *i.e.* without releasing the cyanotoxins into the water. Therefore, dissolved air flotation with pressurised recycle is investigated. With nanofiltration it is intended to remove the cyanotoxins present in water (by natural and/or induced release) down to a safe level for human supply. The complete methodology integrates the two technologies in order to develop a safe treatment sequence that guarantees a good drinking water quality removing both cyanobacteria and cyanotoxins. To reach this objective the two technologies are first studied separately and for each technology the separation mechanisms and the key operating conditions are investigated and optimised.

Therefore, the objectives of this thesis are:

1. To evaluate the DAF capacity for removing intact cyanobacteria and to improve its capacity by
   - ♣ studying the effect of the C/F pre-treatment and analysing the key operating conditions;
   - ♣ evaluating the impact of the water background organic matrixes on C/F/DAF performance;

- ♣ studying the treatment efficiency with the different cyanobacterial forms (laboratory biomass culturing of *M. aeruginosa* cells, cell aggregates (colonies) and *P. rubescens* filaments) and influent concentration (WHO Alerts Levels);

2. To evaluate the NF performance for removing the target cyanotoxins: microcystins and anatoxin-a by
   - ♣ evaluating the pH effect on the membrane charge and membrane performance;
   - ♣ analysing the impact of water background matrix, hardness ions and NOM, on NF performance;
3. To create an efficient safe treatment sequence for cyanobacteria and cyanotoxins effective removal by integrating the former two technologies.

Dissolved air flotation is studied both with and without coagulation and flocculation pre-treatment. When the pre-treatment is used the type and dose of coagulant, velocity gradient and retention time are investigated. For DAF, different pressurised recycle ratios are evaluated. Other parameters, like unicellular, colonial and filamentous cyanobacterial forms and type of organic matter are also studied in this work. The research here included is part of the European Project "Toxic - Barriers against cyanotoxins in drinking water" EVK1-CT-2002-00107 (01/09/2002 – 31/08/2005).

Nanofiltration includes the study of the membrane performance in the presence of different types of salts (mono and divalent salts) and types of natural organic matter (hydrophilic with low molecular weight, hydrophobic with high molecular weight and combination of the two). The cyanotoxins are then added to the water in order to evaluate the interactions between the cyanotoxins, the membrane and the water background inorganic (salts) and organic (NOM) matrix, and their consequences to membrane performance. This investigation is part of a National Project "CIANOTOX – Monitorização de Cianotoxinas e das Condições Desencadeadoras da sua Produção em Águas Superficiais com vista à Optimização das

Condições de Tratamento em ETAs" (01/07/2001 – 30/06/2004), financed by Águas do Algarve, SA.

Finally, the two processes are investigated in sequence, using the same operating conditions optimised for the individual processes. The objective is to always attain a final drinking water quality well below the WHO guideline values for cyanotoxins.

The structure of the thesis follows the research objectives outlined above. This thesis is divided in ten chapters. In the first chapter a brief presentation of the problem is made, the methods are briefing reviewed, and the objectives for the study are set. Chapters 2 to 4 are dedicated to flotation; chapters 5 to 8 are devoted to nanofiltration; and chapter 9 is dedicated to the integration of the two processes. Finally, in chapter 10 the work conclusions are summarised. Each chapter is devised as stand-alone units, and includes a review of the state of art, describes the materials and methods used and discusses the results and conclusions obtained in that part of the work. Chapter 2 compares C/F/DAF and C/F/S performances for removing, without causing damage, cultured cells of *Microcystis aeruginosa*. Two coagulants (alum and an aluminium pre-polymerised coagulant of high basicity (WAC)) and the most relevant operating conditions (velocity gradients for coagulation and flocculation, flocculation retention time, and DAF pressurised recycle ratio) are investigated for two scenarios of influent cell concentration. In chapter 3 the same treatment alternatives for water clarification are compared but now addressing the investigation of the removal of *M. aeruginosa* cells in the presence of different water background matrixes. Chapter 4 evaluates the performance of DAF to remove different cyanobacterial morphologies, namely single cells and colonies of *M. aeruginosa* and filaments of *Planktothrix rubescens*, from clear and natural waters. Chapter 5 investigates the role of membrane charge on NF performance. The zeta potential along the

surface and through the pores of a NF membrane is studied with several electrolyte solutions (including monovalent and divalent hardness ions, KCl, $CaCl_2$ and $MgSO_4$) to investigate the influence of salt type and pH on the charge of the membrane surface and in the membrane pores. Chapter 6 investigates the impact of the water background inorganic matrix on the natural organic matter removal by NF. Natural waters and model solution of hydrophobic high molecular weight NOM and hydrophilic low molecular weight NOM are used to study the chemical and physical aspects of NOM filtration and flux decline with NF membranes and their relation with feed water background inorganic matrix, in particular with water pH and calcium hardness. Chapter 7 studies the influence of feed chemical characteristics (different electrolyte solutions of mono and divalent salts, pH and background NOM) on the microcystins removal by NF. Chapter 8 addresses the removal of both anatoxin-a and microcystins from natural waters by NF, in the presence of calcium and NOM at acid and alkaline conditions. Competitive aspects or interactions between the cyanotoxins (microcystins/anatoxin-a) and or background NOM and inorganics are investigated. In chapter 9 all the results are integrated and the final treatment sequence C/F/DAF + NF is investigated. This chapter leads to the conclusions of the work made in chapter 10.

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CHAPTER 2

COMPARING DISSOLVED AIR FLOTATION AND CONVENTIONAL SEDIMENTATION TO REMOVE CYANOBACTERIAL CELLS OF *MICROCYSTIS AERUGINOSA*

ABSTRACT

Dissolved air flotation (DAF) is generally considered more effective than sedimentation (S) in the treatment of algal-rich water. However, the type and dose of coagulant, as well as the coagulation (C), flocculation (F) and DAF operating conditions are key parameters for removing intact cyanobacterial cells. This study compares C/F/DAF and C/F/S performances for removing, without causing damage, cultured cells of *Microcystis aeruginosa*, a surrogate for overall removal efficiency of cyanobacteria. Two coagulants (alum and an aluminium pre-polymerised coagulant of high basicity (WAC)) and the most relevant operating conditions (velocity gradients for coagulation and flocculation, flocculation retention time, and DAF pressurised recycle ratio) were investigated for two scenarios of influent cell concentration, expressed as chlorophyll *a* (10-35 μg/L and higher than 50 μg/L). Results showed that C/F/DAF is the best process to remove single cells of *M. aeruginosa*, yielding very high chlorophyll *a* removal (93-98%), with no toxin release to water (8-15%), using a low recycle ratio (8%), and lower coagulant doses (3 *vs*. 5 mg/L Al_2O_3 of WAC), slower coagulation

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(380 s^{-1} *vs*. 743 s^{-1}), stronger but shorter flocculation (8 min at 70 s^{-1} *vs*. 15 min at 24 s^{-1}) than C/F/S. WAC performed better than alum, for both processes. For either coagulants cell removal efficiency increased with the influent concentration, although higher doses were necessary to reach the same residuals.

2 COMPARING DISSOLVED AIR FLOTATION AND CONVENTIONAL SEDIMENTATION TO REMOVE CYANOBACTERIAL CELLS OF *MICROCYSTIS AERUGINOSA*

2.1 INTRODUCTION

Cyanobacteria (blue-green algae) have been identified worldwide, posing a significant risk to water supplies when they occur in reservoirs, lakes and rivers used as water sources, due to their ability to produce toxins – as well as taste and odour compounds – as secondary metabolites under particular conditions of growth. These cyanotoxins include hepatotoxic cyclic peptides, neurotoxic alkaloids and dermatotoxins, amongst which the hepatotoxic microcystins (and in particular the microcystin-LR variant) are the most commonly occurring cyanotoxins in water sources. As a result of the increasing concern with their health implications, the World Health Organisation has set a drinking water guideline value of 1.0 μg/L for microcystin-LR. Toxins can occur within the cells (intracellular or cell bound toxins) or be released from cells to water (extracellular or dissolved toxins) under certain conditions of growth and/or external (environmental) stress factors responsible for cell lysis.

Therefore, the removal of cyanobacterial cells without causing cell damage has been and continues to be a problem within the water treatment industry. Although such procedure will not avoid the need for further treatments addressing the removal of dissolved toxins, taste and odour compounds (*e.g.* oxidation, activated carbon, membrane technology (Ribau Teixeira and Rosa (2005), chapter 7) it will definitely improve the overall process effectiveness and economics.

Primary clarification involves either sedimentation or flotation of flocculated water. Conventional drinking water treatment trains include coagulation (C), flocculation (F) and

sedimentation (S). However, algal-rich waters – especially important during a cyanobacterial bloom occurrence – pose problems to sedimentation, due to the algae tendency to float, its small size, low cell density and negative surface charge. An alternative technique for the clarification of algal-rich waters is dissolved air flotation (DAF) (Zabel (1985), Malley and Edzwald (1991), Edzwald *et al.* (1992), Dupre *et al.* (1998), AWWA (2000)).

There seems to be some disagreement in the literature regarding the efficiency of conventional treatment (C/F/S, filtration, chlorination) for cyanobacterial cells removal. Some papers report the occurrence of cell lysis, release of intracellular toxins and taste and odour compounds (Hoffmann (1976), Keijola *et al.* (1988), Himberg *et al.* (1989), Lam *et al.* (1995), Hrudey *et al.* (1999)), while others refer no release of such compounds to the water (Chow *et al.* (1998), Drikas *et al.* (2001)). Some studies report removal efficiencies of *Microcystis* cells between 58 and 90% by the conventional treatment, and showed that such procedure was not effective for extracellular toxin removal (Falconer *et al.* (1989), Velzeboer *et al.* (1995), Chow *et al.* (1999), Hrudey *et al.* (1999)). According to Zabel (1985) and Hrudey *et al.* (1999), floc blanket clarification had shown 76.5% removal of *Microcystis* cells whilst DAF removed 98% in the presence of other algae. The same high DAF removal efficiencies for *Microcystis aeruginosa* and *Anabaena circinalis* (Yan and Jameson (2004)), and for *Chlorella* and *Cyclotella* (Edzwald and Wingler (1990)) have been reported.

It is consensus that algal cells are negatively charged and must be completely destabilised by charge neutralisation to allow maximum treatment efficiency (Malley and Edzwald (1991), Edzwald *et al.* (1992), Mouchet and Bonnélye (1998), Yan and Jameson (2004)). Therefore, there have been some studies regarding the optimisation of the operating conditions of the coagulation/flocculation prior to DAF, namely the flocculation velocity gradient (G) and time

(Malley and Edzwald (1991), Fukushi *et al.* (1995), Odegaard (1995), Valade *et al.* (1996), Vlaski *et al.* (1996), Vlaski *et al.* (1997), Han *et al.* (2001)).

Coagulants such as aluminium sulphate, ferric sulphate, ferric chloride and polymerised coagulants as polyaluminium chloride (PACI) have been successfully used for treating algal-rich waters. Pre-polymerised coagulants have some advantages over metal salt coagulants: better overall treatment efficiency, better floc separation, wider working pH range, lower sensitivity to low temperatures and lower residual metal-ion concentration (Jiang *et al.* (1993), Schofield (2001)).

As far as the DAF operating conditions are concerned, the effectiveness of the pressurised recycle system has been referred as crucial to the success and economy of the DAF process (Edzwald *et al.* (1992), Malley (1995), Johnson *et al.* (1995), Kempeneers *et al.* (2001), Crossley *et al.* (2001), Schofield (2001)).

From the above mentioned literature survey, DAF is generally more effective than sedimentation for treating algal-rich water. However, the type and dose of coagulant, as well as the C/F/DAF operating conditions are key parameters for the overall removal of intact cyanobacterial cells. The objective of this study is to compare C/F/DAF and C/F/S performances for removing cultured cells of *Microcystis aeruginosa*, without cell damaging, *i.e.* without toxin release to water. *M. aeruginosa* is one of the most commonly occurring cyanobacteria in water sources and is a potential producer of microcystins variants. This cyanobacterium grows in laboratory as single cells, being recognised as an ideal surrogate for process removal efficiency assessment of particles of the problematic size range (3-10 μm) (Vlaski *et al.* (1996), Vlaski *et al.* (1997)). The importance of C/F prior to DAF and the

effects of coagulant type (monomeric and pre-polymerised aluminium coagulants), operating conditions (*e.g.* velocity gradients, retention time and pressurised recycle) and influent cell concentration are investigated.

2.2 MATERIALS AND METHODS

2.2.1 CYANOBACTERIAL CELLS

M. aeruginosa supplied by Pasteur Culture Collection (PCC 7820) was grown in laboratory (10 L medium) according to enclosed instructions, *i.e.* BG11 medium at 23-24 ºC under a light regimen of 12 h light, 12 h dark (~5 μM photon m^{-2} s^{-1}). Cultures were harvest at the late exponential phase of growth.

M. aeruginosa is found in nature mostly in colonial form, which is very difficult to produce in laboratory cultures. In fact, these studies were performed with single cells which are a more significant nuisance from the water treatment practice point of view, since the single cell form (3 – 10 μm, spherical cells) regularly penetrates treatment processes and is encountered in the treatment plant effluent (Vlaski *et al.* (1996)). Actually, *M. aeruginosa* single cells are recognised as surrogate for addressing overall particle removal, even for pathogenic microorganisms of similar size characteristics, like *Cryptosporidium parvum* and *Giardia lambia*. The naturally occurring colonial form should be easier to remove.

Treatment experiments were performed with tap water spiked with *M. aeruginosa* cells until a specific concentration of chlorophyll *a* (chl_a) was achieved. The chl_a concentration of the culture was determined, a proper volume was added to tap water and the resulting suspension was analysed to verify the chl_a concentration. At this point, if a proper concentration was

achieved, the experiments started immediately. As already reported by Vlaski *et al.* (1996), spiking similar concentrations of cells from the culture suspension was a difficult task, so it was difficult to continuously provide algae with constant and comparable culture quality.

Two different scenarios of cyanobacterial cells concentration were tested to examine the effect of influent cell concentration on DAF performance, namely *Level 1* scenario corresponding to 10-35 μg/L chl_a in raw water, and *Level 2* with influent values above 50 μg/L chl_a (50-75 μg/L) (initial extracellular microcystin-LR concentrations varied between 1.3 and 42 μg/L). These scenarios correspond to the Alert Levels established by Bartram *et al.* (1999) with the objective of providing the water treatment plant operators and managers with a graduated response to the onset and progress of a cyanobacterial bloom. Alert Level 1 conditions (cyanobacterial biomass of 2,000 cells per mL or 1 μg/L chl_a) require decisions to be made on suitability of treated drinking water, based on the efficiency of water treatment and the concentration of toxin detected. The Alert Level 2 (cyanobacterial biomass of 100,000 cells per mL or 50 μg/L chl_a) describes an established toxic bloom with high biomass. Conditions in this level indicate an increase in the risk of human health effects, and the need for effective water treatment systems (Bartram *et al.* (1999)).

2.2.2 COAGULANTS

Stock solutions of alum ($Al_2(SO_4)_3.18H_2O$, Riedel-deHaen, stock solution with 1000 mg/L Al_2O_3) and WAC (aluminium polyhydroxichlorosulphate with a relative basicity of 60-70%, Elf Atochem, stock solution with 850 mg/L Al_2O_3) were prepared in tap water. In C/F/S experiments the coagulant dose varied between 2 – 20 mg/L Al_2O_3, while in the C/F/DAF experiments it varied between 1 – 7 mg/L Al_2O_3. These coagulant doses correspond to the doses normally used in this kind of experiments and in full-scale operation.

2.2.3 ANALYTICAL METHODS

Samples were analysed for chl_a, turbidity, total and dissolved organic carbon (TOC and DOC), pH, conductivity and extracellular microcystin (MC-LR), all using standard methods of analysis, whenever available.

For chl_a analysis, samples were filtered through GF/F filter paper and the chlorophylls were extracted using 10 mL acetone (90%). The optical densities of the extracts were measured at 665 and 750 nm using a Spectronic Unicam UV300 UV/VIS spectrophotometer and chl_a concentration was computed from Lorenzen (1967) equations.

Turbidity was measured in a HACH 2100N turbidity meter of high resolution (0.001 NTU).

TOC and DOC (after 0.45 μm sample filtration with acrodisk filters, Aquatron. CA, 30 mm) were measured as non-purgeable organic carbon using a Shimadzu TOC 5000A analyser (50 ppb – 4000 ppm).

pH values were measured at 25 ºC using a Whatman WTW pH340 meter, and conductivity at 20 ºC in a Crison GLP32 conductimeter.

Extracellular MC-LR (MC-LR) was first isolated from the intracellular and cell-bound fraction by sample filtration through a Whatman GF/F glass microfiber filter. Microcystins were then extracted from the filtered water samples using an isolute C18 solid phase extraction column, 1 g in a 6 mL reservoir, following the standard operation procedure developed by Meriluoto and Spoof (2005a). The cartridges were first conditioned with 10 mL methanol (75%), followed by 10 mL milli-Q water at a flowrate not exceeding 10 mL/min, without letting it dry during conditioning. The samples were then applied to the cartridge and

the microcystin was eluted with 5 mL methanol (90%) containing 0.1 % trifluoroacetic acid. The methanolic eluate was evaporated at 50-54 ºC in a rotavapor, the residue was resuspended in 500 µL methanol (75%), centrifuged for 10 min at 10,000 x *g*, and 150 µL of supernadant were transferred to HPLC vials for analysis.

Microcystins were analysed by HPLC-PDA using a Dionex Summit system, which includes a high pressure gradient pump Dionex Summit, an autosampler Dionex ASI-100, a column oven Dionex STH-585 and a photo diode-array detector Dionex PDA-100. A C18 column was used (Merck Purospher STAR RP-18 endcapped, 3 µm particles, LiChroCART 55x4 mm). The mobile phase used a gradient of milli-Q water and acetonitrile, both with 0.05% (v/v) of trifluoracetic acid. Chromatograms were analysed between 180 – 900 nm, with a main detection at 238 nm for the typical microcystins spectra (Meriluoto and Spoof (2005b)

2.2.4 COAGULATION/ FLOCCULATION/ SEDIMENTATION EXPERIMENTS

C/F/S experiments were performed in a Jar test unit with four paddles, using 500 mL per sample. The standard experimental procedure included: a) coagulation at a velocity gradient (G_C) of 743 s^{-1} for 2 min; b) flocculation at G_F 24 s^{-1} for 15 min; and c) sedimentation for 20 min. The experiments were performed at room temperature (20±2 ºC). These are typical operating conditions for C/F/S in drinking water treatment (Vlaski *et al.* (1996), AWWA (2000)).

2.2.5 DAF EXPERIMENTS

In the DAF process, bubbles are generated by the release of pressurised recycle water, which has first been air saturated at higher pressure than atmospheric pressure. The air bubbles are

released into the flotation tank and attach themselves to the particles. The bubble-particles rise to the surface of the flotation tank and are then removed as floated sludge. When processing flocculent sludges or algal cells, a pressurised recycle should be used, to avoid subjecting the cells to shearing stresses through the pumps and the pressurised system, which might result in cell lysis (Eckenfelder (2000)). DAF experiments with ($R/Q \neq 0$) and without pressurised recycle ($R/Q = 0$) were performed at room temperature (20±2 ºC) in a laboratory-made flotation cell adapted from de Pinho *et al.* (2000). This apparatus (Figure 2.1) has a 2 L pressure chamber and a 3 L calibrated cylinder, where a paddle can be installed for the C/F prior to DAF (described in section 2.2.6). DAF experimental procedure was as described by Eckenfelder (2000).

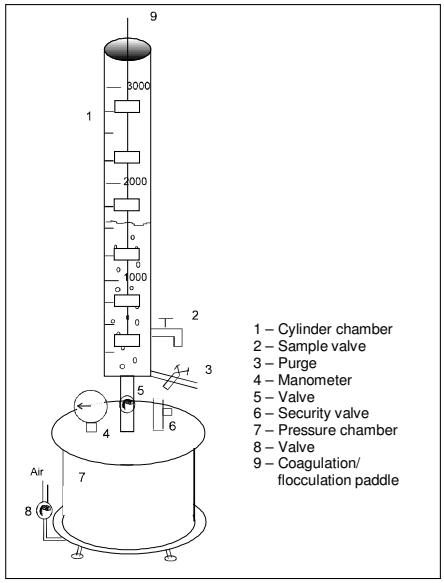

Figure 2.1 Coagulation/flocculation/DAF *apparatus* (adapted from de Pinho *et al.*, 2000).

In the pressurised recycle experiments ($R/Q \neq 0$), the calibrated cylinder was partially filled with tap water spiked with cyanobacterial cells and the pressure chamber with tap water; in experiments without pressurised recycle ($R/Q = 0$), the pressure chamber was filled with the same water used in the calibrated cylinder (tap water spiked with PCC 7820 cells). The released volume was computed from the desired recycle ratio, and the velocity of release

promoted adequate mixing. Applied recycle ratio was 0.5, 0.08 and 0 (without recycle). After a retention period of 8 minutes, the clarified effluent was drawn off through the sampling hose and the above referred parameters were analysed.

In all experiments the relative pressure was 5 bar. In the pressurised recycle experiments a correction factor for dilution (1 + R/Q) was used in computing the removal of all parameters in the clarified water, according to the equation:

$$\text{Removal}\ (\%) = 1 - \frac{C_f}{C_i}\left(1 + R/Q\right) \text{x}\,100$$

where C_i and C_f are the initial concentrations and R/Q the recycle ratio. Experiments were made in duplicate or triplicate.

2.2.6 COAGULATION/ FLOCCULATION/ DAF EXPERIMENTS

The experimental procedure for the C/F/DAF experiments followed Eckenfelder (2000) standard experimental procedure, but before the release of the recycle water volume, the paddle was installed in the cylinder, and a rapid (coagulation) and a slow (flocculation) mixing were performed. The experiments proceeded then as previously described. Two sets of operating conditions were studied, one with the typical conditions of C/F prior to sedimentation (G_C 743 s^{-1} for 2 min, G_F 24 s^{-1} for 15 min, see section 2.2.4) and another using typical conditions of C/F prior to DAF, *i.e.* with slower coagulation (G_C 380 s^{-1} for 2 min) and stronger but shorter flocculation (G_F 70.0 s^{-1} for 8 min). Both sets used the same DAF retention time of 8 min and two pressurised recycle ratios were tested, 0.5 and 0.08. C/F/DAF operating conditions studied are typical values for water treatment (Edzwald *et al.* (1992), Vlaski *et al.* (1996), Hedberg *et al.* (1998), Crossley *et al.* (2001)). Experiments were made in duplicate or triplicate.

2.3 RESULTS AND DISCUSSION

2.3.1 DAF EXPERIMENTS

Figure 2.2 and Table 2.1 show the results of DAF experiments with and without pressurised recycle (R/Q ≠ 0 and R/Q = 0, respectively), for the two scenarios studied of influent cell concentration.

Table 2.1 DAF removal efficiencies of turbidity, chl_a and extracellular MC-LR from tap water spiked with *M. aeruginosa* (PCC 7820) cells and comparison with natural flotation performance (Level 1: influent concentration 10 – 35 μg/L; Level 2: influent concentration > 50 μg/L).

	Removal (%)					
	Level 1		Level 2			Level 2
	R/Q = 0	R/Q = 0.5	R/Q = 0	R/Q = 0.08	R/Q = 0.5	Natural flotation
Turbidity	14.1	0.4	43.5	11.6	9.5	4.1
Chl_a	15.7	24.7	18.0	23.5	19.2	17.6
MC-LR	2.2	6.2	0.3	9.5	18.0	16.9

NOTE: Removal efficiencies relative to R/Q ≠ 0 include the correction factor for dilution.

As expected, DAF without coagulant addition has no advantages over the natural flotation – it presents low cell removal (expressed as turbidity and chl_a), and almost no removal of dissolved organics (DOC and extracellular MC-LR) (Figure 2.2 and Table 2.1). In addition, pressurised recycle does not improve DAF performance for particle removal, which indicates that the observed low removal efficiencies cannot be related with cell floc destruction but rather with its inexistence. In fact, as previously mentioned, the *M. aeruginosa* cultures used in these experiments grow as single cells, and not as the cell aggregates observed in nature. Nevertheless, removal of extracellular MC-LR seems to indicate the disadvantages of using DAF without pressurised recycle, probably due to cell damaging effects. Although low, removal increases with cell influent concentration, expressed by the two chl_a levels. Edzwald *et al.* (1992) found the same poor DAF performance in similar studies with no flocculation (no coagulant addition), whereas Zabel (1985) referred algae removals of 10 to 20% when a flotation plant was operated without coagulant addition.

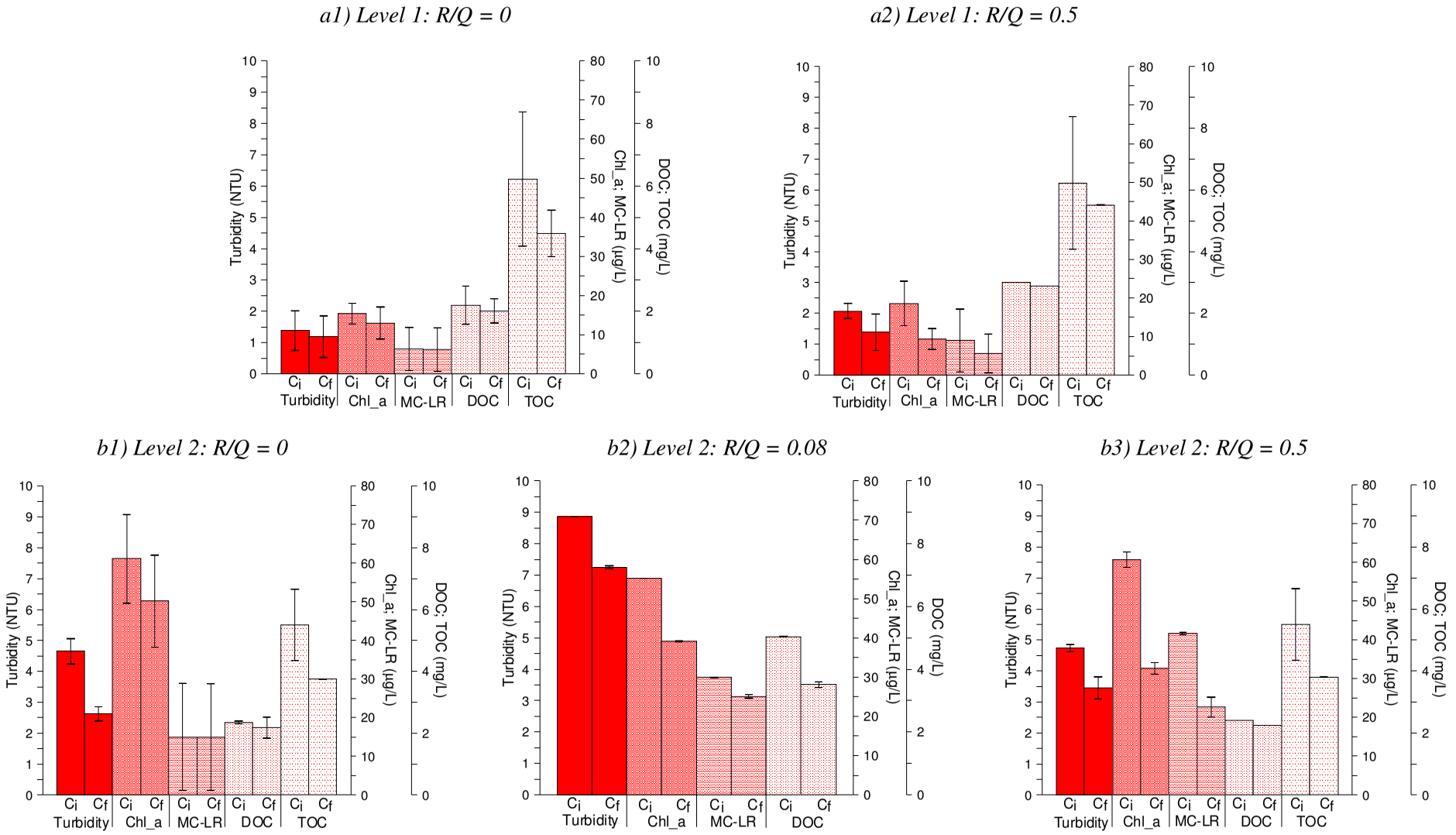

Figure 2.2 DAF results of *M. aeruginosa* (PCC 7820) cultured cells, with and without pressurised recycle (C_i , C_f are the initial and final concentrations respectively, Level 1: C_i = 10-35 µg/L chl_a; Level 2: C_i >50 µg/L chl_a).

These results can be attributed to the lack of destabilised particles – low to non charged particles and hydrophobic particles – necessary for the effectiveness of flotation process (Malley and Edzwald (1991)) and the slightly negative surface charge of air bubbles (Edzwald (1993)). Algae stability is related to electrostatic repulsive interactions (electronegative for a pH range of 2.5 – 11.5), steric effects due to water bound to cell surface, and to adsorbed macromolecules or extracellular organic matter (Edzwald (1993)). According to Malley and Edzwald (1991), particle destabilisation is even more important for efficient DAF than large floc size. In fact, particle destabilisation is a pre-requisite for floc formation. Yan and Jameson (2004) observed that the optimum algae removal efficiency occurred at a point where the surface charge of the cyanobacterial cells (*M. aeruginosa* and *Anabaena circinalis*) was almost completely neutralised, which indicates that charge neutralisation was a very important factor for the attachment of polymer chains onto algal cells. In addition, *Microcystis sp* cells (except certain filamentous forms) are fairly small in size (usually with 3 to 7 μm diameter), which implies a low particle-air bubble collision efficiency (Yan and Jameson (2004)). To improve cell-bubble contact, the algae size has to be increased to larger than 10 μm by coagulation and/or flocculation. Therefore, two different types of coagulants (monomeric and pre-polimerised aluminium salts) were tested to achieve particle destabilisation and size increase.

2.3.2 COAGULATION/ FLOCCULATION/ SEDIMENTATION EXPERIMENTS

Data obtained in C/F/S experiments with the two coagulants and scenarios of influent *M. aeruginosa* cells studied show that WAC is more efficient than alum for cell removal (expressed by turbidity and chl_a, Figures 2.3a and 2.3b). Although the optimal dose does not differ clearly (*ca*.7 mg Al_2O_3/L for alum and 5 mg Al_2O_3/L for WAC) alum cannot reach the low turbidity residuals attained with WAC, and when the same Al_2O_3 dose is used,

significantly lower residuals are obtained with WAC. For example, using 5 mg/L Al_2O_3, the residual turbidity obtained with WAC is *ca.* 1/3 the value obtained with alum, for both influent cell concentrations (the turbidity is 0.65 NTU with alum and 0.22 NTU with WAC in Level 1, and 1.5 NTU with alum and 0.52 NTU with WAC in Level 2).

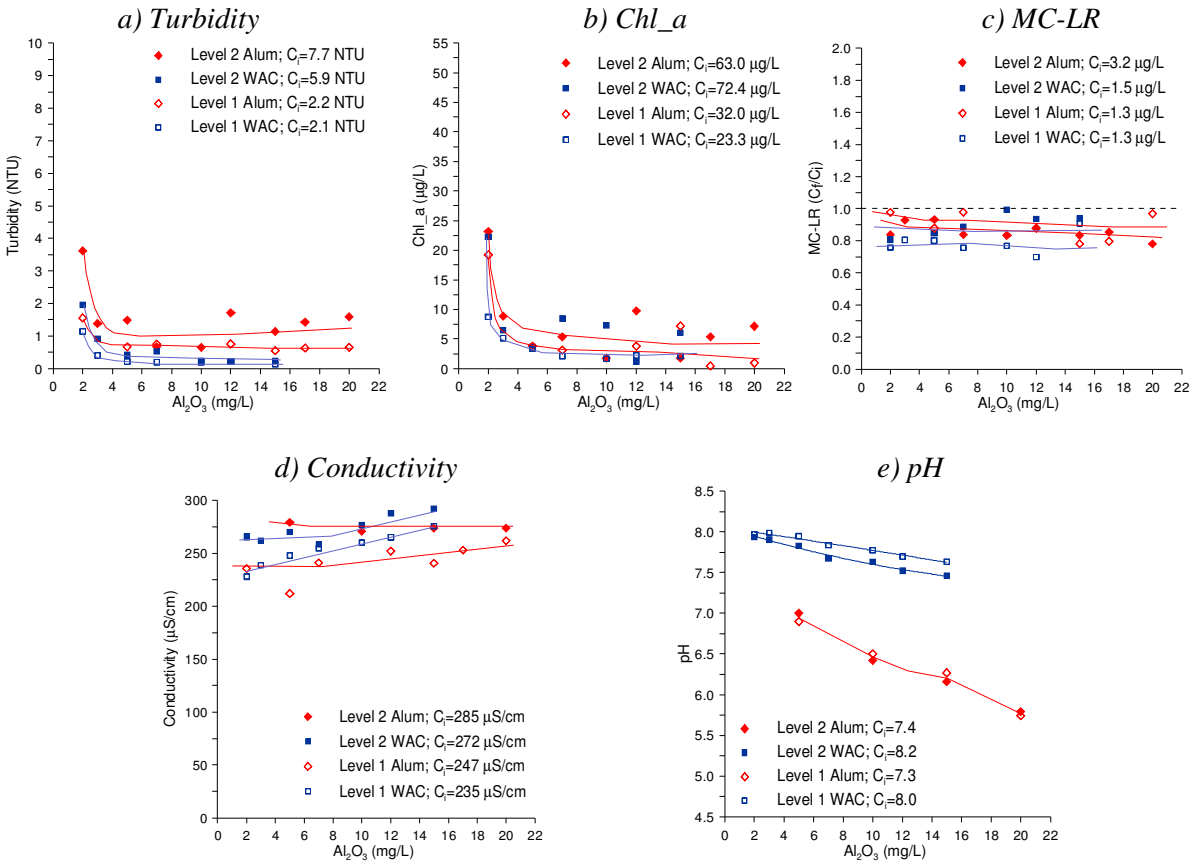

Figure 2.3 C/F/S results of *M. aeruginosa* (PCC 7820) cultured cells, with alum and WAC (Level 1: C_i = 10-35 µg/L chl_a; Level 2: C_i >50 µg/L chl_a; coagulation at 20±2 ºC, 743 s^{-1} for 2 min; flocculation at 24 s^{-1} for 15 min; sedimentation for 20 min).

It is also evident that, when using alum, the influent cell concentration affects both the removal efficiency and the residual concentration. As reported in literature for other parameters (*e.g.* turbidity, NOM) (Campinas *et al.* (2000)), C/F/S removal efficiency of cyanobacterial biomass increases with its influent concentration, although higher coagulant doses are required to achieve the same residuals (Figures 2.3a and 2.3b). The same behaviour was already obtained by Widrig *et al.* (1996) with model waters containing algal-derived

organic matter like *Microcystis*. Unlike alum, WAC can overcome the effect of the influent concentration, yielding similar turbidity residuals for both feed concentrations. Moreover, alum overdosing has a negative effect on cell removal for coagulant doses above 10 mg/L (particularly in terms of turbidity). Neither toxin release to water was observed in the tested range of coagulant dose (2 – 20 mg/L Al_2O_3), and nor the benefits associated to the enhanced coagulation on natural organic matter removal (*i.e.* MC-LR concentration does not decrease with the coagulant dose, Figure 2.3c). If enhanced coagulation occurred, the toxin removal was expected to be higher for alum than WAC, since alum further decreased the pH (and no significant differences in conductivity were obtained, Figures 2.3d and 2.3e). Its ability to minimise the water pH reduction, and to cope with raw water fluctuations are definitive advantages of the pre-polymerised coagulants (like WAC) over the conventional monomeric coagulants (like alum).

Compared to alum, WAC produces bigger flocs with better settling characteristics (Figure 2.4), which is related to the dominant coagulation mechanisms. WAC is a pre-polymerised aluminium coagulant with a cation highly charged and partially neutralised, so coagulation is mostly by charge neutralisation (Koether *et al.* (1997)). Pre-polymerisation of the metal salt coagulant is principally to enhance the charge interaction mechanism of colloid destabilisation as a consequence of the slowing down the hydrolysis of the metal salt (Jiang *et al.* (1993)), which explains the lower water pH decrease (Figure 2.3e). In turn, since influent turbidity is low, the main coagulation mechanism for alum must be sweep coagulation, as there is charge neutralisation but not enough particles to originate big flocs. This type of mechanism occurs when the aluminium salt concentrations exceeds the correspondent saturation value, and a significant decrease in the water pH is observed (Figure 2.3e) (Koether *et al.* (1997)). The same mechanisms were proposed by Jiang *et al.* (1993) to explain the removal of particles

and/or algal cells by C/F/S using ferric sulphate, aluminium sulphate and a pre-polymerised coagulant.

a) 7820 WAC flocs, 5 mg/L Al_2O_3 *b)* 7820 Alum floc, 5 mg/L Al_2O_3

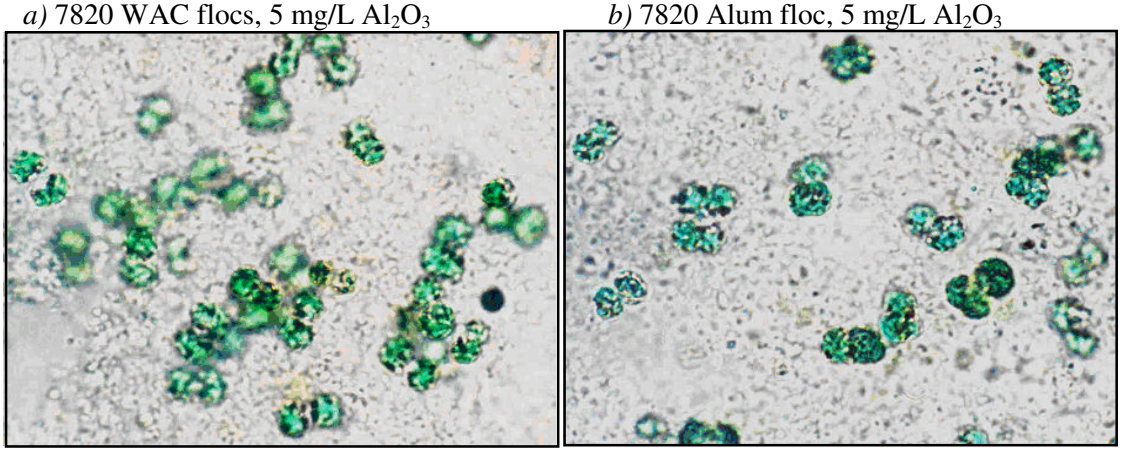

Figure 2.4 Flocs of *M. aeruginosa* (PCC 7820) cultured cells formed by WAC **(a)** and alum **(b)** addition (C/F/S using 5 mg/L Al_2O_3).

Data in Figure 2.3c show that MC-LR is not removed by C/F/S but most important, there is no release of MC-LR to water in the studied range of coagulant dose, as found by other authors (Keijola *et al.* (1988), Velzeboer *et al.* (1995), Chow *et al.* (1999), Hrudey *et al.* (1999)). For instance, Chow *et al.* (1999) reported that the aluminium sulphate dose, at concentrations used in water treatment practise (11 mg/L Al_2O_3), did not cause lysis of the *M. aeruginosa* cultured cells nor increased the amount of dissolved MC-LR in the water. They also concluded that the mechanical stirring action (200 rpm in coagulation and 20 rpm in flocculation), which the cyanobacterial cells may encounter in conventional water treatment, did not damage the integrity of the *M. aeruginosa* cultured cells used in the study and no release of MC-LR occurred. In turn, Lam *et al.* (1995) concluded that treatments using high alum doses (34 − 52 mg/L Al_2O_3) appeared to control the cyanobacterial bloom mainly by cell-coagulation and sedimentation, but with a little increase in extracellular MC-LR concentration in water.

2.3.3 COAGULATION/ FLOCCULATION/ DAF EXPERIMENTS

2.3.3.1 EFFECTS OF COAGULANTS TYPE

Figure 2.5 shows the effects of the coagulant type (alum and WAC) and dose on C/F/DAF performance.

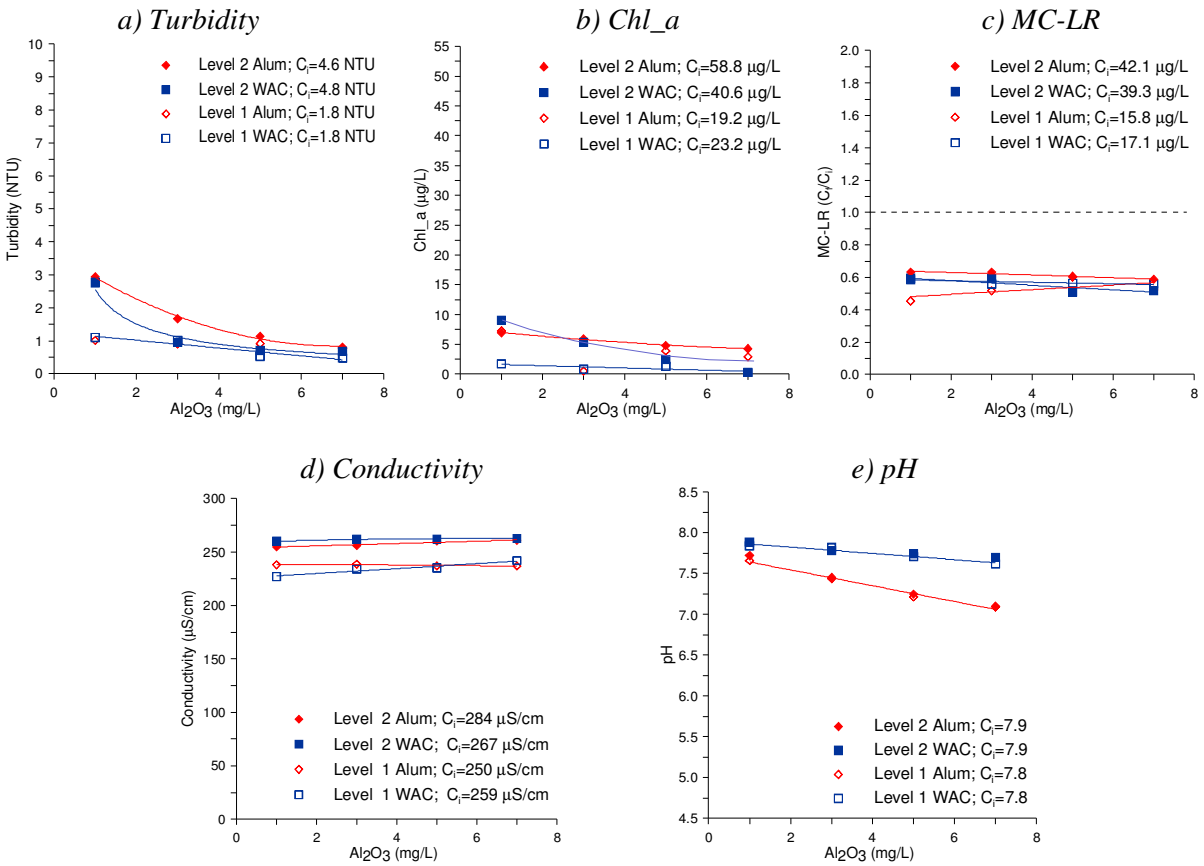

Figure 2.5 C/F/DAF results of *M. aeruginosa* (PCC 7820) cultured cells, with alum and WAC (Level 1: C_i = 10-35 µg/L chl_a; Level 2: C_i >50 µg/L chl_a; coagulation at 20±2 ºC, 743 s^{-1} for 2 min; flocculation at 24 s^{-1} for 15 min; DAF with R/Q = 0.5, 8 min).

As already found in C/F/S experiments, WAC is more efficient than alum for removing turbidity and chl_a, and has a superior ability to cope with raw water quality changes, yielding similar turbidity and chl_a residuals for both feed concentrations (Figures 2.5a and 2.5b). For 5 mg/L Al_2O_3, chl_a residual obtained with WAC is half the value obtained with alum (2.4 *vs.* 4.8 µg/L chl_a) for Level 2 of influent cell concentration and 1/3 for Level 1 (1.3 *vs.* 3.9 µg/L chl_a, Figure 2.5b). Chl_a removal efficiencies varied between 80 – 99% for WAC and 69 – 89% for alum in the coagulant range of 3 – 7 mg/L Al_2O_3. Similar results were found by

Chung *et al.* (2000) in a pilot and full-scale plant using polyaluminium chloride as coagulant. Kempeneers *et al.* (2001) reported an overall chl_a removal of 74.5% during ten years of operation of a C/F/DAF full-scale plant, using alum/alum + activated silica/powdered activated carbon as coagulant and an effluent quality below 5 μg/L chl_a, when treating water with low chl_a concentration and not exceeding 10 μg/L for higher influent chl_a concentrations. Zabel (1985) and Vlaski *et al.* (1996) obtained similar results with *Microcystis*. Residual turbidity below 1 NTU (except with alum in Level 2: 1.7 NTU) is achieved for coagulant doses higher than 3 mg/L Al_2O_3 (Figure 2.5a). Similar results were obtained by Johnson *et al.* (1995), Valade *et al.* (1996) and Chung *et al.* (2000).

As previously reported by Kempeneers *et al.* (2001), C/F/DAF removal efficiency of cyanobacterial mass increases with its influent concentration, although higher coagulant doses are required to achieve the same residuals (Figures 2.5a and 2.5b). In C/F/DAF experiments, coagulant overdosing effects are not observed due to the coagulant range studied (not exceeding 7 mg/L Al_2O_3).

Most important, there is no toxin increase in water after C/F/DAF using both coagulants. Actually, removal efficiencies of extracellular MC-LR vary between 12 – 24% for WAC and 5 – 22% for alum, higher removal being observed for higher influent cell concentration and with increasing coagulant dose (Figure 2.5c).

Sedimentation and DAF efficiencies for *M. aeruginosa* cells removal can be directly compared (Figure 2.3 *vs*. Figure 2.5) once the same C/F operating conditions (G_C, G_F and retention time) were used in both C/F/S and C/F/DAF previous experiments. Sedimentation and DAF are equivalent processes as far as turbidity removal and residuals are concerned, but

DAF presents a superior efficiency for chl_a removal (*e.g.* in Level 2 with WAC, chl_a removal by sedimentation is *ca.* 69 – 94% and 77 – 99% using DAF) and residuals, particularly evident for low coagulant doses. Both processes seem not to promote cell damage under the operating conditions tested as the dissolved MC-LR does not increase in any studied case. WAC always achieves better removals than alum for both processes and levels of influent cell concentration.

These results lead to the investigation of WAC C/F/DAF performance under more cost effective operating conditions, since the literature indicates as good or even better DAF results under lower coagulation velocity gradients, shorter flocculation times and lower pressurised recycle ratios (Edzwald *et al.* (1992), Valade *et al.* (1996), Vlaski *et al.* (1997), Hedberg *et al.* (1998), Scriven *et al.* (1999)).

2.3.3.2 Effects of Velocity Gradient, Retention Time and Pressurised Recycle

Results of G variation on C/F/DAF removal efficiencies for particles (turbidity and chl_a) and dissolved organics (MC-LR and DOC) are presented in Figure 2.6. These results show no effect on MC-LR release to water, but better performances with G_C 380 s^{-1} and G_F 70 s^{-1}, *i.e.* lower residuals (turbidity is *ca.* 0.7 NTU, chl_a 2.2 µg/L and DOC 1.9 mg/L) and lower optimal coagulant dose (2-3 mg/L Al_2O_3).

As expected from literature data, better results are obtained with weaker coagulation mixing, and stronger but shorter flocculation. Actually, strong flocculation mixing and short flocculation time result in strong and small size floc particles. But large flocs are not necessary for flotation since bubbles and particles need to be attached, and particle-bubble agglomerate density should be reduced to less than water (Edzwald (1995), Odegaard (1995)).

Literature agrees on the flocculation time reduction from 20 – 30 minutes in the conventional sequence (C/F/S) to 5 – 10 minutes in the DAF sequence (C/F/DAF) (Malley and Edzwald (1991), Fukushi *et al.* (1995), Valade *et al.* (1996), Vlaski *et al.* (1996), Han *et al.* (2001)). Edzwald *et al.* (1992) results showed that good flotation performance was achieved with either 8 or 16 min for flocculation, so shorter times 5 – 8 min should be adequate.

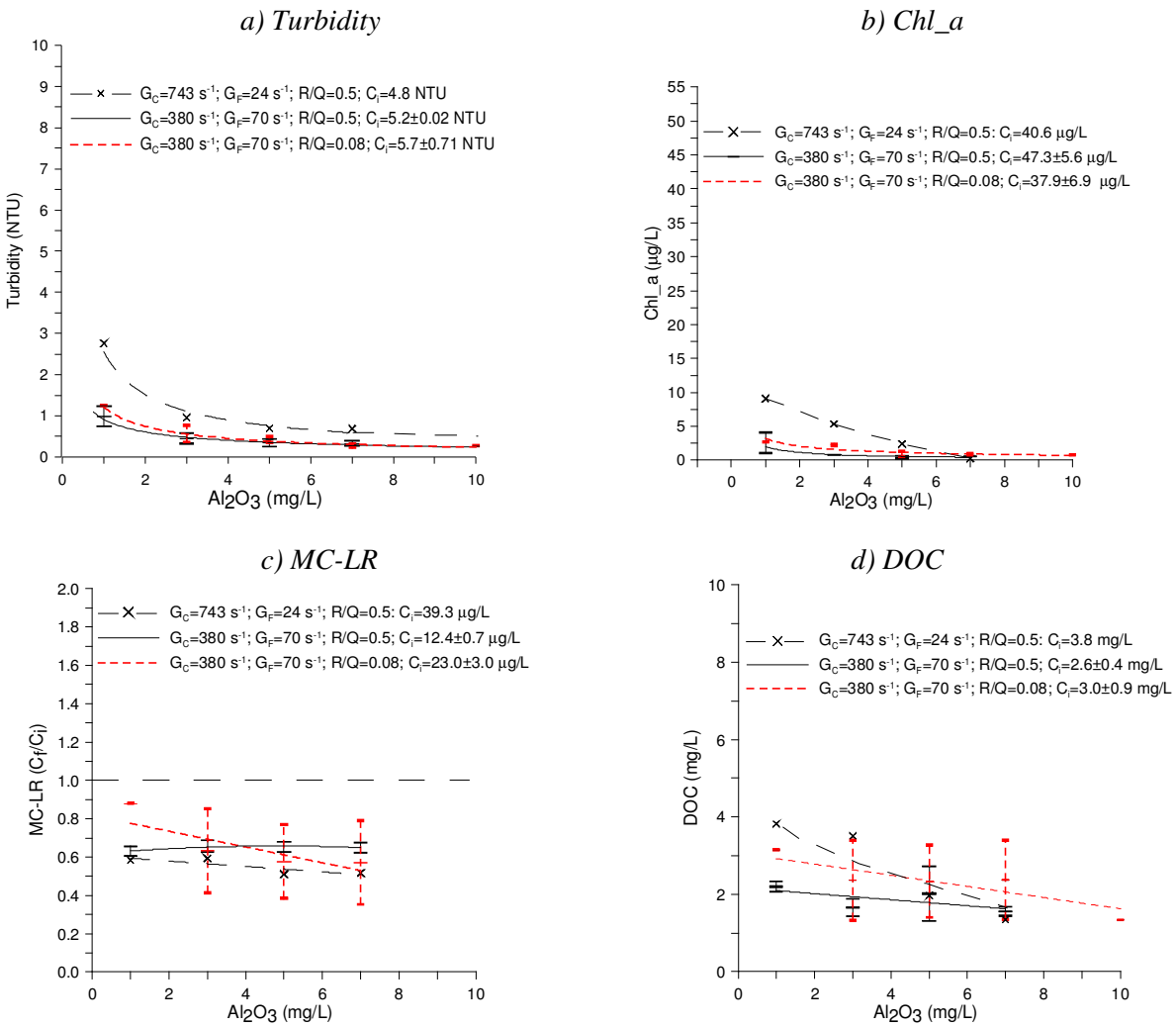

Figure 2.6 Effects of velocity gradients (G_C and G_F) and pressurised recycle ratio (R/Q) on C/F/DAF performance (WAC; coagulation at 20±2 ºC, 743 s^{-1} or 380 s^{-1} for 2 min; flocculation at 24 s^{-1} for 15 min or at 70.0 s^{-1} for 8 min; R/Q at 0.5 or 0.08; DAF for 8 min).

In terms of flocculation velocity gradient (G_F), values between 30 and 80 s^{-1} have been proposed. Odegaard (1995) demonstrated that the optimum G_F value for F/S was 20 – 40 s^{-1}

compared to 60 – 80 s^{-1} for F/DAF systems. Valade *et al.* (1996) concluded that the flocculation operating conditions (retention time of 5 and 20 min and G_F of 30 and 70 s^{-1}) had only slight effects on DAF performance. In addition, they also concluded that DAF plants could be designed with flocculation times as low as 5 min, and high G_F resulted in lower turbidity and particle counts. However, some authors disagree on the efficiency of these small size flocs in the C/F/DAF process. Fukushi et al. (1995) agreed that long flocculation is not needed, but they considered that larger flocs have a much higher collision efficiency, hence larger flocs should be prepared for effective DAF. Furthermore, Vlaski *et al.* (1997) concluded that the increase of the larger floc size fraction achieved by low flocculation G values resulted in relative floc density decrease, and in higher DAF efficiencies. Zabel (1985), Hedberg *et al.* (1998) and Scriven *et al.* (1999) presented G_F values between 70 – 80 s^{-1} for high algae and turbidity removal efficiencies.

The highest pressurised recycle ratio studied (0.5) yields similar to lower residuals, but equal optimal WAC dose (3 mg/L Al_2O_3) (Figure 2.6). To understand whether these results are related to higher removal efficiencies at high R/Q values or just due to dilution effects Figure 2.7 shows the removal efficiencies corrected for dilution.

Data corrected for dilution show that there is no performance improvement with increasing recycle, so the lowest value tested (0.08) should be adequate, as found earlier by Edzwald *et al.* (1992). Edzwald *et al.* (1992) studied different recycle ratios from 2 to 10% and verified that 8% is a good value in terms of clay, fulvic acids and algae removals. They concluded on the existence of a minimum recycle needed for effective DAF treatment that increased with influent raw (or flocculated) water turbidity. According to Vlaski *et al.* (1996, 1997), with recycle ratios as low as 8%, the volume of bubbles far exceeds the volume of particles, and

higher recycle ratio conditions result in increase of the mean bubble size to theoretically less efficient sizes. Kempeneers *et al.* (2001) used a 6% recycle ratio, and Schofield (2001) recommended values between 6 and 10%. Regarding the air saturation pressures, values of 400 – 600 kPa have been recommended (Zabel (1985), Malley and Edzwald (1991), Edzwald (1995), Schofield (2001)).

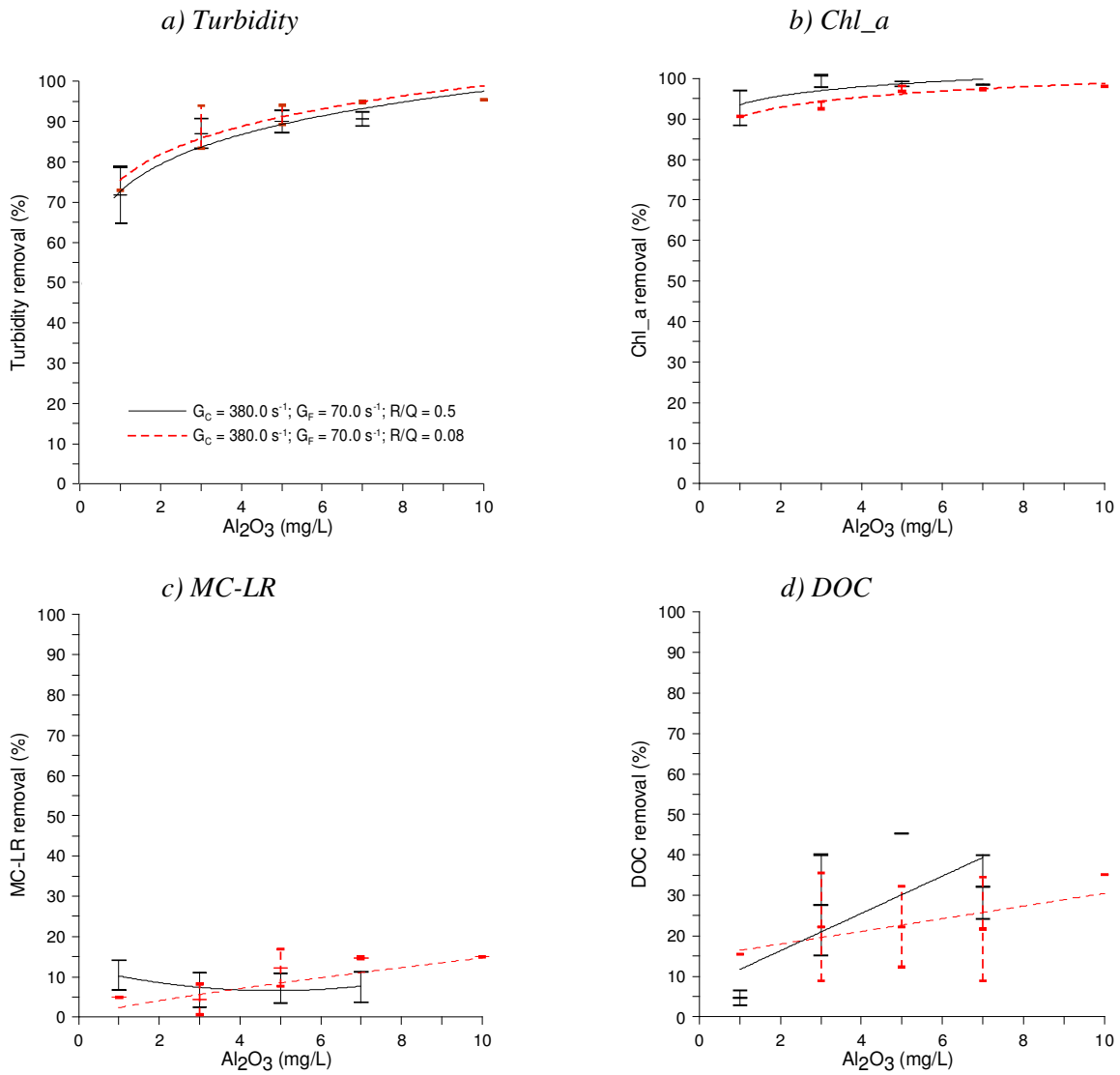

Figure 2.7 C/F/DAF removal efficiencies at 0.08 and 0.5 R/Q corrected for dilution (WAC; coagulation at 20±2 ºC, 380 s^{-1} for 2 min; flocculation at 70.0 s^{-1} for 8 min; R/Q at 0.5 or 0.08; DAF for 8 min).

2.4 CONCLUSIONS

This study compared dissolved air flotation and sedimentation performances to remove, without cell damage, single cells of *M. aeruginosa*, a surrogate for overall removal efficiency of cyanobacteria.

The results emphasised the importance of the coagulation/flocculation on the particle destabilisation and size increase required for cell-bubble contact enhancement, and subsequent formation of flocs of strength and size adequate for effective DAF treatment. The pre-polymerised coagulant of high basicity (WAC) performed better than alum, for both clarification processes (sedimentation and DAF). WAC achieved higher removal efficiencies, lower pH decrease, and lower residuals and optimal dose. In addition, it showed a superior ability to cope with the influent cell concentration increase. For both coagulants, cell removal efficiency increased with the influent concentration, although higher doses were required to reach the same residuals.

Both treatment processes, C/F/S and C/F/DAF, could efficiently remove *M. aeruginosa* cells with no toxin release to water, under the operating conditions tested, *i.e.* coagulation with 2 – 20 mg/L Al_2O_3, at 380 s^{-1} or 743 s^{-1} for 2 min, flocculation at 24 s^{-1} for 15 min or at 70 s^{-1} during 8 min, 15 min sedimentation or 8 min DAF at 0.5 or 0.08 recycle ratio, and 5 bar relative pressure. However, C/F/DAF performed better than C/F/S. It yielded very high chlorophyll a removal (93 – 98%) with no toxin release to water using low recycle ratios (8%), lower coagulant doses (3 *vs.* 5 mg/L Al_2O_3 of WAC), slower coagulation (380 s^{-1} *vs.* 743 s^{-1}), stronger but shorter flocculation (8 min at 70 s^{-1} *vs.* 15 min at 24 s^{-1}) than C/F/S. The best set of C/F/DAF operating conditions indicated that strong and small flocs and a minimum recycle were needed for effective treatment.

Similar studies on the impact of the water background organic matrix and evaluating the removal of filamentous cyanobacteria and of *M. aeruginosa* cell aggregates (closer resembling the naturally occurring colonies) are in progress.

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CHAPTER 3

REMOVAL OF *MICROCYSTIS AERUGINOSA* FROM NATURAL WATERS BY DISSOLVED AIR FLOTATION AND CONVENTIONAL WATER TREATMENT

ABSTRACT

Coagulation/flocculation (C/F) /dissolved air flotation (DAF) and C/F/sedimentation (S) experiments were performed using natural waters to evaluate the influence of the water background organic matrix on the *Microcystis aeruginosa* removal. Two natural waters from Alcantarilha Water Treatment Plant (Algarve, Portugal), raw water (RW) and ozonated water (OW), spiked with cultured cells of *M. aeruginosa* were used since preozonation is the process that mostly affect the molecular weight and hydrophilicity of the natural organic matter (NOM). Results showed that NOM had an important influence on the cyanobacterial cells removal, since high coagulant doses were necessary to achieve the same residuals obtained for waters with low NOM content. Such results were related with the need of higher coagulant doses to destabilise the particles present in the water. C/F/DAF process showed the best efficiencies for algae removal, higher than 90%, and the lowest residuals for lower coagulant dose were obtained with this process. Microcystins were practically not removed from the water but no release of toxins was found. OW showed higher removals than RW, especially for C/F/S. These results were attributed to the preozonation effect on the C/F since it causes particle destabilisation therefore promoting coagulation and flocculation.

3 REMOVAL OF *MICROCYSTIS AERUGINOSA* FROM NATURAL WATERS BY DISSOLVED AIR FLOTATION AND CONVENTIONAL WATER TREATMENT

3.1 INTRODUCTION

Cyanobacteria (blue-green algae) are members of freshwater phytoplankton in surface waters. They are of concern due to their ability to produce taste and odours compounds, as well as a wide range of toxins, which have a hepatotoxic or neurotoxic behaviour, being dangerous to animal and human heath. They also can form mass growths, or blooms that accumulate in eutrophic waters many of which are for drinking water consumption. Therefore, the removal of cyanobacterial cells without lising them (*i.e.*, without releasing intracellular metabolites like toxins, taste and odour compounds) would significantly reduce the concentration of these metabolites in the finished drinking water, a main goal of the water treatment processes.

Alternative survey on the removal efficiency of the conventional water treatment process for algae or cyanobacteria removal has been made. Some authors reported that the conventional treatment (coagulation/ flocculation/ sedimentation (C/F/S), filtration, chlorination) may cause cell lysis and release intracellular toxins (Hoffmann (1976), Keijola *et al.* (1988), Himberg *et al.* (1989), Lam *et al.* (1995)), while others observed no release of these compounds to the water (Chow *et al.* (1998), Hrudey *et al.* (1999), Drikas *et al.* (2001)). However, all of them agree on the ineffectiveness of the conventional treatment to remove toxins (Keijola *et al.* (1988), Himberg *et al.* (1989), Ando *et al.* (1992), Chow *et al.* (1998), Hrudey *et al.* (1999)). Since algae are low-density particles and some can float, dissolved air flotation (DAF) has proven to be more effective for treating algal-rich water than the conventional clarification by settling. Bauer *et al.* (1998) demonstrated DAF efficiency for treating algal rich waters from the Thames river in a full-scale plant. DAF showed better

results in reducing the algal load onto subsequent filtration than the precipitator clarifiers. Results from Vlaski *et al.* (1997), Chung *et al.* (2000), Kempeneers *et al.* (2001) and Kwon *et al.* (2004) demonstrated that DAF process could reach higher efficiencies for improving river water quality with high content of chlorophyll *a* when compared with conventional treatments.

Also a previous study (Ribau Teixeira and Rosa (2005), chapter 2) compared the removal of cultured cells of *M. aeruginosa* from tap water by C/F/DAF and by conventional C/F/S process. Results showed that both treatment processes, C/F/S and C/F/DAF, can remove *M. aeruginosa* cells from the water, but DAF process presented higher chl_a removals (69 – 94% with C/F/S and 77 – 99% with C/F/DAF). The pre-treatment C/F was essential for DAF process. The cells were not damaged by either C/F/S or C/F/DAF, as no release of microcystins to the water was observed.

However, in the mentioned studies the influence of the naturally occurring organic matter (NOM) onto cyanobacterial cells removal by DAF is not fully addressed. In fact, C/F is likely the most critical step for algae removal (Vlaski *et al.* (1997)) and NOM has a very strong influence on coagulation performance, since NOM adsorbs onto natural particles and acts as a particle-stabilising agent in surface waters. As in coagulation, particles must be destabilised by surface charge neutralisation or adsorption and interparticle bridging (Jiang and Graham (1996), AWWA (2000)), NOM is one important factor to be considered in terms of algae removal.

The aim of the present study is to evaluate the efficiency of the water clarification processes, namely C/F/S and C/F/DAF, on the cyanobacterial cells and cyanotoxins removal using

natural waters. It is also intended to evaluate the effect of the preozonation (NOM type) on the coagulation/ flocculation of the cyanobacterial cells since the oxidation of NOM may cause destabilisation and enhance coagulation/ flocculation. DAF experiments are also performed for comparison purpose.

M. aeruginosa is found in nature mostly in colonial form, which is very difficult to produce in laboratory conditions. The experiments presented here were made using single cells which is a more significant nuisance from the water treatment practice point of view, because the single cell form usually penetrates treatment processes and is encountered in treatment plant effluent (Vlaski *et al.* (1996)). The colonial form should be easier to remove and will be addressed in the following chapter.

3.2 MATERIALS AND METHODS

3.2.1 NATURAL WATER SAMPLES

Raw water (RW) and ozonated water (OW) (after preozonation) from Alcantarilha Water Treatment Plant (WTP), Algarve, Portugal, were the natural waters used in the experiments. Since 2000, this WTP supplies water to *ca.* half million people in southern Portugal (Algarve), and was designed to treat up to 3 m^3/s (*ca.* 1 million people by the year 2020) of surface water from Funcho Dam reservoir (2 km^2 and 43.4 hm^3). These are moderately hard waters with the characteristics presented in Table 3.1.

Table 3.1 Characteristics of the natural waters used in the experiments.

Water type	pH	Conductivity (μS/cm)	Turbidity (NTU)	DOC (mg C/L)	UV_{254nm} (1/cm)	SUVA (L/(m.mg))
RW	7.3	322	4.02	3.85	0.065	1.69
OW	7.3	318	3.60	3.56	0.032	0.87

SUVA: specific UV absorbance, defined as the UV absorbance expressed per meter of absorbance per unit concentration of DOC in mg/L.

These waters were chosen since they represent two types of NOM – ozonation decreases NOM molecular weight and hydrophobicity as shown by SUVA parameters. In addition, preozonation can assist in particulate matter removal by altering the surface characteristics of the solids and enhancing bubble attachment (Schofield (2001)) and with the ozone dose normally used with natural waters some cells can pass the ozonation process (Daldorph (1998)).

3.2.2 CYANOBACTERIAL CELLS

The experiments were performed with cultures of *Microcystis aeruginosa* supplied by Pasteur Culture Collection (PCC7820) and maintained in laboratory, as detailed in chapter 2.

RW and OW were spiked with PCC7820 cells until a specific concentration of chlorophyll *a* (chl_a) was achieved, namely Alert Level 2 established by Bartram *et al.* (1999). Spiking similar concentrations of cells from the culture suspension is a difficult task, making it difficult to continuously provide algae with constant and comparable quality of culture (Vlaski *et al.* (1996)). Alert Level 2 corresponds to the worst scenario (cyanobacterial biomass 100,000 cells per mL or 50 μg/L chl_a) and describes a toxic bloom with high biomass and possibly also localised scum (Bartram *et al.* (1999)). The natural waters spiked with *M. aeruginosa* stayed overnight at room temperature before the treatment trials.

3.2.3 ANALYTICAL METHODS

Samples were analysed for turbidity, chl_a, dissolved organic carbon (DOC), UV_{254nm} absorbance, pH, conductivity and extracellular microcystin-LR (MC-LR) the dominant variant produced by *M. aeruginosa* PCC7820. Turbidity was measured in a HACH 2100N

turbidity meter of high resolution (0.001 NTU), chl_a and UV_{254nm} using a Spectronic Unicam UV300 UV/VIS spectrophotometer, DOC in a Shimadzu TOC 5000A analyser (50 ppb – 4000 ppm), pH (at 25 ºC) using a Whatman WTW pH340 meter, and conductivity in a Crison GLP32 conductimeter, all using standard methods for analysis. Microcystins were analysed by HPLC-PDA using a Dionex Summit system, which includes a high pressure gradient pump Dionex Summit, an autosampler Dionex ASI-100, a column oven Dionex STH-585 and a photo diode-array detector Dionex PDA-100. A C18 column was used (Merck Purospher STAR RP-18 endcapped, 3 µm particles, LiChroCART 55x4 mm). The mobile phase used a gradient of milli-Q water and acetonitrile, both with 0.05% (v/v) of trifluoracetic acid. Chromatograms were analysed between 180 – 900 nm, with a main detection at 238 nm for the typical microcystins spectra (Meriluoto and Spoof (2005a)).

Extracellular MC-LR (MC-LR) was first isolated from the intracellular and cell-bound fraction by sample filtration through a Whataman GF/F glass microfiber filter. Microcystins were then extracted from the filtered water samples using an isolute C18 solid phase extraction column, 1 g in a 6 mL reservoir, following the standard operation procedure developed by Meriluoto and Spoof (2005b) with some deviations. The cartridges were first conditioned with 10 mL methanol (75%), followed by 10 mL milli-Q water at a flowrate not exceeding 10 mL/min, without letting it dry during conditioning. The samples were then applied to the cartridge and the microcystin was eluted with 5 mL methanol (90%) containing 0.1 % trifluoroacetic acid. The methanolic elute was evaporated at 50-54 ºC in a rotavapor, resuspended in 500 µL methanol (75%), centrifuged for 10 min at 10,000 x *g*, and 150 µL of supernadant were transferred to HPLC vials for analysis.

3.2.4 COAGULATION/ FLOCCULATION/ SEDIMENTATION EXPERIMENTS

The coagulant tested was WAC (aluminium polyhydroxichlorosulphate), a pre-polymerised aluminium coagulant with a relative basicity of 60-70% (Elf Atochem, stock solution with 850 mg/L Al_2O_3), in the concentrations range between 2 and 12 mg/L of Al_2O_3. Such coagulant doses correspond to the usual doses for this kind of experiments and used in full-scale operation. WAC was chosen since it showed the best results, as presented in Ribau Teixeira and Rosa (2005) (chapter 2).

C/F/S experiments were performed in a Jar test unit with four paddles and 500 mL of each sample. The operating conditions for gradient velocity (G) and retention time were: a) coagulation at G_C 743 s^{-1} for 2 min; b) flocculation at G_F 24 s^{-1} for 15 min; and c) sedimentation for 20 min. These are the usual conditions for C/F/S in water treatment (Vlaski *et al.* (1996), AWWA (2000)).

3.2.5 COAGULATION/ FLOCCULATION/ DAF EXPERIMENTS

C/F/DAF experiments were performed in a laboratory-made flotation cell, adapted from De Pinho *et al.* (2000) and already presented in Ribau Teixeira and Rosa (2005) (chapter 2). The experimental procedure for the flotation experiments was as described in Eckenfelder (2000) and Ribau Teixeira and Rosa (2005). In this *apparatus* a paddle for C/F was installed. The operating conditions studied were: a) coagulation at G_C 380 s^{-1} for 2 min, using 2 – 12 mg/L Al_2O_3 of WAC; b) flocculation at G_F 70 s^{-1} for 8 min; and c) DAF for 8 min. The applied recycle ratio (R/Q) was 0.08. The studied values of G, R/Q and retention times correspond to the ones optimised in earlier studies using tap water spiked with *M. aeruginosa* cells (Ribau Teixeira and Rosa (2005), chapter 2) and referred in the literature (Edzwald *et al.* (1992),

Vlaski *et al.* (1996), Hedberg *et al.* (1998), Crossley *et al.* (2001)). Experiments were made in duplicate.

All experiments used a relative pressure of 5 bar. For computing the removal of all parameters in the clarified water, a correction factor for dilution (1 + R/Q) was used.

3.2.6 DAF EXPERIMENTS

DAF experiments used the same *apparatus* referred for C/F/DAF experiments, but with no paddle installed. The experimental procedure was as described in Eckenfelder (2000) and Ribau Teixeira and Rosa (2005) (chapter 2).

The applied pressure and recycle were 5 bar and 0.08, respectively, in all experiments. A correction factor for dilution (1 + R/Q) was used in computing the removal of all parameters in the clarified water. Experiments were made in duplicate. These experiments were performed for comparison purposes with the others studied treatment processes.

3.3 RESULTS AND DISCUSSION

3.3.1 COAGULATION/ FLOCCULATION/ SEDIMENTATION EXPERIMENTS

Figure 3.1 shows the variation of turbidity, chl_a, MC-LR, DOC and UV_{254nm} in the C/F/S clarified water with the coagulant dose added to the natural waters (RW and OW) spiked with *M. aeruginosa*.

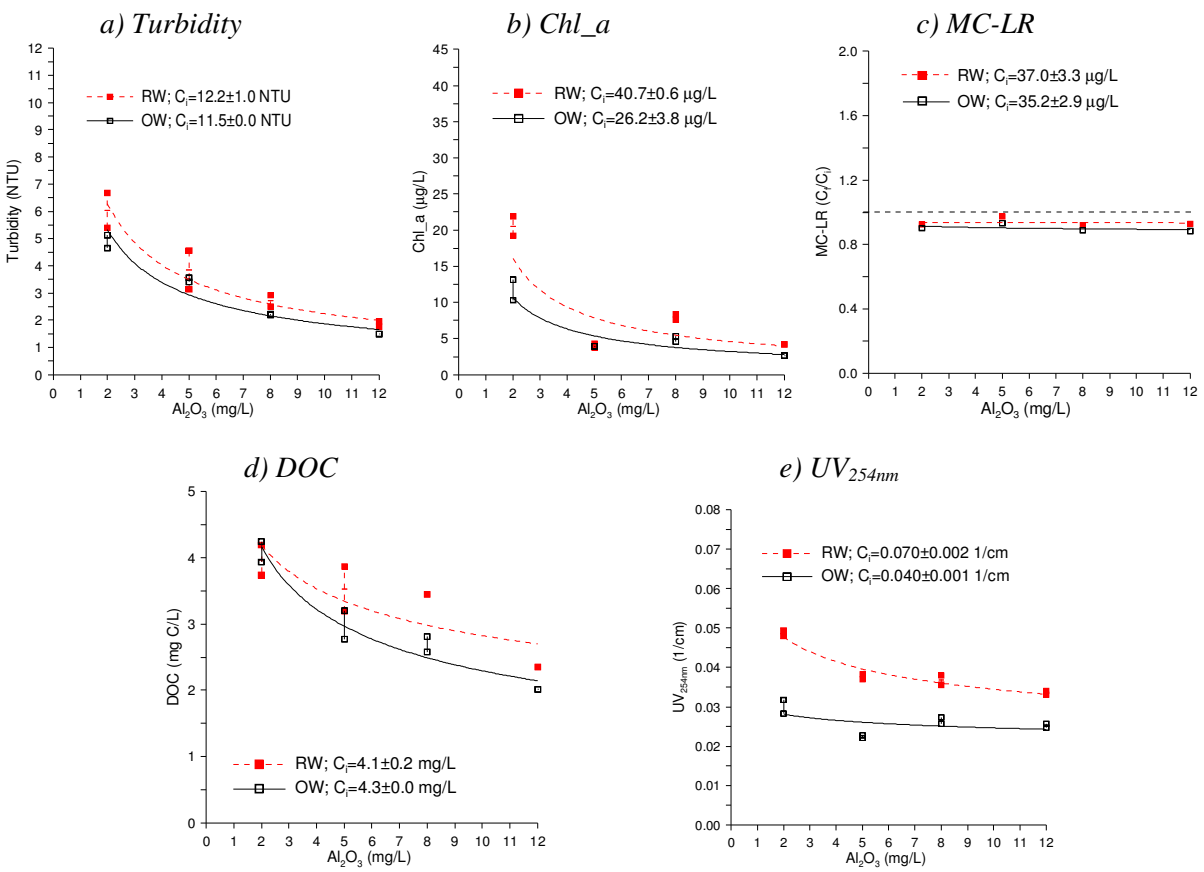

Figure 3.1 Results from the C/F/S experiments: **a)** turbidity, **b)** chl_a, **c)** MC-LR, **d)** DOC and **e)** UV_{254nm} (C_i, C_f are the initial and final concentrations, respectively).

As expected results show, except for MC-LR, an increase of the clarified water quality with the coagulant dose added to water, for all the parameters and for the two types of water (Figures 3.1a, 3.1b, 3.1d and 3.1e). To reach residuals of 1.9 NTU in RW and 1.5 NTU in OW, it is necessary to add 12 mg/L of Al_2O_3, and the final concentration for chl_a is 4.2 µg/L and 2.7 µg/L, respectively for RW and OW, with these initial concentrations (Figures 3.1a and 3.1b). MC-LR concentration does not vary with the coagulant dose added. MC-LR is practically not removed by C/F/S (2.5 – 7.9% for RW and 6.8 – 11.7% for OW) but most important, there is no release of MC-LR with the tested operating conditions (Figure 3.1c). Similar results had already been obtained using tap water (Ribau Teixeira and Rosa (2005), chapter 2) and also by other authors (Velzeboer *et al.* (1995), Chow *et al.* (1999)). NOM

(DOC and UV_{254nm}) residuals of *ca.* 2.1 and 1.9 mg C/L and 0.035 and 0.025 1/cm of UV_{254nm}, respectively for RW and OW, were obtained with 12 mg/L of Al_2O_3.

Clarified OW presents better quality than clarified RW, as well as has higher removal efficiencies (except for UV_{254nm}), despite the relatively low differences between these two types of water (Figure 3.1). In fact, ozonation oxidises the NOM present in the water into lower molecular weight and more polar species, which can lead to an increase in the C/F/S clarification process as referred by AWWA (2000). Preozonation has been shown to improve clarification efficiency by a mechanism that causes particle destabilisation and flocculation (AWWA (2000)). Most particles in raw drinking water are negatively charged due to the adsorption of NOM on the particles surface and NOM in bulk solution exerts an appreciable coagulant demand. According to AWWA (2000) the preoxidation benefits for the coagulation and flocculation include: oxidation of adsorbed organics to more polar forms, making the particles less stable and more amenable to aggregate; alteration of the configuration of the adsorbed organics so they bind more effectively to coagulants; and oxidation of organics in bulk solution to form carboxylic acid functional groups that bind metals, resulting in a precipitation of the organic material. It is also reported that preozonation for organics removal is variable and appears to be site specific, since it selectively modifies fractions of the NOM matrix (Owen *et al.* (1995), Widrig *et al.* (1996)). Widrig *et al.* (1996) demonstrated the benefits of preozonation on DOC removal, showing an increase between 5 to 15% of DOC removal with preozonation beyond the highest removals for coagulation alone. In addition, Amy *et al.* (1992) referred that coagulation removed humic and high molecular weight organic matter more efficiently than it removed non-humic and low molecular weight organic matter. These results justify that the observed UV_{254nm} variation is higher than that of DOC, because the first measures the aromaticity/hydrophobicity, while the second measures the

concentration of dissolved carbon containing molecules. The performance of conventional treatments may degrade with changes in the nature of the raw water in consequence of the inability to control the nature of the coagulant species formed during dilution under prevailing raw water conditions and in competition with other reactions (Jiang and Graham (1996)). Therefore, the removal efficiencies of NOM depend on the type and dose of coagulant, coagulation pH, temperature, and raw water quality characteristics.

Comparing the results obtained with the natural waters (RW, OW, Figure 3.1) and with the clear water (tap water, results from Ribau Teixeira and Rosa (2005), chapter 2), it is possible to see the NOM influence on the C/F/S performance (Table 3.2). The presence of NOM decreases the removal efficiency of the process, and to achieve the highest removal efficiencies it is necessary to add more coagulant (Table 3.2). In fact, more coagulant dose is necessary to neutralise the NOM and the cyanobacteria charges (destabilise all the particles present in solution) for effective C/F. These observations lead to the conclusion that natural occurring NOM in surface waters may result in lower cyanobacteria removal efficiencies as already reported by Ma and Liu (2002), and/or in a higher consumption of coagulant.

Table 3.2 Comparison between the C/F/S removal efficiencies (%) for the optimal coagulant doses for RW / OW and tap water (TW).

Dose of coagulant ($mg/L\ Al_2O_3$)	Turbidity			Chl_a			MC-LR			DOC		
	TW[#]	RW	OW	TW[#]	RW	OW	TW[#]	RW	OW	TW[#]	RW	OW
5 *	92.8	65.7	69.8	95.0	90.3	87.0	6.0	2.6	6.8	-	32.8	47.3
12 [+]	89.5	85.9	87.0	90.6	89.5	88.1	6.4	7.3	11.7	-	58.0	64.5

[#] Results from Ribau Teixeira and Rosa (2005), chapter 2;
* Optimal coagulant dose for TW (Ribau Teixeira and Rosa (2005), chapter 2) and [+] optimal coagulant dose for RW / OW.

3.3.2 COAGULATION/ FLOCCULATION/ DAF EXPERIMENTS

Figure 3.2 shows the C/F/DAF performance (in terms of turbidity, chl_a, MC-LR, DOC and UV_{254nm}) on the clarification of RW and OW. Results obtained in DAF experiments (with no coagulant addition) are also presented for comparison purposes.

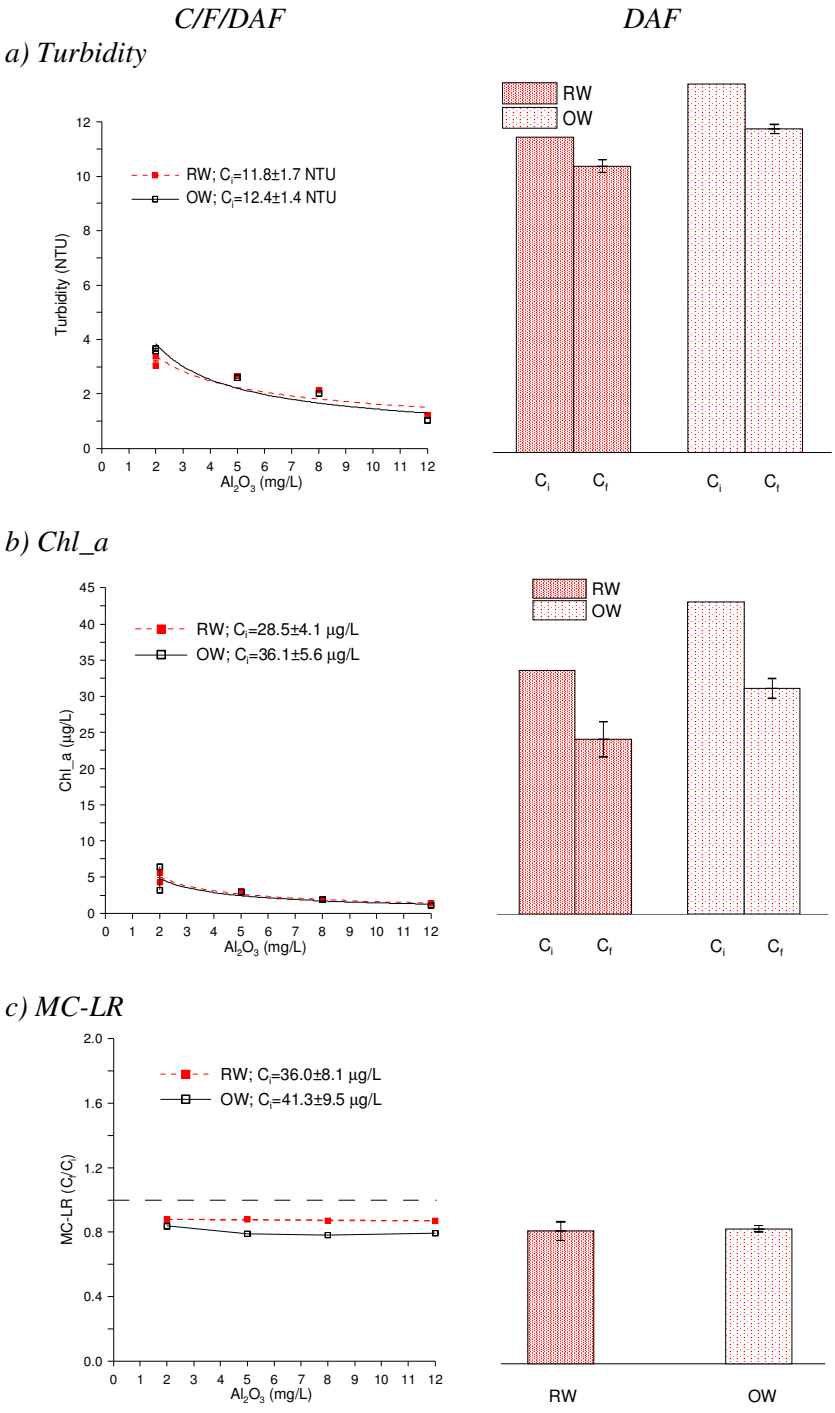

Figure 3.2 Results from the C/F/DAF (symbol chart) and DAF (bar chart) experiments: **a)** turbidity, **b)** chl_a, **c)** MC-LR, **d)** DOC and **e)** UV_{254nm} (C_i , C_f are the initial and final concentrations).

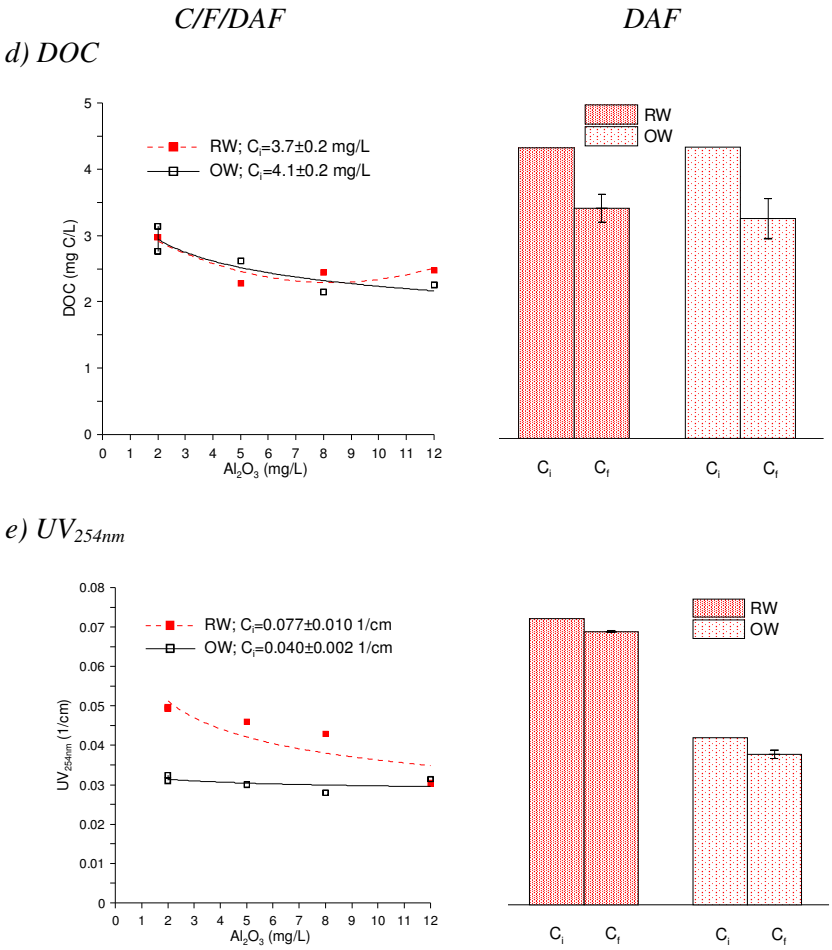

Figure 3.2 (cont.) Results from the C/F/DAF (symbol chart) and DAF (bar chart) experiments: **a)** turbidity, **b)** chl_a, **c)** MC-LR, **d)** DOC and **e)** UV_{254nm} (C_i , C_f are the initial and final concentrations).

As already obtained for C/F/S (section 3.3.1), C/F/DAF results show a decrease of residual concentrations with the coagulant dose added to the water for all the parameters studied except for MC-LR (Figures 3.2a, 3.2b, 3.2d and 3.2e). As previously found with tap water spiked with *M. aeruginosa* cells (Ribau Teixeira and Rosa (2005), chapter 2), coagulant addition does not improve MC-LR removal but, most important, there is no release of MC-LR in the studied range of coagulant added to water (Figure 3.2c). Residuals of 2.0 NTU, 1.9 µg/L chl_a, 2.4 and 2.2 mg C/L and 0.043 and 0.028 1/cm, respectively for RW and OW, are obtained for a lower coagulant dose (8 mg/L Al_2O_3) than what was necessary for clarification by C/F/S (12 mg/L Al_2O_3). Therefore, C/F/DAF can reach lower residuals than the C/F/S process for the same coagulant dose (Figure 3.1 *vs.* Figure 3.2). In the same line, Kempeneers

et al. (2001) showed results of effluent quality below 5 μg/L of chl_a when the influent had low chl_a concentration and not exceeding 10 μg/L for higher influent chl_a concentrations, in a full-scale plant using alum/alum and activated silica/powered activated carbon, during ten years of operation. Similar results were obtained by Chung *et al.* (2000) in a pilot and full-scale plant using polyaluminium chloride as coagulant, and by Zabel (1985) and Vlaski *et al.* (1996) but using alum and $FeCl_3$ in waters containing *M. aeruginosa*. Hargesheimer and Watson (1996) also reported better particle removal efficiencies by DAF than by conventional gravity sedimentation (the turbidity in the clarified water by DAF was 20 – 50% lower than in the conventional gravity sedimentation, and chl_a removal efficiencies were 73% by DAF and 57% by sedimentation), while for TOC the removal efficiencies by the two processes were comparable, 13% by DAF and 10% by sedimentation.

Comparing the C/F/DAF and DAF results (Figure 3.2), it is evident the need of C/F prior to DAF process for cyanobacterial removal. As expected DAF removal efficiencies are very low for all parameters studied since in this process particles are not destabilised, condition necessary for the effectiveness of flotation process, and the air bubbles have a surface charge slightly negative (Malley and Edzwald (1991), Yan and Jameson (2004), Ribau Teixeira and Rosa (2005)). For DAF effectiveness, particle destabilisation is very important, even more than the flocs size (Malley and Edzwald (1991)), so coagulation conditions that produce hydrophobic flocs or particles of little or with no charge are required for efficient DAF.

In C/F/DAF process there is no significant differences between RW and OW for turbidity, chl_a, MC-LR and DOC (Figure 3.2). Furthermore, RW DOC are quite similar from OW DOC (3.7 and 4.1 mg C/L, respectively for RW and OW, Figure 3.2d), and as already explained less coagulant dose is necessary in C/F/DAF to reach the same residuals of C/F/S.

Therefore, C/F/DAF is less influenced by NOM type than C/F/S, where clarified OW presents better quality than RW, which may be related with the denser / heavier flocs necessary for C/F/S effectiveness.

Furthermore, the coagulant dose necessary to treat natural waters (like RW and OW) increases when the water contains NOM (Table 3.3). Such results show the influence of NOM on the coagulation effectiveness. As explained by Jiang and Graham (1996) and Ma and Liu (2002) in the presence of NOM, the coagulant reacts first with the free natural organic acids, and then, if the coagulant dose is high enough to neutralise the surface charges of the organic materials, the coagulant can take part in the electro-neutralisation and bridging process. Therefore, cyanobacterial cells removal by C/F/DAF depends on the water background NOM (RW or OW), being highest for the latter.

Table 3.3 Comparison between the C/F/DAF removal efficiencies (%) for the optimal coagulant doses for RW / OW and tap water (TW).

Dose of coagulant ($mg/L\ Al_2O_3$)	Turbidity			Chl_a			MC-LR			DOC		
	TW[#]	RW	OW	TW[#]	RW	OW	TW[#]	RW	OW	TW[#]	RW	OW
2 [*]	78.2	66.7	65.4	92.3	83.6	83.0	7.7	5.2	9.7	20.3	18.1	25.6
8 [+]	95.0	82.8	84.2	97.3	91.8	95.2	14.8	6.7	15.8	21.7	24.3	46.6

[#] Results from Ribau Teixeira and Rosa (2005), chapter 2;
[*] Optimal coagulant dose for TW (Ribau Teixeira and Rosa (2005), chapter 2) and [+] optimal coagulant dose for RW / OW.

3.4 CONCLUSIONS

This study showed the influence of the water background NOM on the removal of the cyanobacterial cells of *Microcystis aeruginosa* by two treatment processes, namely C/F/S and C/F/DAF. The presence of NOM (raw and ozonated water) exerted an important coagulant demand, higher coagulant doses being necessary to destabilise the particles present in the water and to achieve the same residuals obtained in experiments performed with water with very low NOM content (tap water). Comparing C/F/S and C/F/DAF, C/F/DAF showed the

best removal efficiencies of *M. aeruginosa* cells (higher than 90% expressed by chl_a) and lower residuals were obtained with lower coagulant dose. Both processes could not achieve significant removal efficiencies of extracellular MC-LR in the studied range of coagulation dose but, most important, no release of microcystin was observed.

Pre-ozonated water containing *M. aeruginosa* cells presented the highest removal efficiencies (except for UV_{254nm}). In fact, preozonation benefited the coagulation and flocculation due to the oxidation of adsorbed organics to more polar forms, alteration of the configuration of the adsorbed organics, and oxidation of organics to form carboxylic acid functional groups. As UV_{254nm} measures the aromatic/hydrophobic compounds, removal efficiencies were higher for higher concentrations, as in raw water. Such influence was much higher for C/F/S process than for C/F/DAF process, which is an advantage of C/F/DAF over C/F/S process (C/F/DAF is less influenced by NOM type).

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CHAPTER 4

THE ABILITY OF DISSOLVED AIR FLOTATION TO REMOVE CYANOBACTERIAL SINGLE CELLS, COLONIES (*MICROCYSTIS AERUGINOSA*) AND FILAMENTS (*PLANKTOTHRIX RUBESCENS*)

ABSTRACT

The removal of single cells, colonies and filamentous cyanobacteria from water by dissolved air flotation (DAF) was evaluated. The influence of the natural organic matter on the overall efficiency was also addressed. Experiments were performed using tap water and raw water from Alcantarilha Water Treatment Plant Works spiked with *Microcystis aeruginosa* cells and cell aggregates, and *Planktothrix rubescens* filaments. As expected, coagulation / flocculation enhances the DAF efficiency, since flotation needs particle destabilisation. Cell removal measured by chlorophyll *a* and intracellular microcystin-LR were high for both types of waters (>89%). No release of MC-LR was obtained for all the studied cyanobacteria morphologies. For *P. rubescens*, results with RW were quite similar to those obtained with tap water, since DOC initial concentrations were very high due to the growth medium contribution, and which represents a different scenario from the natural waters with *P. rubescens* blooms. It seems that with a pre-polymerised coagulant the main coagulation mechanism was charge neutralisation.

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4 THE ABILITY OF DISSOLVED AIR FLOTATION TO REMOVE CYANOBACTERIAL SINGLE CELLS, COLONIES (*MICROCYSTIS AERUGINOSA*) AND FILAMENTS (*PLANKTOTHRIX RUBESCENS*)

4.1 INTRODUCTION

Cyanobacteria (blue-green algae) have been identified worldwide, posing a significant risk to water supplies when they occur in reservoirs, lakes and rivers used as water sources, due to their ability to produce toxins – as well as taste and odour compounds – as secondary metabolites under particular conditions of growth. The most commonly occurring group of cyanobacterial hepatotoxins in fresh water are microcystins, which are potentially produced by common genera of cyanobacteria like *Microcystis*, *Planktothrix* and *Anabaena* (Sivonnen and Jones (1999)). *Microcystis* are unicellular or colonial, while *Planktothrix* and *Anabaena* are natural occurring filamentous cyanobacteria. Toxins can occur within the cells (intracellular or cell bound toxins) or be released from cells to water (extracellular or dissolved toxins) under certain conditions of growth and/or external (environmental) stress factors responsible for cell lysis. As a result of the increasing concern with their health implications, the World Health Organisation (WHO) has set a drinking water guideline value of 1.0 μg/L for microcystin-LR (MC-LR).

Regarding the efficiency of conventional treatment (coagulation (C)/ flocculation (F)/ sedimentation (S), filtration, chlorination) for cyanobacterial cells removal, there seems to be some disagreement in the literature. Some papers report the occurrence of cell lysis, release of intracellular toxins and taste and odour compounds (Himberg *et al.* (1989), Lam *et al.* (1995), Hrudey *et al.* (1999)) while others refer no release of such compounds to the water (Chow *et al.* (1998), Drikas *et al.* (2001)). Some studies report removal efficiencies of *Microcystis* cells between 58% and 90% by the conventional treatment, and showed that such procedure was

not effective for extracellular toxin removal (Falconer *et al.* (1989), Velzeboer *et al.* (1995), Chow *et al.* (1999)). According to Zabel (1985) and Hrudey *et al.* (1999), floc blanket clarification had shown 76.5% removal of *Microcystis* cells whilst dissolved air flotation (DAF) removed 98% in the presence of other algae. DAF is generally more effective than C/F/S for treating cyanobacterial rich waters (Ribau Teixeira and Rosa (2005), chapter 2).

For cyanobacterium *Planktothrix sp*, there are few water treatment studies. Most of the studies with *Planktothrix* are related with their distribution and seasonal dynamics, growth rate and factors that affect the growth, like light or nutrients. However, some authors reported that *Planktothrix sp* produce high concentrations of microcystins bringing problems to the water treatment. For filamentous cyanobacteria, several authors agree on the removal efficiencies achieved by the conventional water treatment processes, but some report damage of cells and release of toxins to the water. Lahti *et al.* (2001) studied raw and treated drinking waters of several Finnish surface waters and found that the most abundant cyanobacterial species was *Planktothrix agardhii*. The highest microcystin concentration measured in raw water was almost 10 μg/L, while in treated water, when microcystins were detected, the concentrations were clearly below the WHO guideline value. Schmidt *et al.* (2002) investigated the natural occurrence of microcystins and *Planktothrix rubescens* in a reservoir and the relation with the removal of microcystin by the water treatment works (WTW). For the conventional flocculation and filtration, results showed high levels of safety, even when there was a significant increase in the number of cells in raw water. However, an increase in the extracellular microcystins was found in the clarified water during this treatment process, implying a potential risk of further microcystin release. Hoeger *et al.* (2005) studied two treatment plants, one in Switzerland and the other in Germany, for *P. rubescens* and microcystin removal. The water treatment system from Lake Zurich (preozonation and sand

filtration) was effective on removing microcystins and cyanobacterial filaments from the raw water, despite the high cell densities and microcystins concentration in the water intake and the observed release of microcystins. In Germany, the treatment sequence (flocculation, filtration, chlorination) was not as efficient as in Zurich, but no release of microcystins was observed. Chow *et al.* (1998), using ferric chloride as the coagulant, observed no increase of microcystin in water after C/F/S, although it appeared that *Anabaena circinalis* was susceptible to chemicals. However, Velzeboer *et al.* (1995) found that aluminium sulphate seemed not to cause cell lysis of cultured *A. circinalis*, at the concentrations and conditions that normally occur in a WTW.

Regarding cyanobacterial morphologies, some authors referred no significant differences between the removal of *Microcystis* cells or colonies and *Anabaena* filaments in a DAF unit (Yan and Jameson (2004)), while others reported removal efficiencies of 40 – 80% for *Microcystis,* 90 – 100% for *Anabaena* and only 30% for *Planktothrix* in a Belgian DAF plant (Hrudey *et al.* (1999)). The same high DAF removal efficiencies (98%) for *Microcystis aeruginosa* and *Anabaena circinalis* (Yan and Jameson (2004)), and for *Chlorella* and *Cyclotella* (Edzwald and Wingler (1990)) have been reported by other authors.

The objective of this study was to evaluate the DAF efficiency to remove different cyanobacterial morphologies, namely single cells and colonies (using *Microcystis aeruginosa*), and filaments (using *Planktothrix rubescens*), from clear and natural waters.

4.2 MATERIAL AND METHODS

4.2.1 CYANOBACTERIAL CULTURES

M. aeruginosa supplied by Pasteur Culture Collection (PCC 7820) was grown in laboratory (10 L medium, Figure 4.1) according to enclosed instructions, *i.e.* BG11 medium at 23 – 24 ºC under a light regimen of 12 hours light, 12 hours dark (~5 μM photon m^{-2} s^{-1}). These cultures contain individual cells or pair of cells, as shown in Figure 4.2. Cultures were harvested at the late exponential phase of growth.

Cell aggregates of *M. aeruginosa* were produced by growth media manipulation, namely by increasing the calcium and magnesium concentrations, as suggested by Menaia (2003), since no natural bloom occurred during the experiments. After 2-2.5 months, *M. aeruginosa* cell aggregates could be seen by visual and microscopic inspection (Figure 4.3). *M. aeruginosa* is found in nature mostly in colonial form, which is very difficult to produce in laboratory cultures. The cultured material differs physically from naturally occurring field populations of *M. aeruginosa* for it is made up of small, regular colonies, and many single cells and pairs of cells contain much less mucilage (mucopolysaccharied acid) than natural material. The field populations may contain very large (macroscopic) colonies, and greater amounts of mucilage surrounding them (Sivonnen and Jones (1999), Drikas *et al.* (2001)).

Figure 4.1 Cultures of *Microcystis aeruginosa* (PCC7820) cells.

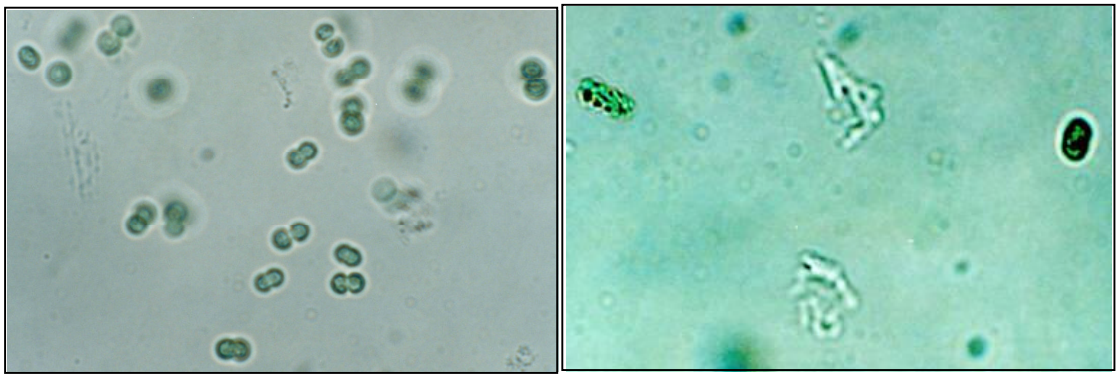

Figure 4.2 *M. aeruginosa* (PCC7820) single cells or pair of cells (amplification: *ca.*1000x).

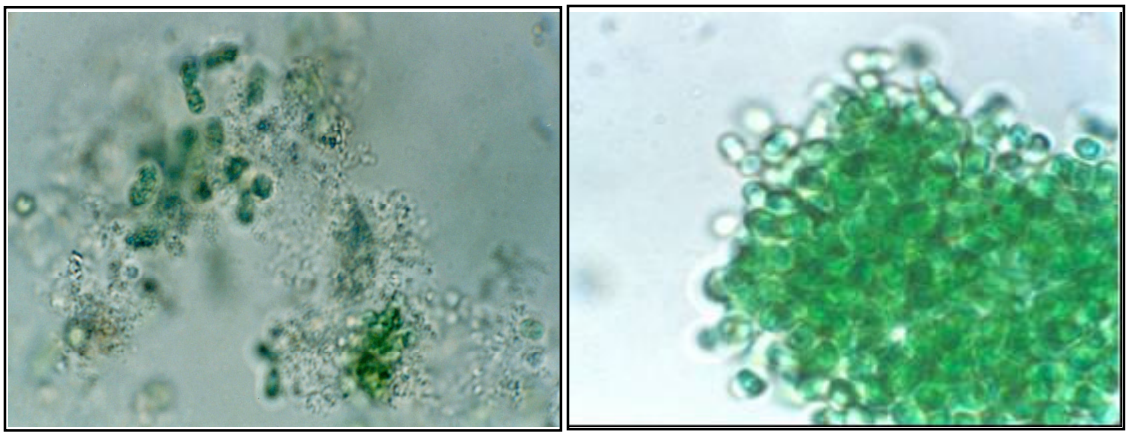

Figure 4.3 *M. aeruginosa* cell aggregates (amplification: *ca.* 1000x).

P. rubescens filaments were supplied by DVGW-Technologiezentrum Wasser Karlsruhe (TZW), within TOXIC European Project "Barriers against cyanobacteria in drinking water", and maintained in laboratory according to TZW instructions (Figure 4.4). The growth media basically contains stock solution (KNO_3, K_2HPO_4, $MgSO_4 \cdot 7H_2O$), soil extract, micronutrient solution and vitamins.

Figure 4.4 Culture of *Planktothrix rubescens*.

Treatment experiments were performed using tap water (TW) and raw water (RW) spiked with *M. aeruginosa* cells or cell aggregates and with *P. rubescens* until a specific concentration of chlorophyll *a* (chl_a) was achieved, namely the Alert Level 2 from Bartram *et al.* (1999). The Alert Level 2 (cyanobacterial biomass of 100,000 cells per ml or 50 µg/l chl_a) describes an established toxic bloom with high biomass. Conditions in this level indicate an increase in the risk of human health effects, and the need for effective water treatment systems (Bartram *et al.* (1999)). These solutions stayed overnight at room temperature before use. As found by other authors (Vlaski *et al.* (1996)) spiking similar concentrations of cells from the culture suspension was a difficult task, so it was difficult to continuously provide cyanobacteria with constant and comparable culture quality.

4.2.2 NATURAL WATER SAMPLES

Raw water from Alcantarilha WTW was the natural water used in these experiments. Since 2000, this WTP supplies water to *ca.* half million people in southern Portugal (Algarve), and was designed to treat up to 3 m^3/s (*ca.* 1 million people by year 2020) of surface water from Funcho Dam reservoir (2 km^2 and 43.4 hm^3). The characteristics of these moderately hard waters are presented in Table 4.1.

Table 4.1 Characteristics of the raw water used in the experiments before spiking with PCC7820 cells or cell aggregates, and *P. rubescens* filaments.

Experiments with:	pH (20ºC)	Conductivity (µS/cm)	Turbidity (NTU)	DOC (mg C/L)	UV_{254nm} (1/cm)	SUVA (L/(m.mg))
Single cells of *M. aeruginosa*	7.3	322	4.02	3.85	0.065	1.69
Cell aggregates of *M. aeruginosa*	7.2	338	1.46	3.22	0.040	1.24
Filaments of *P. rubescens*	7.4	325	-	3.40	-	-

SUVA: specific UV absorbance, defined as the UV absorbance expressed per meter of absorbance per unit concentration of DOC in mg/L

4.2.3 ANALYTICAL METHODS

Samples were analysed for chl_a, dissolved organic carbon (DOC), pH, conductivity, extracellular (extra MC-LR) and intracellular or cell bound microcystin-LR (intra MC-LR), the dominant toxin variant. Chl_a was measured using a Spectronic Unicam UV300 UV/VIS spectrophotometer, DOC in a Shimadzu TOC 5000A analyser (50 ppb – 4000 ppm), pH (at 25 ºC) using a Whatman WTW pH340 meter, and conductivity in a Crison GLP32 conductimeter, all using standard methods of analysis. Microcystins were extracted as described below and analysed by HPLC-PDA using Dionex Summit system, which includes a high pressure gradient pump Dionex Summit, an autosampler Dionex ASI-100, a column oven Dionex STH-585 and a photo diode-array detector Dionex PDA-100. A C18 column was used (Merck Purospher STAR RP-18 endcapped, 3 µm particles, LiChroCART 55x4 mm). The mobile phase used a gradient of milli-Q water and acetonitrile, both with 0.05% (v/v) of trifluoroacetic acid. Chromatograms were analysed between 180 - 900 nm, with a main detection at 238 nm for the typical microcystins spectra.

For the extraction of intra MC-LR, samples were filtered through a GF/F glass microfiber, that stayed during 18-24h in the freezer (-20 ºC, in the dark) in 20 mL methanol 75% (v/v). After this period, the filters were washed with a small volume of methanol 75%, and centrifuged (6,000 x *g*, 10 min). The supernadant was collected and evaporated in a rotavapor (50-54 °C), the residue was resuspended in 500 µL methanol 75% and centrifuged again

during 10 min, at 10,000 x *g*. Then, 150 µL of supernatant were transferred to a vial, and either analysed immediately on HPLC-PDA or remained in the freezer (-20 ºC, in the dark) until analysis. Extra MC-LR was extracted from the aqueous sample using an isolute C18 solid phase extraction column, 1 g in a 6 mL reservoir following the standard operation procedure developed by Meriluoto and Spoof (2005). The cartridges were first conditioned with 10 mL methanol 75% followed by 10 mL milli-Q water at a flowrate not exceeding 10 mL/min, without letting it dry during conditioning. The samples were then applied to the cartridge and the microcystin was eluted with 5 mL methanol 90% containing 0.1% trifluoroacetic acid. The methanolic elute was evaporated at 50 ºC in a rotavapor, resuspended in 500 µL 75% methanol, centrifuged for 10 min at 10,000 x *g* and 150 µL of supernadant were transferred to HPLC vials for analysis.

4.2.4 DAF AND COAGULATION/ FLOCCULATION/ DAF EXPERIMENTS

DAF and C/F/DAF experiments were carried out in the laboratory-made flotation *apparatus* described by Ribau Teixeira and Rosa (2005) (chapter 2), which also describe the standard experimental procedure used. The operating conditions used corresponded to the ones that gave the best results in C/F/DAF experiments with *M. aeruginosa* single cells (Ribau Teixeira and Rosa (2005), chapter 2) namely: i) coagulation at a velocity gradient (G_C) of 380 s^{-1} for 2 min, using the pre-polymerised aluminium coagulant WAC (aluminium polyhydroxichlorosulphate with a relative basicity of 60-70%, Elf Atochem, stock solution with 850 mg/L Al_2O_3), the dose varying between 2–10 mg/L of Al_2O_3 in the experiments with TW, being 2 and 8 mg/L Al_2O_3 in RW experiments with *M. aeruginosa* cultures (the optimal doses obtained by Ribau Teixeira and Rosa (2005), chapter 2, and chapter 3) and 2 mg/L Al_2O_3 for experiments with *P. rubescens*, ii) flocculation at G_F of 70 s^{-1} for 8 min; iii) DAF during 8 min, using 5 bar of relative pressure and 0.08 of pressurised recycle ratio (R/Q). All

experiments were made at room temperature (20±2 ºC). A correction factor for dilution (1+R/Q) was used in computing the removal efficiency (R) of all parameters in the clarified water. Experiments were made in duplicate.

4.3 RESULTS AND DISCUSSION

Figures 4.5 and 4.6 show the results of DAF and C/F/DAF performance on removing *M. aeruginosa* single cells and cell aggregates from TW and Figure 4.7 shows C/F/DAF results using RW. Figure 4.8 shows the same results for *P. rubescens*.

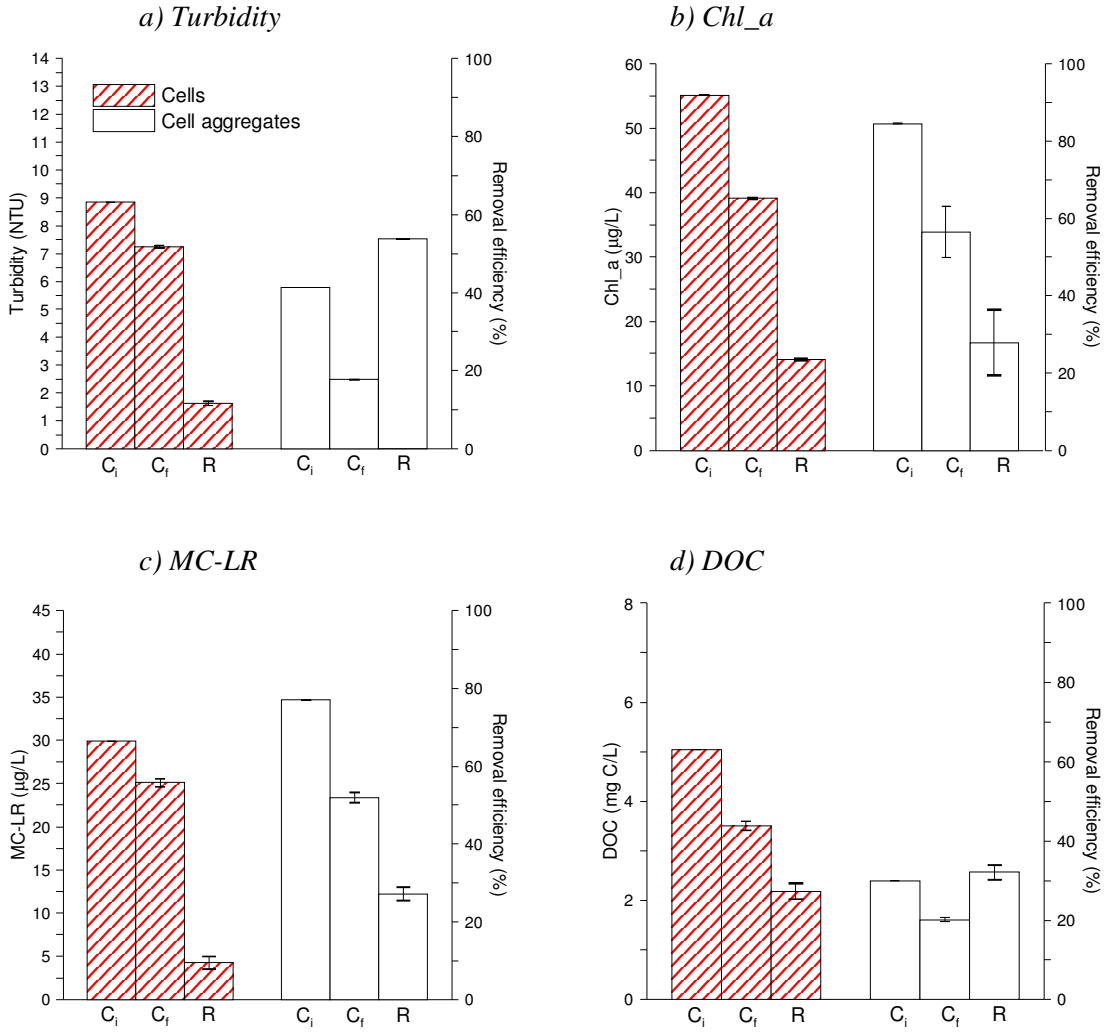

Figure 4.5 DAF performance in the removal of *M. aeruginosa* cultured single cells and cell aggregates from TW (C_i , C_f are the initial and the final concentrations, respectively; R is the removal efficiency).

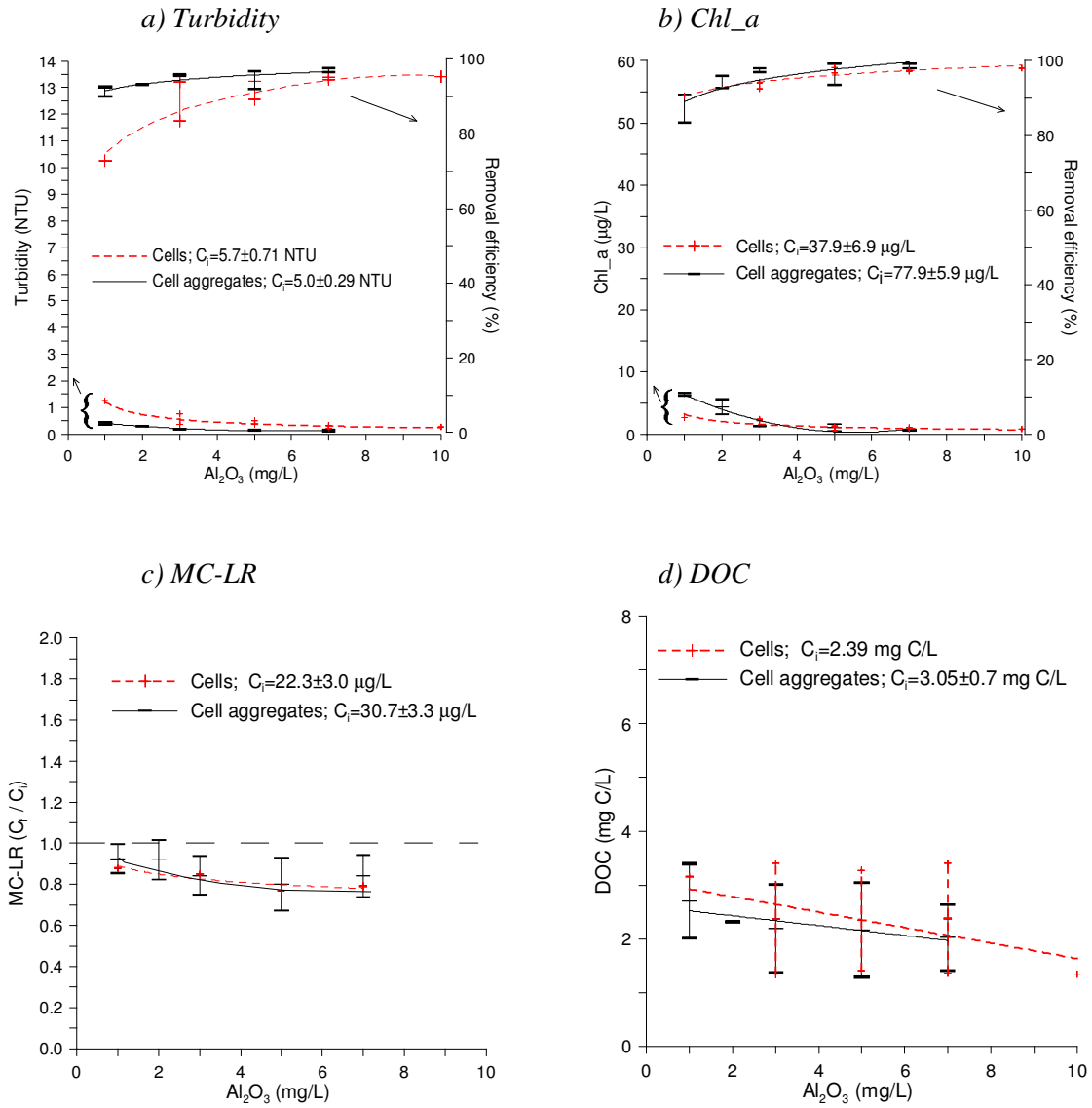

Figure 4.6 C/F/DAF performance in the removal of *M. aeruginosa* cultured single cells and cell aggregates from TW (C_i , C_f are the initial and the final concentrations, respectively).

M. aeruginosa results demonstrate that for the same chl_a concentration, cell aggregation affects turbidity, which is the usual on-line parameter of the unit operation for solid liquid separation. When cell aggregates are present, turbidity is lower for the same chl_a concentration (more evident in the experiments using TW, Figures 4.5, 4.6). Since the method used to quantify chl_a demands sample filtration, more cells are quantified when aggregation

takes place because the concentration is higher than when single cells are present. Therefore, in naturally occurring cyanobacterial blooms, chl_a is a better indicator for the overall removal efficiency of cyanobacteria.

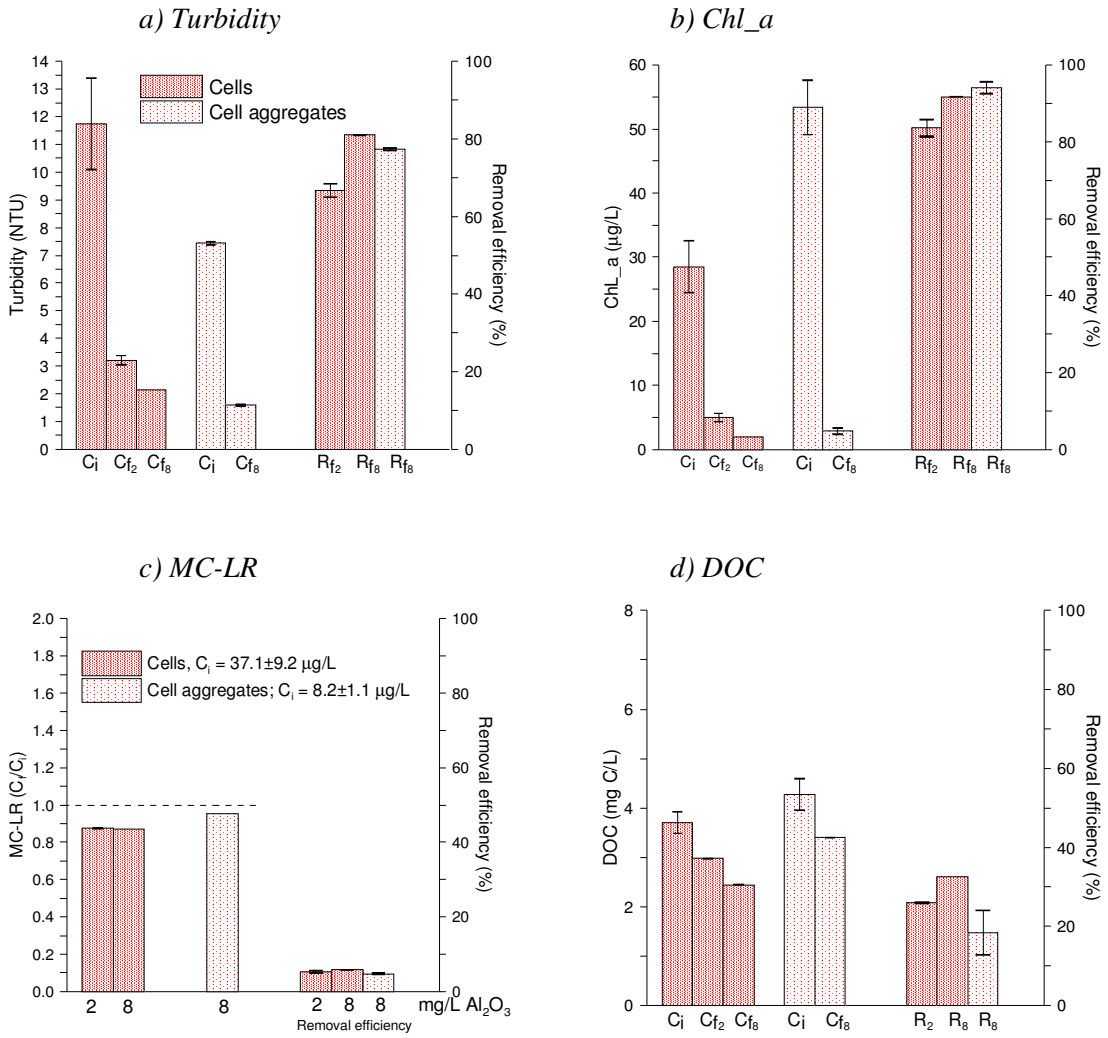

Figure 4.7 C/F/DAF performance in the removal of *M. aeruginosa* cultured single cells and cell aggregates from RW (C_i and C_f are the initial and the final concentrations, respectively with 2 and 8 mg/L Al_2O_3 of coagulant; R is the removal efficiency).

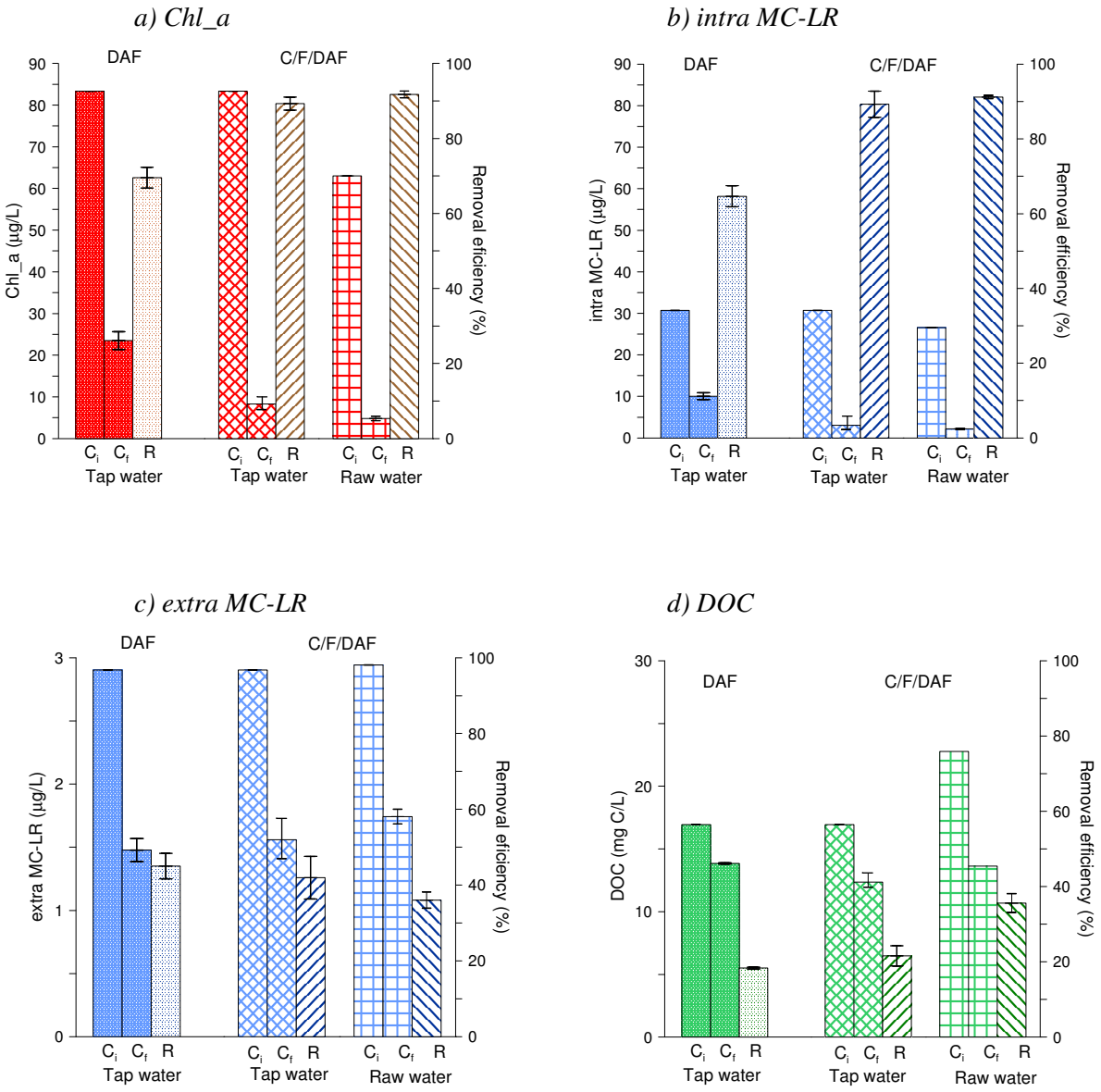

Figure 4.8 Results of DAF and C/F/DAF experiments on removing *P. rubescens* filaments.

Results also show relatively high *P. rubescens* removal efficiencies achieved by DAF, higher than those observed with *M. aeruginosa* cells (70% of chl_a removal for *P. rubescens* in TW, Figure 4.8a, *vs.* 24% of chl_a for *M. aeruginosa* in TW, Figure 4.5b). In fact, *P. rubescens* is a filamentous cyanobacteria, producing long filaments, whereas *Microcystis sp* cells usually have diameters between 3 and 7 µm (Yan and Jameson (2004)). Thus, the collision efficiency between the cyanobacterial filaments and the air bubbles in flotation should be higher than in the *M. aeruginosa*, and *P. rubescens* is better removed by DAF (without coagulant addition).

When using the C/F/DAF process the removal efficiency increases significantly, *ca.* 90-98% (as chl_a) for *M. aeruginosa* (Figure 4.6b) and 89% for both chl_a and intra-MC-LR for *P. rubescens* (Figures 4.8a, 4.8b). Since cyanobacterial stability is related to electrostatic repulsive interactions, steric effects and adsorbed macromolecules or extracellular organic matter (Edzwald (1993)), particle destabilisation is very important and coagulation conditions that produce flocs or particles of little or no charge should be provided for efficient DAF (Malley and Edzwald (1991), Ribau Teixeira and Rosa (2005), chapter 2). C/F therefore enhances cyanobacteria removal by DAF and better residuals are achieved. The same explanation based on the cyanobacterial size and subsequent collision efficiency may be addressed to the higher C/F/DAF removal efficiencies achieved with *P. rubescens* when compared with *M. aeruginosa*.

For *M. aeruginosa* cell, aggregation seemed not to significantly influence the particle removal nor the coagulant dose added, when coagulation is already optimised (Figures 4.6a, 4.6b, 4.6d, and Figures 4.7a, 4.7b, 4.7d). The increase observed in the chl_a removal from 92 to 94% in TW and 92 to 95% in RW (respectively, with 2 mg/L Al_2O_3 for TW and 8 mg/L Al_2O_3 for RW) is not significant to conclude about the effect of the aggregation in the overall efficiency, particularly because the influent cell concentration (as chl_a) was higher for the cell aggregates suspension compared to the single cells. As previously reported (Ribau Teixeira and Rosa (2005), chapter 2), the increase in the influent concentration promotes the cyanobacterial removal. However, when the C/F pre-treatment is not used, cell aggregation has an obvious positive impact on the cyanobacterial removal (Figures 4.5a, 4.5b, 4.5d). Aggregation increases the attachment opportunities between particles (cyanobacteria) and air bubbles when aggregates are present, being best transported to the water surface. As

mentioned by Vlaski *et al.* (1996) single cells regularly penetrate treatment processes and are encountered in treated water.

For *P. rubescens*, intra MC-LR removal efficiencies match the chl_a removals and they are high, particularly for C/F/DAF process (respectively 89% and 90% in TW, and 91% and 92% in RW, Figures 4.8a and 4.8b). Again, the high removal efficiencies of cyanobacterial mass are related with the high influent concentrations both in TW and RW (Kempeneers *et al.* (2001), Ribau Teixeira and Rosa (2005), chapter 2). Yan and Jameson (2004) observed no significant differences in the flocculation behaviour of the two types of cyanobacteria: *M. aeruginosa* (individual spherical cells or colonies) and *A. circinalis* (filamentous), but the initial cyanobacterial concentrations were not specified so presumably they had the same conditions for both cultures.

As with *M. aeruginosa* (Figures 4.6c, 4.7c), with *P. rubescens* there is no release of toxins to water and no significant difference is observed between C/F/DAF and DAF for removing extra MC-LR, *ca.* 43% in TW for both processes and 36% for C/F/DAF with RW (Figure 4.8c). Such unexpected removal efficiencies are much higher than those obtained with *M. aeruginosa* single cells or even with cell aggregates (Figures 4.6c, 4.7c, and Velzeboer *et al.* (1995), Hrudey *et al.* (1999), Drikas *et al.* (2001)), and may be related to dissolved toxin adsorption onto cell filaments of *P. rubescens* and/or onto DOC substances, but it needs further investigation. In fact, DOC initial concentration of TW after spiking with *P. rubescens* filaments (17.0 mg C/L) is much higher than the initial DOC concentration of TW spiked with *M. aeruginosa* cells and cell aggregates (2.1 – 3.9 mg C/L). This is due to the growth medium contribution of the *P. rubescens* culture and represents a different scenario from the natural waters with *P. rubescens* blooms. Despite this high initial concentration, in both processes

when using TW, DOC removals are similar to those found for *M. aeruginosa* cells experiments (20%, Figure 4.6d) and slightly lower for DAF (18%, Figure 4.8d) than for C/F/DAF (19 – 24%, Figure 4.8d).

Coagulation mechanisms for removing cyanobacteria may also be discussed based on TW and RW results. Bernhardt and Clasen (1991) reported that coagulation of algal cells that are smooth and more or less spherical occurs largely by charge neutralisation, while filamentous algae, large algae or species with bristles on their cell surface could be dealt by sweep coagulation. However, for WAC pre-polymerised coagulant, coagulation is mostly by charge neutralisation (Koether *et al.* (1997)). Pre-polymerisation of the metal salt coagulant is principally to enhance the charge interaction mechanism of colloid destabilisation as a consequence of the slowing down the hydrolysis of the metal salt (Jiang and Graham (1996)). From these results it seems that the main mechanism is charge neutralisation for all the studied cyanobacterial morphologies. For *P. rubescens*, RW has similar DOC concentration but lower cyanobacterial concentration than TW (22.8 mg C/L and 63.0 µg/L chl_a for RW, and 17.0 mg C/L and 83.3 µg/L chl_a for TW), because of the experimental problems related with spiking similar volumes of *P. rubescens* filaments. Therefore, for the same coagulant dose added to water, similar to higher cell removal is achieved for waters with lower concentration (RW) (Jiang and Graham (1996)) since less competition takes place between particle cells for the coagulant charge neutralisation. If sweep coagulation was the main mechanism, higher coagulant dose should be needed for entrapment of the filaments to achieve such high removals and increasing concentration of compounds in the water requires no compulsory increasing of the coagulant dose (Jiang and Graham (1996)). However, the mechanism involved in the *P. rubescens* and DOC removal needs further investigation, e.g. by increasing the coagulant dose.

4.4 CONCLUSIONS

The present study investigated the removal of different cyanobacterial morphologies by DAF from clear (TW) and natural water (RW). Results showed that C/F enhances DAF efficiency due to the need of particle destabilisation and subsequent flocculation. When using DAF, without coagulant addition, higher removals of *M. aeruginosa* cell aggregates and *P. rubescens* filaments were obtained compared with *M. aeruginosa* cells. Both processes showed high cell removal efficiencies for all cyanobacterial morphologies (86 – 93% of intra MC-LR *vs*. 88 – 98% of chl_a), together with no release of MC-LR to water, with the operating conditions tested. For the filamentous *P. rubescens* significant extra MC-LR removal (34 – 48%) was obtained. These high extra MC-LR removal efficiencies were attributed to the adsorption onto DOC substances and/or filaments since DOC initial concentrations were very high due to growth medium contribution. *P. rubescens* results with RW were quite similar to those obtained with tap water, due to the high initial DOC concentrations which represents a different scenario from the natural waters with *P. rubescens* blooms. It seemed that with a pre-polymerised coagulant the main coagulation mechanism for cyanobacterial removal is charge neutralisation. C/F/DAF was as much or even more efficient for removing *P. rubescens* filaments (89% in TW – 92% in RW as chl_a) than *M. aeruginosa* cells or cell aggregates (84% in RW – 93% in TW), probably due to its higher initial concentrations and experimental limitations.

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CHAPTER 5
THE ROLE OF MEMBRANE CHARGE ON NANOFILTRATION PERFORMANCE

ABSTRACT

The zeta potential along the surface and through the pores of a commercial nanofiltration membrane was studied with several electrolyte solutions (including monovalent and divalent hardness ions, KCl, $CaCl_2$ and $MgSO_4$) to investigate the influence of salt type and pH on the charge of the membrane surface and in the membrane pores. The membrane negative charge increased with increasing pH, the membrane surface being more negatively charged than the pores, but having the same isolectric point (pH 4.2 ± 0.2). The membrane was less negatively charged in the presence of divalent salts. The membrane performance evaluated in terms of flux and retentions showed an inverse behaviour: flux decreased with pH, whereas retention increased. Results were explained by membrane charge variation and its effect on membrane pore size and by electroviscous and osmotic effects. For higher salt concentrations (higher ionic strength) flux and retention decrease, this decrease being more pronounced for the highly rejected $MgSO_4$ salt. Negative proton retentions were obtained at low pH due to the behaviour of the more mobile co-ion H^+ in a mixture of electrolytes.

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5 THE ROLE OF MEMBRANE CHARGE ON NANOFILTRATION PERFORMANCE

5.1 INTRODUCTION

Separation in nanofiltration primarily occurs due to steric hindrance and membrane solute interactions (Mulder (1997), Rosa and de Pinho (1994), Nyström *et al.* (1995), Rosa (1995), Chaufer *et al.* (1996), Schaep *et al.* (1998), Peeters *et al.* (1999), Childress and Elimelech (2000)). Steric hindrance and non-electrostatic membrane-solute interactions (*e.g.* Van-der-Waals forces) are mostly responsible for the retention of uncharged molecules, and their transport takes place by convection due to a pressure difference and by diffusion due to a concentration gradient across the membrane (Rosa and de Pinho (1994), Rosa (1995), Chaufer *et al.* (1996), Mulder (1997)). In addition, neutral molecules also interact with membrane charge, mainly through polarity effects. As found by Van der Bruggen *et al.* (1999), polarity decreases retention which can be explained by electrostatic interaction directing the dipole towards the membrane. For charged compounds both steric hindrance and electrostatic interactions are responsible for separation (Nyström *et al.* (1995), Chaufer *et al.* (1996), Mulder (1997), Schaep *et al.* (1998), Peeters *et al.* (1999), Childress and Elimelech (2000)). Another important parameter in the transport process through the membrane is the membrane charge along the surface and through the pores (Childress and Elimelech (2000), Schaep and Vandecasteele (2001)). Membranes in contact with an aqueous solution acquire an electric charge by some mechanisms: dissociation of surface functional groups, adsorption of ions from the solutions, and adsorption of polyelectrolytes, ionic surfactants and macromolecules (Elimelech *et al.* (1994)). This charging mechanism can take place on the exterior membrane surface and on the interior pore surface of the membrane, because of the distribution of ions in solution to maintain the electroneutrality of the system (Schaep and Vandecasteele (2001)). The ion separation resulting from the electrostatic interactions between ions and membrane

surface charge is based on the Donnan exclusion mechanism (Schaep *et al.* (1998), Childress and Elimelech (2000)). In this mechanism the co-ions (which have the same charge of the membrane) are repulsed by the membrane surface and to satisfy the electroneutrality condition, an equivalent number of counter-ions is retained which results in salt retention.

Additionally, solution chemistry of natural waters, in particular pH and hardness cations, has a significant effect on the membrane charge and on the characteristics of the molecules in the solution. The pH protonate and deprotonate the functional groups of the membrane and of the molecules in solution, over its range. This will change the membrane charge and the size of the membrane pores with consequences in the NF and UF performance (Oak *et al.* (1997), Childress and Elimelech (2000), Schaep and Vandecasteele (2001), Ribau Teixeira and Rosa (2002)). For hardness cations interactions with the NF membranes will take place, so they could have a marked effect on fouling and NF performance (Childress and Elimelech (1996)). Because of these complex interactions that could develop between the membrane and the inorganics and organics in solution, the determination of the membrane charge and particular its variation with the ionic composition of the water is essential to understand the fouling mechanisms and to increase the membrane performance (Childress and Elimelech (1996)).

The objective of this work is to investigate the role of membrane charge on NF performance to produce drinking water from moderately hard natural waters (300-400 μS/cm). NF experiments and streaming potential measurements were carried out with several electrolytes at different pH values. The chemical species selected include monovalent ions (K^+, Cl^-), divalent hardness cations (Ca^{2+}, Mg^{2+}) and divalent anion (SO_4^{2-}).

5.2 MATERIAL AND METHODS

5.2.1 MEMBRANE AND CHEMICALS

The NFT50 membrane investigated is a thin film composite RO/NF membrane of polypiperazine amide on a polysulphone microporous support and a polyester support, from Alfa Laval, with a retention of magnesium sulphate higher than 99% (according to the manufacturer). Polypiperazine contains carboxylic and amine functional groups (Her *et al.* (2000), Schaep and Vandecasteele (2001)).

Neutral solutes of increasing molar mass were used for membrane characterisation, namely ethanol, glycerol, DL-phenylalanine, D-glucose, sucrose, raffinose (pentahydrate) and PEG 1500. All reagents were *pro analysis* grade from Merck.

In the permeation experiments using electrolytes, certified analytical grade potassium chloride (KCl), calcium chloride ($CaCl_2$) and magnesium sulphate ($MgSO_4$) salts were used. KOH and HCl were used for adjusting the solution pH. The water conductivity was below 1 μS/cm.

5.2.2 ANALYTICAL METHODS

The organic solutes were analysed for total organic carbon using a Shimadzu total organic carbon analyser, model TOC 5000A. The salts were analysed by conductivity using a Crison GLP 32. The pH was analysed in a Whatman WTW pH340 at 25ºC and the permeate fluxes were determined by weight (analytical balance Shimadzu, model BX 620S).

5.2.3 STREAMING POTENTIAL MEASUREMENTS

The streaming potential measurements through membrane pores were carried out in a flat-sheet flow module made of polycarbonate (Nyström *et al.* (1994), Pihlajamäki (1998)) to suit

a membrane sample area of 21.6 cm^2, fitted with reversible silver / silver chloride electrodes above and below the membrane.

The streaming potential measurements along the surface were performed in a module where two pieces of the membrane to be investigated were attached into the module and they were separated from each other by a gasket (height 0.1 mm, Pihlajamäki and Nyström (1995)). The normal passage of permeate was closed so that the electrolyte solution could only flow through this channel. The electrodes were placed close to the ends of the flow channel (Pihlajamäki and Nyström (1995)).

Prior to measurements, a new membrane was washed by circulating deionised water (conductivity < 1 μS/cm) for half an hour without pressure. The membranes were stabilized at a constant pressure (1 bar) with the electrolyte solution. The pH was adjusted in the range 3.7 – 8.3, by adding small amounts of KOH and HCl. Temperature was maintained at 25 ºC using a water bath. Streaming potential was measured at five different pressures, in the range of 0.2 – 1 bar.

Streaming potential measurements were performed with KCl (1 mM), $CaCl_2$ (0.1 mM and 1 mM) and $MgSO_4$ (1 mM).

The apparent zeta potential, ξ, was determined from the slope of the ΔE (streaming potential) *versus* ΔP (pressure) plots using the Helmholtz-Smoluchowski equation (Smoluchowski (1905)):

$$\xi = \frac{\Delta E}{\Delta P} \frac{\eta \kappa}{\varepsilon_o \varepsilon_r}$$

where η and κ are the viscosity of the permeate and the conductivity of the solution, respectively, ε_o is the permittivity of vacuum and ε_r the dielectric constant of the medium.

5.2.4 NANOFILTRATION EXPERIMENTS

The performance of the NFT50 membrane was evaluated using a plate-and-frame unit, Lab-unit M20, from Danish Separation Systems (membrane area of 0.0360 m^2 up to 0.720 m^2; maximum pressure 80 bar; maximum flow 18 L/min and constant temperature maintained by a heat exchanger).

In this work one pair of membranes (360 cm^2) was tested. The permeate and the concentrate were both recycled to the feed tank. The transmembrane pressure was 10 bar, controlled by a back-pressure regulator and monitored with pressure gauges (at the entrance and at the exit of the module). The circulating velocity was 8 L/min (value recommended by the manufacturer as necessary for concentration polarisation control, corresponding to a Reynolds number of 965) and the temperature was maintained at 25 ºC during the experiments. In all runs, a stabilisation time of 10 minutes was used.

After installing the membranes in the plate-and-frame module and prior to the nanofiltration experiments, the membranes were compacted during 12 h by permeating deionised water at 30 bar.

After compaction, the membrane was characterised both in terms of hydraulic permeability and selectivity (*i.e.* cut-off (g/mol)). The membrane hydraulic permeability was determined through the permeation of pure water at transmembrane pressures ranging from 5 to 25 bar (with 5 bar increments). Membrane cut-off was obtained by permeating dilute solutions (*ca.*

30-50 mg/L) of neutral solutes of increasing molar mass, in the range 46 − 1500 g/mol (section 6.2.1).

NF performance with the electrolytes KCl (1 mM), $CaCl_2$ (0.1 mM, 1 mM, 10 mM) and $MgSO_4$ (1 mM, 10 mM) was first evaluated at natural pH (pH ≈ 6.2), and with a mixture of KCl (1 mM) and $CaCl_2$ (1 mM) (natural pH ≈ 5.3). The solution pH was then adjusted to higher or lower values.

Between each NF run, membranes were washed until the pure water flux reached 90% of the initial value measured after compaction and the bulk conductivity was similar to that of deionised water. Each value presented is the average of triplicate runs.

5.3 RESULTS AND DISCUSSION

5.3.1 MEMBRANE CHARACTERISATION

The water flux measurements showed that the flux increases linearly with the transmembrane pressure yielding a hydraulic permeability of 5.9 kg/(h·m^2·bar) at 25 ºC (R^2 = 0.9995). The permeate flux of the neutral solutes varied between 98.5% and 100% of the pure water flux. As expected due to the very dilute solutions and the hydrodynamics in the feed chamber (Re 965), those values indicate little to no concentration polarisation, so the apparent retention is similar to the intrinsic retention.

Figure 5.1 shows the sieving curve obtained with the neutral solutes and the corresponding pore radius obtained by curve-fitting. The sieving curve indicates that this is a tight NF membrane with a cut-off of 150 g/mol and an effective pore radius of 0.43 nm. The curve-

fitting of log(f/(1-f)), where f is defined as retention, *vs.* solute molar mass was intersected by the 91% retention line (log(0.91/(1-0.91))=1) and yielded the cut-off (Figure 5.1b) (Rosa (1995), Rosa and de Pinho (1995)). The membrane pore radius was determined by fitting the experimental data of dilute solutions of organic neutral solutes by an integrated transport model developed by Rosa and de Pinho (Rosa and de Pinho (1994), Rosa (1995)) for ultrafiltration and nanofiltration. This model integrates the mass transfer in the fluid phase adjacent to the membrane (not important for these experimental data since there is little to no concentration polarisation, as previously discussed) with the transport phenomena through the membrane described by a steric pore flow model (SPFM). In this SPFM, the effective pore radius is an overall parameter related to the membrane morphological structure (*i.e.* polymer molecular arrangement) responsible for the diffusive and convective steric hindrances and for the membrane-solute-water chemical interactions. This is a simplified approach to transport in NF membranes to easily calculate an affective membrane pore radius, although in reality there is a pore size distribution in NF membranes.

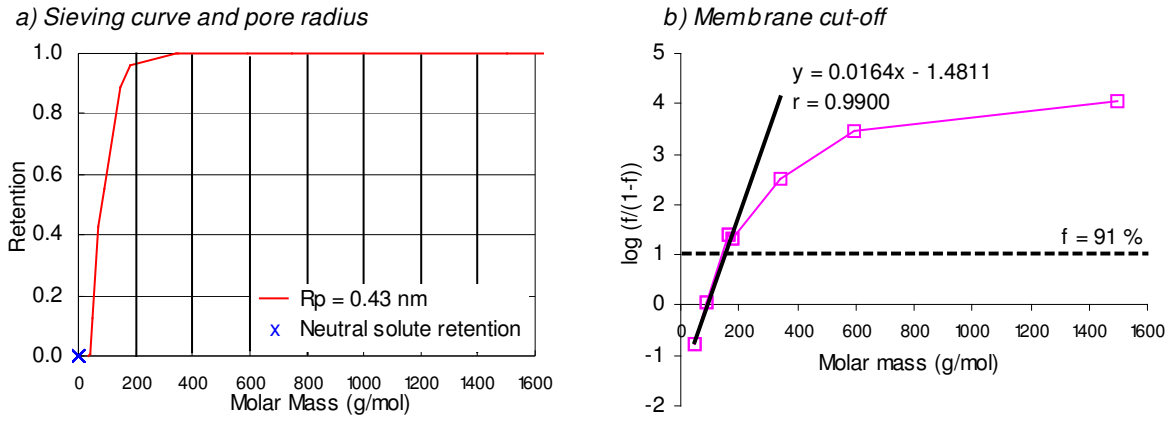

Figure 5.1 a) Sieving curve of NFT50 membrane and membrane effective pore radius obtained by curve-fitting using the SPFM, and **b)** Determination of molecular weight cut-off.

5.3.2 STREAMING POTENTIAL MEASUREMENTS

The experimental data for calculating the membrane zeta potential along the surface and through the pores in the pH range 4.0-8.3, with the electrolytes 1 mM KCl, 0.1 mM $CaCl_2$, 1 mM $CaCl_2$ and 1 mM $MgSO_4$ are shown in Figure 5.2.

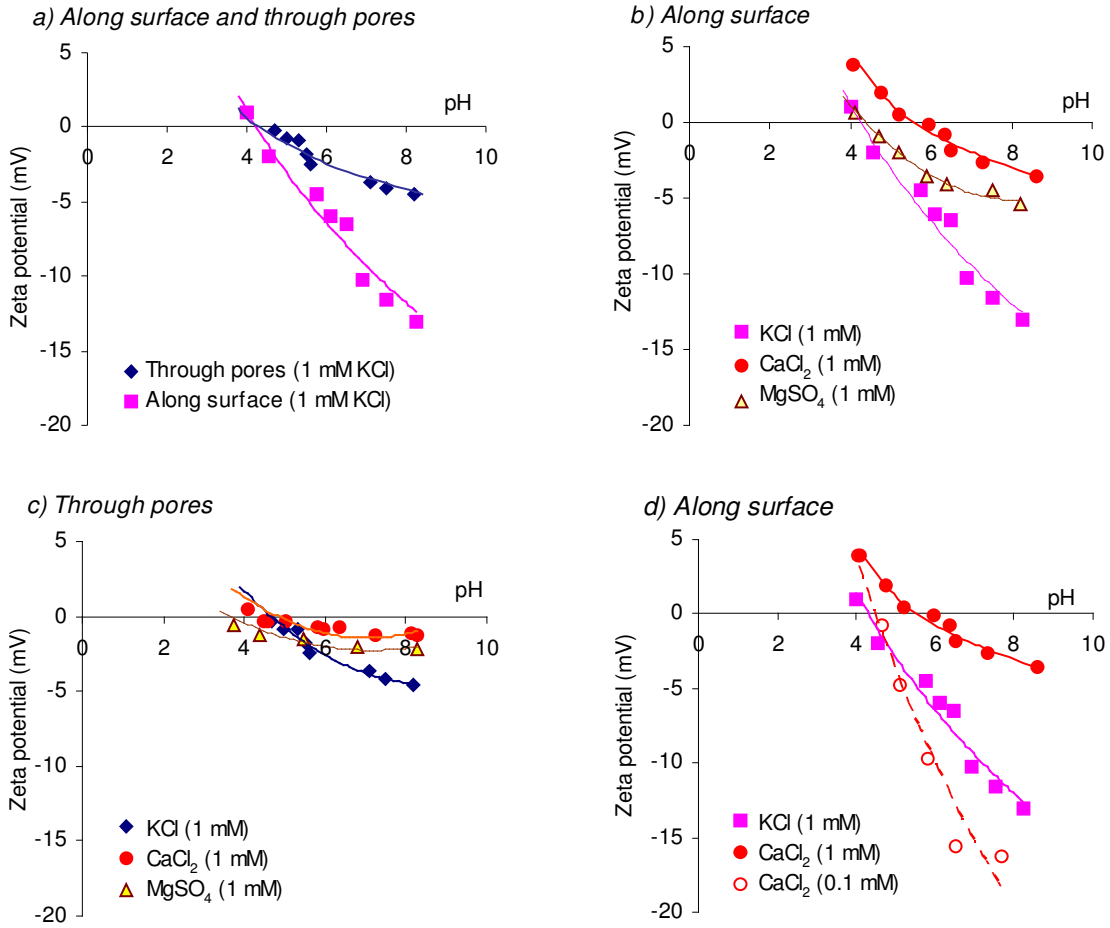

Figure 5.2 Streaming potential measurements in the pH range 4.0-8.3: **a)** along the surface and through the pores for clean membranes, **b)** along the surface in the presence of divalent cations Ca^{2+} and Mg^{2+}, **c)** through the pores in the presence of divalent cations Ca^{2+} and Mg^{2+}, and **d)** along the surface in the presence of two concentrations of $CaCl_2$.

Results show that the membrane surface has a slightly positive ξ-potential at the lowest pH (pH 4), passes through an isoelectric point (i.e.p.) at pH 4.2 ± 0.2 and is negatively charged above this pH. The surface and the pores are negatively charged in the pH range 4.4 to 8.3 and their negative charge increases with the pH. Both measurements (along surface and through

pores) give the same i.e.p., pH ~ 4.2 ± 0.2, but the membrane surface is more negatively charged than the pores (Figure 5.2a).

ξ-potential curves of this shape are characteristic of amphoteric surfaces, or surfaces with both acidic and basic functional groups, carboxylic and amine groups, respectively, in NFT50 membrane. If the membrane has an overall neutral behaviour (*i.e.* acidic and basic groups are equivalent), the membrane could vary between positively charged at acid pH, slightly positively charged to neutral at acid-neutral pH (i.e.p. - ΔpH), neutral to slightly negatively charged at neutral-basic pH ((i.e.p. + ΔpH) and negatively charged at basic pH. For the plateau of zero charge (i.e.p. ± ΔpH), membrane has no net charge, it behaves like a nonpolar surface to which anions (Cl^- and/or OH^-) can adsorb. According to Elimelech *et al.* (1994), anions can approach more closely to nonpolar or hydrophobic surfaces because they are less hydrated than cations. In this process a surface will acquire a negative electrokinetic potential due to the presence of anions beyond the plane shear. If the membrane has an overall acidic behaviour (*i.e.* more acidic than basic (amine) groups), the membrane could vary between positively charged at very acid pH, neutral at acid pH, negatively charged at acid-neutral pH and very negatively charged at basic pH. In both cases (overall neutral behaviour or acidic behaviour), the membrane surface charge is positive at acid pH (charge decreases with the pH increase) and is negative at basic pH (charge increases with the pH increase).

Figure 5.2b presents zeta potential *vs.* pH for 1 mM $CaCl_2$ and 1 mM $MgSO_4$ along the surface. As found by other authors with NF membranes (Childress and Elimelech (1996), Peeters *et al.* (1999), Hagmeyer and Gimbel (1999)) for NFT50 membrane the divalent ions have an important effect on the surface charge (Figure 5.2b). In the presence of divalent cation (Ca^{2+}), the membrane is more positively charged over the entire pH range and the i.e.p.

shifts from 4.2 to 5-6. Since the NFT50 membrane is negatively charged above pH 4.2, complex formation of the Ca^{2+} ion with the membrane is electrostatically favourable (Childress and Elimelech (1996)). The Ca^{2+} adsorption on the membrane surface reduces its negative charge, yielding a net positive charge for pH below 5-6. On the other hand, when both divalent cations (Mg^{2+}) and anions (SO_4^{2-}) are present in solution, the effect of the divalent anion is opposite to the effect of the divalent cation (Childress and Elimelech (1996)), Mg^{2+} adsorbs less than Ca^{2+}, therefore the zeta potential curve is intermediate between the curves obtained for KCl and $CaCl_2$. In fact, the ionic strength increases from KCl solution to both $MgSO_4$ and $CaCl_2$ electrolytes. The increase of the ionic strength produces a shielding effect responsible for the decrease of the membrane negative charge along the surface.

This effect is well illustrated in Figure 5.2d where the ionic strength increases from $CaCl_2$ 0.1 mM to KCl 1 mM and to $CaCl_2$ 1 mM. The same phenomena discussed for the membrane surface apply to the pores (Figure 5.2c) but in a much less extension due to steric hindrance for ion penetration through the membrane pores, particularly important for divalent anions and cations.

5.3.3 NF PERFORMANCE

5.3.3.1 MONO AND DIVALENT SALTS

Figure 5.3 shows a flux decrease and a retention increase with the pH for KCl and the divalent salts $CaCl_2$ and $MgSO_4$. The flux decrease is higher for divalent salts than for monovalent salt and there is no significant difference between $CaCl_2$ and $MgSO_4$. According to Nyström *et al.* (1995) and to Elimelech and co-workers (Childress and Elimelech (1996), Faibish *et al.*

(1998), Childress and Elimelech (2000)), this behaviour may be caused by several mechanisms: membrane pore size, electroviscous effect and osmotic pressure gradient.

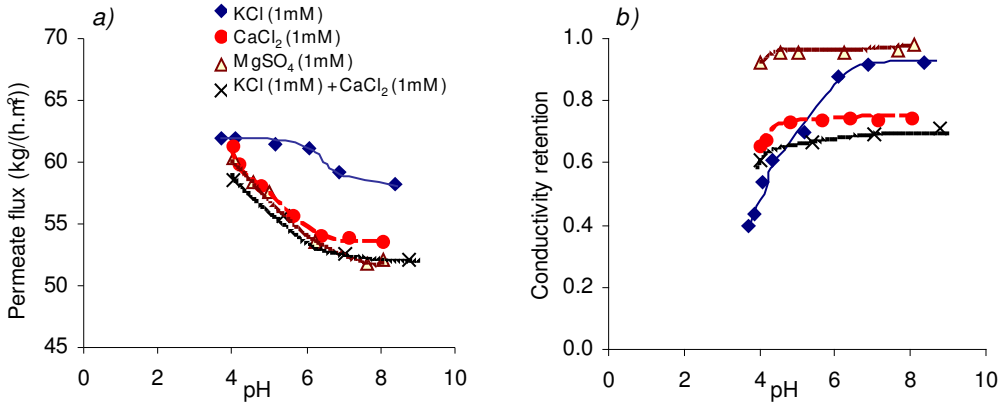

Figure 5.3 Flux **(a)** and conductivity retention **(b)** of NFT50 membrane as a function of pH for KCl, $CaCl_2$ and $MgSO_4$ (1 mM).

The pore size of the membrane is reduced at high pH values because the negatively charged groups on the membrane pore surface (Figure 5.2c) adopt an extended conformation due to electrostatic repulsion between them. This expanded conformation reduces the pore size (or pore volume) of the membrane and causes a decrease in flux and a retention increase (Oak *et al.* (1997), Childress and Elimelech (2000), Schaep and Vandecasteele (2001), Ribau Teixeira and Rosa (2002)). This effect is particularly important for KCl, for which the zeta potential varies significantly through the pores; on the contrary, the zeta potential through pores in the presence of divalent salts ($CaCl_2$ and $MgSO_4$) is almost constant with pH, (slightly) negative in the entire pH range (Figure 5.2c). Figure 5.4 proves this effect of pore narrowing in the presence of $CaCl_2$ and, particularly, KCl since retention of neutral solutes increases in the presence of these electrolytes, being higher for KCl. The observed differences are small due to the solution pH (5.3) which is still close to membrane i.e.p. and far from the maximum membrane negative charge.

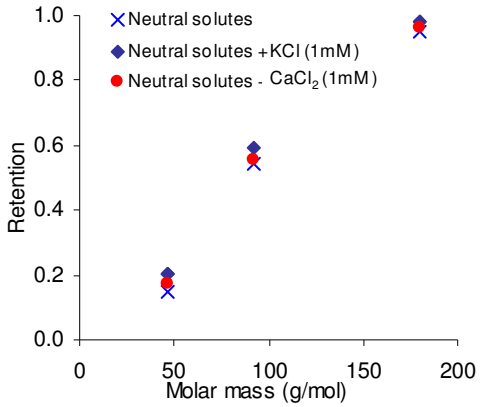

Figure 5.4 Retention of neutral solutes in the presence of KCl and $CaCl_2$ (1 mM, pH ≈ 5.3).

The osmotic pressure near the membrane surface increases at high pH because of the high retentions. The increase in osmotic pressure results in a decrease in net driving pressure which leads to a decrease in water flux (operating pressure was kept constant). This effect is particularly important for the divalent salts, highly rejected by the membrane.

For all salts, retention increases with pH following the increase of membrane negative charge (Figure 5.3b). Around the i.e.p., the membrane is uncharged, there is no electrostatic repulsion, the ion transport is hindered by the size of the ions and salt retentions are low (probably to the minimum value). At pH *ca.* 5 the membrane pore is weakly negatively charged (Figure 5.2c) and the retention order is: KCl < $CaCl_2$ < $MgSO_4$. Since membrane negative charge is very weak, there is no change in the conformation of the pores as explained above, so the observed retention order is consistent with the size of the salts. At pH above 5, the membrane exhibits a negative charge (Figure 5.2) which causes Cl^- and SO_4^{2-} co-ion repulsion leading to high retentions. In addition, the membrane ξ-potential for KCl is more negative than for $CaCl_2$ and $MgSO_4$ (Figure 5.2b and 5.2c), so the extended conformation of the pores (that reduces the pore size) will increase the retention of the KCl. Besides this, the obtained retentions $MgSO_4$ > KCl > $CaCl_2$ follow the Donnan exclusion mechanism. The

lower retentions of $CaCl_2$ compared to KCl correspond to the increasing of the order of cation charge density, *i.e.* the attraction forces on the cations become progressively stronger. On the other hand, the higher $MgSO_4$ retentions compared to KCl correspond to the increase of the anion charge density, *i.e.* the anion repulsion forces become stronger.

When the electrolyte mixture of KCl and $CaCl_2$ is used, both flux and retention decrease compared to values obtained with single solutions (Figure 5.3), due to the increase of the electrolyte concentration responsible for higher ionic strength and stronger attraction forces of the cations (as further studied below). However, the curve shape is very similar to the one found for single $CaCl_2$, which emphasises the importance of the Ca^{2+} adsorption onto the membrane surface on the membrane overall performance.

The variation of retentions and the membrane ξ-potential (along surface and through the pores) have opposite shape with pH: i) for $CaCl_2$ and $MgSO_4$ membrane charge does not increase significantly with pH above 5 and retentions remain constant; ii) for KCl the negative membrane charge increases with pH as well as the retentions.

Figure 5.5 shows a negative retention of H^+ ions when the membrane is positively charged, *i.e.* at pH below 4.0 for KCl and $MgSO_4$ electrolytes and at pH ranging from 5 to 6 for $CaCl_2$ (Figure 5.2b). Negative proton retentions have been referred by some authors (Hagmeyer and Gimbel (1999), Childress and Elimelech (2000), Tanninen and Nyström (2002)) and were attributed to the higher mobility of the H^+ co-ions compared to other cations in solution (the membrane is positively charged).

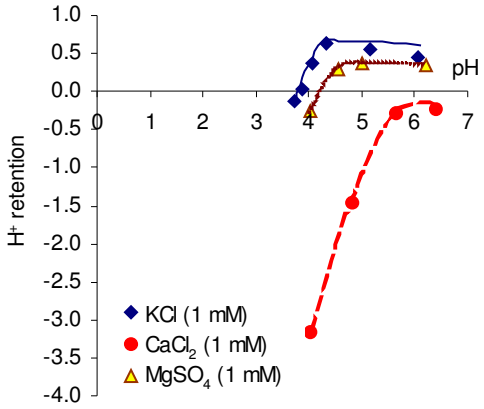

Figure 5.5 Retention of H^+ as a function of pH.

5.3.3.2 DIVALENT SALT CONCENTRATION

Figure 5.6 shows the effect of divalent salt concentration on the NF performance at different pH values.

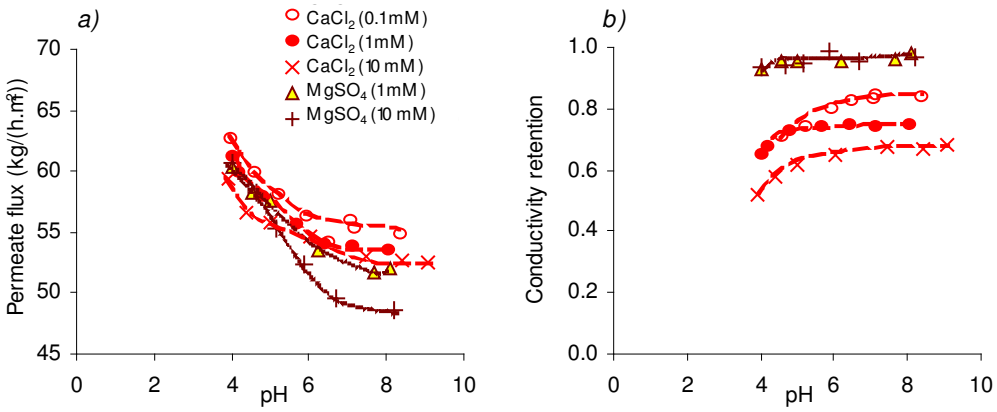

Figure 5.6 Flux **(a)** and conductivity retention **(b)** of NFT50 membrane as a function of pH at different concentrations of $CaCl_2$ and $MgSO_4$.

As expected due to osmotic effects, higher concentrations yield higher flux decrease, this effect being more pronounced for the highly rejected $MgSO_4$ salt.

The salt retentions decrease as the electrolyte concentration increases. This can be explained by considering the thickness of the double layer formed in the pores of the top-layer when the membrane is contacted with the salt solution. The increasing salt concentration (higher ionic

strength) produces a decrease of the thickness of the double layer, resulting in a lower retention. With increase in salt concentration the shielding effect is stronger, leading to a decrease of the membrane repulsion forces of the anions. As reported in similar studies (Afonso and de Pinho (2000), Labbez *et al.* (2002)), these effects are more evident for Cl^- ions than for SO_4^{2-}, since the latter has a higher charge density, being rejected by the membrane even at high concentrations.

5.4 CONCLUSIONS

This study demonstrates the importance of divalent hardness salts ($CaCl_2$ and $MgSO_4$) on the NF performance of natural waters.

Streaming potential measurements along surface and through pores were made with several electrolyte solutions at different pH to investigate the charge of the membrane surface and pore. The results obtained fully explain the NF performance with pH evaluated in terms of both flux and retentions. The maximum flux and minimum retention were obtained at the i.e.p. (4.2±0.2), for uncharged membrane. With the pH increase, the membrane negative charge increased, therefore the flux decreased whereas the retention increased. For divalent hardness ions ($CaCl_2$ and $MgSO_4$), the membrane was less negatively charged and the flux decreased further. The shape of the salt retention curves corresponded to the shape of the membrane charge curves, *e.g.* for $CaCl_2$ electrolyte and pH above i.e.p., when the membrane was less negatively charged, the electrolyte retention presented the lowest values. The results were explained by membrane pore size, electroviscous effect and osmotic pressure mechanisms.

The divalent salt concentration increase produced a retention decrease, which was related to a higher ionic strength of the more concentrated solutions and to a lower zeta potential of the membrane (the membrane was less negatively charged for solutions with higher ionic strength).

At low pH, negative proton retention was observed due to the behaviour of the more mobile H^+ co-ion in a mixture of electrolytes.

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CHAPTER 6

THE IMPACT OF THE WATER BACKGROUND INORGANIC MATRIX ON THE NATURAL ORGANIC MATTER REMOVAL BY NANOFILTRATION

ABSTRACT

The impact of the water background inorganic matrix (pH and calcium hardness) on the natural organic matter (NOM) removal by nanofiltration was investigated. Pre-treated surface water and two NOM model substances of different molecular weight and hydrophilicity (salicylic acid and purified Aldrich humic acid, 2 –3 mg C/L) were nanofiltered using a polypiperazine amide membrane. Results showed that the background inorganic matrix greatly influenced the NOM removal by a negatively charged membrane, regardless of the type of NOM, which caused no effect on the results. Flux showed no significant variation with running time and recovery rate, but it decreased with pH and especially in the presence of calcium. Such results were related to the low DOC concentration and the low flux that minimised the concentration polarisation, and to the pH effect on the membrane and NOM charges. The pH increase caused pore narrowing, larger NOM hydrodynamic radius and stronger repulsions between the negatively-charged membrane and NOM functional groups, yielding higher rejections which, in turn, increased the osmotic gradient with a subsequent

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flux decline. Flux and rejections decreased further in the presence of 1 mM Ca^{2+} which reduced the membrane negative charge, and decreased the sieving effects and increased the chemical interactions.

6 THE IMPACT OF THE WATER BACKGROUND INORGANIC MATRIX ON THE NATURAL ORGANIC MATTER REMOVAL BY NANOFILTRATION

6.1 INTRODUCTION

Membrane processes are increasingly used in drinking water treatment to meet more stringent water quality regulations. Nanofiltration (NF) is one of the membrane water treatment processes that removes multivalent ions, small hazardous microcontaminants (*e.g.*, pesticides, toxins, endocrine disruptors) and natural organic matter (NOM) from surface water (Hong and Elimelech (1997), Schäfer *et al.* (1998)). The removal of NOM from drinking water is of great importance due to its potential to form disinfection by-products when waters are disinfected with chlorine (Pomes *et al.* (1999), Siddiqui *et al.* (2000)) and to promote biofilm growth in water distribution networks. NOM is also considered one of the major cause of NF fouling during the membrane filtration of surface waters (Nyström *et al.* (1995), Nilson and DiGiano (1996), Hong and Elimelech (1997), Schäfer *et al.* (1998), Cho *et al.* (1999)).

NOM is a heterogeneous organic mixture with slightly water-soluble compounds. It is one of the main constituents of natural surface waters and may be divided into hydrophobic and hydrophilic compounds, being humic substances part of the hydrophobic compounds. Humic substances are refractory anionic polyelectrolytes (due to the dissociation of the carboxylic (and phenolic) functional groups) of low to moderate molecular weight (Stumm (1992)). At high ionic strength or low pH, humic substances have a small hydrodynamic radius in solution (more spherocolloidal) and at low ionic strength or high pH, humic substances have a large hydrodynamic radius (more linear) due to the intermolecular charge repulsion (Stumm (1992)).

NF membranes are able to effectively remove NOM through a combination of size exclusion and physical-chemical interactions as electrostatic repulsion and adsorption (Cho *et al.* (1999), Amy and Cho (1999)). The NOM fouling, that causes flux decline, may be reduced by controlling various physical and chemical parameters such as pH, ionic strength and calcium concentration as well as permeation rate and hydrodynamic conditions (crossflow velocity and channel configuration), which allow working under the critical flux (Jucker and Clark (1994), Nyström *et al.* (1995), Nilson and DiGiano (1996), Cho *et al.* (1999), Kilduff *et al.* (2004), Seidel and Elimelech (2002)). At high ionic strength and low pH or in the presence of calcium, a severe flux decline occurs due to the dense thick fouling layer that develops on the membrane surface. At low ionic strength, high pH and absence of divalent ions, a loose thin fouling layer develops on the membrane surface and a lower flux decline is obtained. Rejection of charged solutes increases due to the thick double layer nearly overlapping with the membrane pores (Jucker and Clark (1994), Braghetta *et al.* (1997)).

Membrane characteristics (membrane charge, pore size and water permeability) and NOM characteristics (molecular weight and hydrophobicity) also play an important role in the fouling process. Elimelech and co-workers (Elimelech *et al.* (1994), Childress and Elimelech (1996), Hong and Elimelech (1997)) concluded that humic substances affect the membrane charge through adsorption onto the membrane surface. The negatively charged functional groups of humic substances dominate the membrane surface charge. It is common to associate the increase in negative charge and the increase of hydrophilicity with the decrease of adsorptive fouling by NOM (Jucker and Clark (1994), Nilson and DiGiano (1996), Hong and Elimelech (1997), Kimura *et al.* (2003)).

Chellam and Taylor (2001) reported that operating conditions such as feed water recovery had a significant negative impact on the rejection of total hardness and total trihalomethanes (THM) by NF membranes. A decrease in rejection with the increase in recovery was also obtained by Reiss *et al.* (1999) for NOM and total dissolved solids.

While extensive research has been done using different sources and types of humic acids, many authors (Hong and Elimelech (1997), Schäfer *et al.* (1998), Amy and Cho (1999), Seidel and Elimelech (2002)) studied concentrations higher than those normally used in NF technology or even present in clear surface water (2 − 5 mg C/L) (EPA (1999)). In fact, NF membranes always follow a pre-treatment so the NOM content is already reduced when the water is nanofiltered (Her *et al.* (2000), Peltier *et al.* (2002)).

The aim of this paper is to study the chemical and physical aspects of NOM filtration and flux decline with NF membranes and their relation with feed water background inorganic matrix, in particular with water pH and calcium hardness. A contribution to the understanding of the mechanisms of NF performance for drinking water production from clear surface water with low NOM content and moderate hardness is intended. Natural waters and model solutions of hydrophobic high molecular weight NOM (>50 kDa, purified Aldrich humic acid) and hydrophilic low molecular weight NOM (salicylic acid) are investigated.

6.2 MATERIALS AND METHODS

6.2.1 NATURAL WATER SAMPLES

Decanted water (DW) from Alcantarilha Water Treatment Plant (WTP), Algarve, Portugal, was used in this study. DW was collected after ozonation/ coagulation (C)/ flocculation (F)/ sedimentation (S). The samples presented a low NOM content with an apparent molecular

weight mainly below 15 kDa (Ribau Teixeira (2001)). The characteristics of DW are presented in Table 6.1. Since 2000, this WTP supplies water to *ca.* half million people in southern Portugal (Algarve), and was designed to treat up to 3 m^3/s (*ca.* 1 million people by the year 2020) of surface water from the Funcho Dam reservoir (2 km^2 and 43.4 hm^3).

Based on DW specific UV absorbance (SUVA) values (0.92 L/(m.mg), Table 6.1), the dissolved organic carbon (DOC) was largely composed of non-humic materials; the organic matter was relatively hydrophilic, less aromatic, and of a lower molecular weight compared to waters with higher SUVA values (Edzwald and Van Benschoten (1990)). Such values must be due to the pre-ozonation, which may slightly decrease the contaminants concentration (their concentration is mostly removed by C/F/S), but especially decreases their size and molecular weight.

6.2.2 CHEMICALS AND NOM MODEL SUBSTANCES

Deionised water (DI) was used for the preparation of all stock solutions and membrane performance experiments. Table 6.1 presents the characteristics of the DI used. Certified analytical grade potassium chloride (KCl) and calcium chloride ($CaCl_2$) salts were used. KOH and HCl were used for adjusting the pH solution.

Two different types of organic matter were chosen as NOM model substances: salicylic acid (SA) and Aldrich humic acid (AHA), for they represent hydrophilic, low molecular weight organic matter *vs.* hydrophobic, high molecular weight organic matter. These NOM model substances were obtained from commercial sources and have been used to provide consistent experimental conditions (Hong and Elimelech (1997), Schäfer *et al.* (1998), Yeh and Wang (2004)). The salicylic acid is a certified analytical grade from Merck (> 99.0% purity) with a

low molecular weight (138.12 g/mol) and was used without any purification. Stock solutions (1 g/L) of commercial AHA were prepared by dissolving it in DI and raising the pH to 8 through the addition of NaOH. AHA was then purified through a repeated precipitation with HCl to remove bound iron and decrease the ash content as described in Hong and Elimelech (1997). After five repeated cleaning procedures a dialysis step against DI water was performed to further purify the acid-cleaned precipitate (Hong and Elimelech (1997)). The molecular weight of purified AHA should be higher than 50 kDa since it was purified by a dialysis membrane with a molecular cut-off of 50 kDa. The model NOM carbon content was determined and stock solutions of 2 − 3 mg C/L were prepared in accordance with the reported data for Alcantarilha's WTP DW (Table 6.1) (Rosa *et al.* (2005)). Table 6.1 shows the characteristics of the NOM solutions used in the experiments. Model NOM solutions contained always 1 mM KCl as background electrolyte. Experiments with these model solutions were performed at different pH values (from 4 to 9), with and without 1 mM of $CaCl_2$ to investigate the interactions between NOM and the hardness calcium ion. Decanted water was used without any addition of KCl or $CaCl_2$.

Table 6.1 Characteristics of the water samples used in the experiments.

Type of water	pH	Conductivity (μS/cm)	DOC (mg C/L)	UV_{254nm} (1/cm)	SUVA (L/(m.mg))
DW	6.93	342.0	2.17	0.020	0.92
DI	6.06	2.7	0.63	0.002	0.003
SA + 1 mM KCl	4.08	98.2	2.74	0.007	0.26
SA + 1 mM KCl + $CaCl_2$	4.30	225.0	2.81	0.011	0.39
AHA + 1 mM KCl	5.09	92.4	2.33	0.106	5.10
AHA + 1 mM KCl + $CaCl_2$	5.14	243.0	2.14	0.121	5.65

SUVA: specific UV absorbance, defined as the UV absorbance expressed per meter of absorbance per unit concentration of DOC in mg C/L.

6.2.3 ANALYTICAL METHODS

Samples were analysed for pH (at 25 ºC, using a Whatman WTW pH340 meter), conductivity (Crison GLP32 conductimeter), dissolved organic carbon (Shimadzu TOC 5000A analyser,

50 ppb – 4000 ppm) and UV_{245nm} absorbance (Spectronic Unicam UV300 UV/VIS spectrophotometer) using standard methods of analysis (Clesceri *et al.* (1998)). The permeate fluxes were determined by weight (analytical balance Shimadzu, model BX 620S).

A linear regression was found between SA concentration and DOC ($R^2 = 0.9998$), while AHA correlated linearly with both DOC ($R^2 = 0.9596$) and UV_{254nm} ($R^2 = 0.9994$).

6.2.4 MEMBRANE

The investigated NFT50 membrane is a thin film composite NF/RO membrane of polypiperazine amide on a polysulfone microporous support and a polyester support (Alfa Laval). In a previous study (Ribau Teixeira *et al.* (2005), chapter 5), this membrane was characterised by having an hydraulic permeability of 5.9 kg/($h \cdot m^2 \cdot bar$) at 25ºC ($R^2 = 0.9995$), a molecular weight cut-off of 150 g/mol and a pore radius of 0.43 nm.

Zeta potential measurements were also performed and related with membrane performance in the presence of the hardness cations usual in natural waters, namely $CaCl_2$ and $MgSO_4$ (Ribau Teixeira *et al.* (2005), chapter 5). The membrane surface was slightly positive at pH 4 (1 mV), passed through an isoelectric point (i.e.p.) at pH 4.2 ± 0.2 and was negatively charged above this pH (up to 8.3). The surface charge was *ca.* -13 mV at pH 8.3 with a background electrolyte of 1 mM KCl. For divalent hardness ions (1 mM $CaCl_2$ and 1 mM $MgSO_4$), the membrane was less negatively charged (-3.5 mV and -5.4 mV at pH ~ 8, respectively for $CaCl_2$ and $MgSO_4$). The shape of the salt rejection curves corresponded to the shape of the membrane charge curves, *e.g.* for $CaCl_2$ electrolyte and pH above i.e.p., when the membrane was less negatively charged, the electrolyte rejection presented the lowest value. Further information can be found in Ribau Teixeira *et al.* (2005) (chapter 5).

6.2.5 PERMEATION EXPERIMENTS

The performance of the NFT50 membrane was evaluated using a M20 plate and frame unit, from Danish Separation Systems (membrane area of 0.0360 m^2 up to 0.720 m^2; maximum pressure 80 bar; maximum flow 18 L/min and constant temperature maintained by a heat exchanger). In this study the membrane area tested was 0.0720 m^2.

The membranes were first compacted and were then stabilised with DI until a steady permeate flux with DI was achieved at the pressure and crossflow velocity to be used in the experiments, 10 bar and 8 L/min, respectively. The model NOM solution was then prepared and added to the feed reservoir. After 10 minutes of stabilisation at 10 bar, 8 L/min of crossflow velocity and 25ºC the permeate flux was measured and samples from the feed reservoir and the permeate were taken to serve as baseline for flux and rejection at 0% recovery rate.

The experimental procedure consisted of concentration runs, for it was intended to simulate the industrial NF operation at different water recovery rates, defined as the ratio between the permeate and the initial feed volumes. In these runs, permeate was not recycled to the feed reservoir until a stipulated permeate volume was obtained. At this time, permeate was recycled to the feed reservoir during a stabilisation period, after which the flux was measured and feed and permeate samples were collected and the run followed to the next recovery rate. These samples from the feed and permeate solutions taken at stipulated recovery rates were analysed to determine the NOM (DOC and UV_{254nm}) and the salt (by conductivity measurements) rejections. Flux measurements were taken continuously during the experimental time. Between each NF run, membranes were washed until the pure water flux

reached 90% of the initial value measured after compaction and the feed conductivity was similar to that of DI.

6.3 RESULTS

Figure 6.1 shows the NF performance (fluxes, and conductivity and NOM rejections) with the DW from Alcantarilha WTP at different pH values and water recovery rate. Figures 6.2 and 6.3 present similar data obtained respectively with SA and AHA model solutions, with and without $CaCl_2$ (1 mM). Figure 6.4 depicts the variation of flux and salt rejection with the pH at 0% and 90% water recovery rate for DW and NOM model solutions in comparison with analogous data obtained in a previous work Ribau Teixeira *et al.* (2005) (chapter 5) for the same electrolyte solutions (1 mM KCl eletrolyte, with and without 1 mM $CaCl_2$) in the absence of NOM.

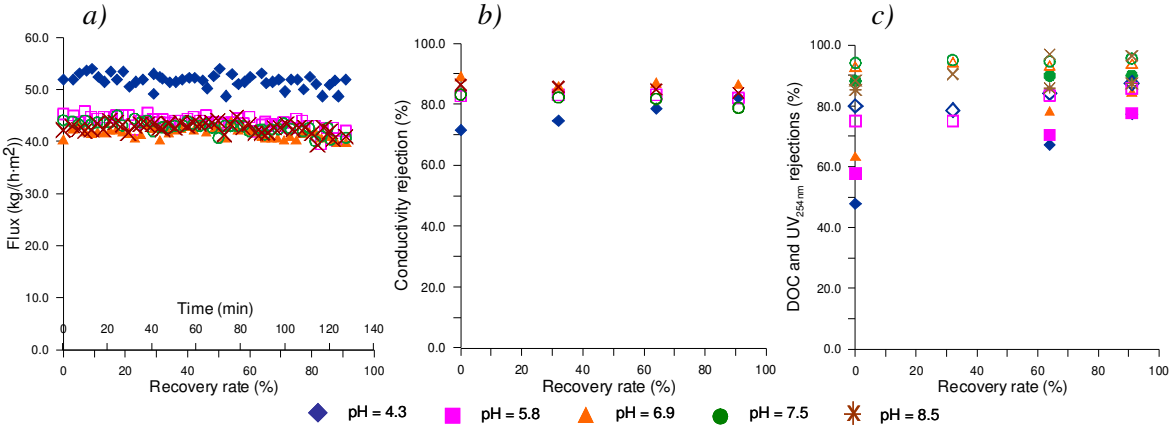

Figure 6.1 NF performance with DW at different pH values and water recovery rates: **a)** flux, **b)** conductivity rejection and **c)** DOC (filled symbols) and UV_{254nm} (empty symbols) rejections (10 bar, 25 ºC, Initial concentration (C_i) = 2.1 – 3.3 mg C/L).

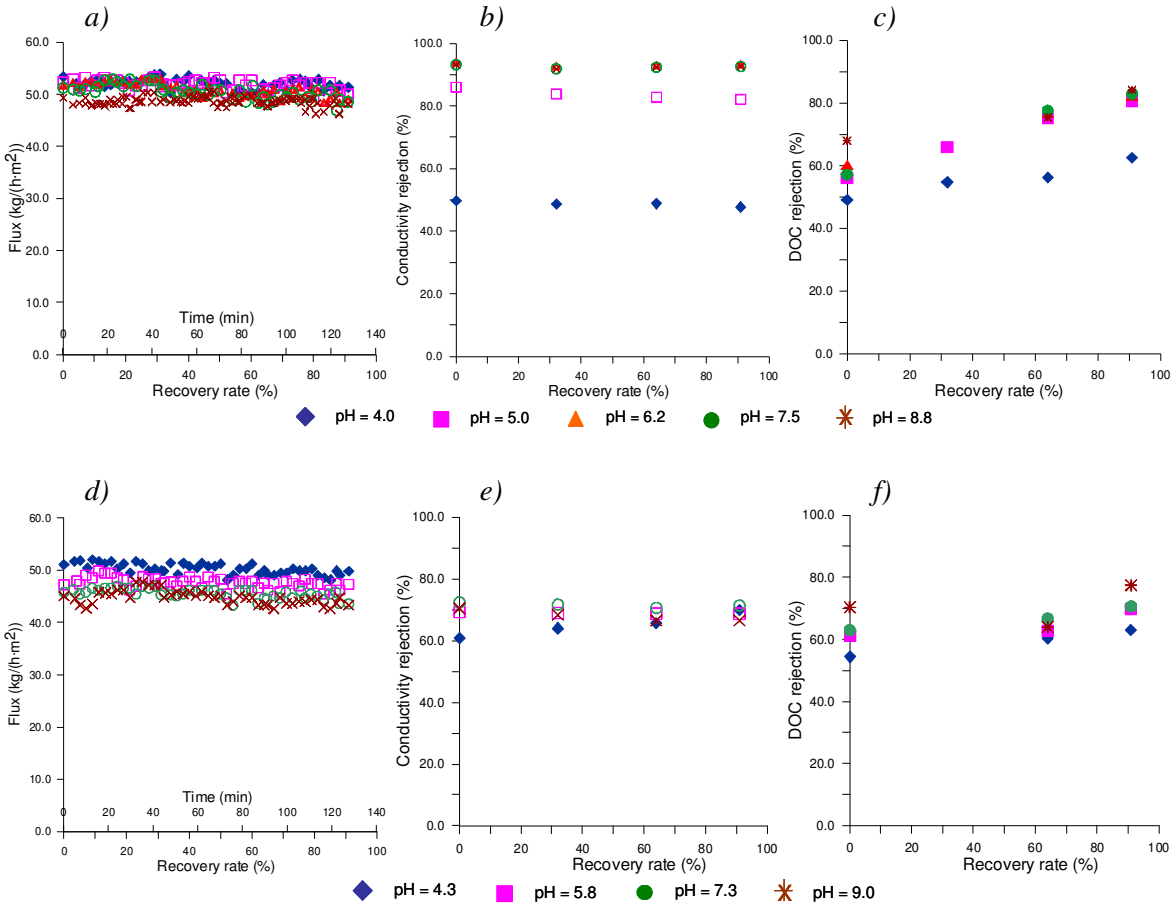

Figure 6.2 NF performance with SA solution at different pH values and water recovery rates: **a)** flux, **b)** conductivity rejection and **c)** DOC rejection for SA + 1 mM KCl; and **d)** flux, **e)** conductivity rejection and **f)** DOC rejections for SA + 1 mM KCl + 1 mM $CaCl_2$ (10 bar, 25ºC, C_i = 2.6 – 2.9 mg C/L).

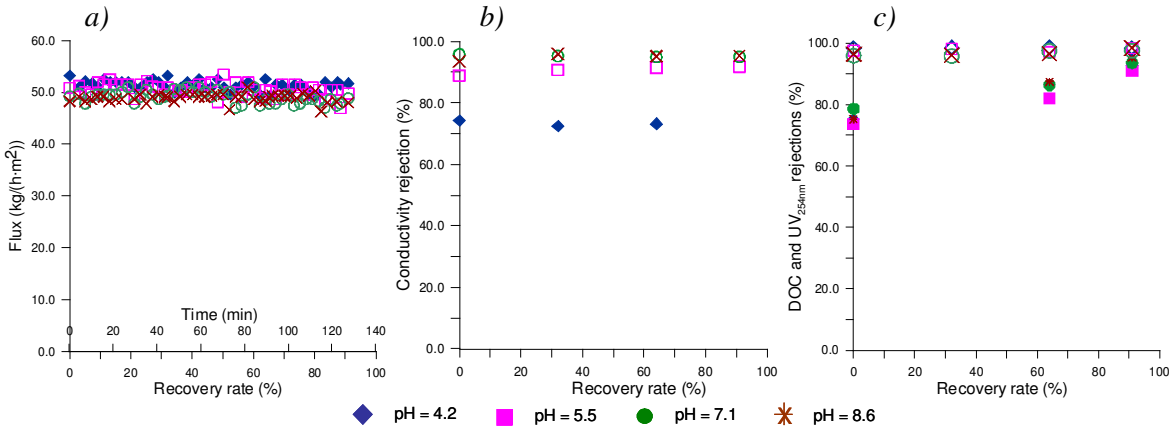

Figure 6.3 NF performance with AHA solution at different pH values and water recovery rates: **a)** flux, **b)** conductivity rejection and **c)** DOC (filled symbols) and UV_{254nm} (empty symbols) rejections for AHA + 1 mM KCl; and **d)** flux, **e)** conductivity rejection and **f)** DOC (filled symbols) and UV_{254nm} (empty symbols) rejections for AHA + 1 mM KCl + 1 mM $CaCl_2$ (10 bar, 25ºC, C_i = 2.0 – 2.6 mg C/L)).

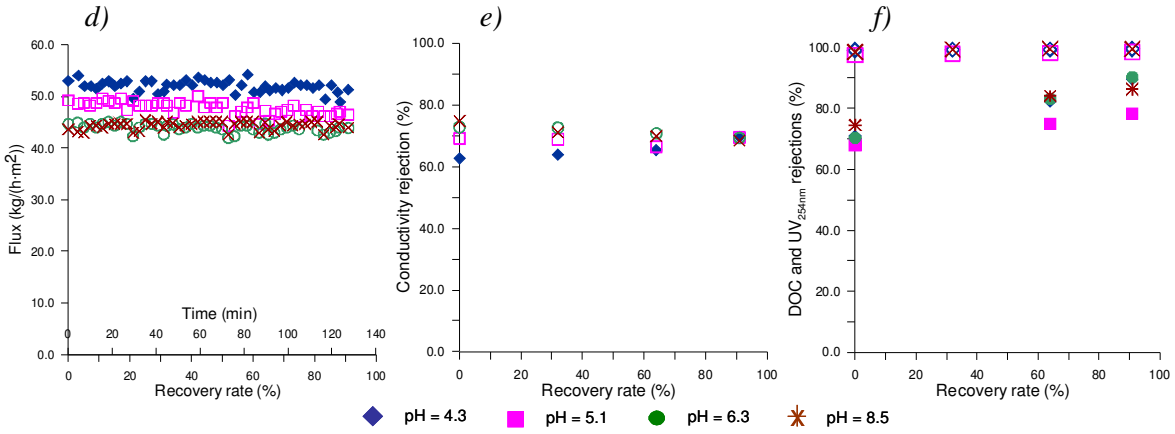

Figure 6.3 (cont.) NF performance with AHA solution at different pH values and water recovery rates: **a)** flux, **b)** conductivity rejection and **c)** DOC (filled symbols) and UV_{254nm} (empty symbols) rejections for AHA + 1 mM KCl; and **d)** flux, **e)** conductivity rejection and **f)** DOC (filled symbols) and UV_{254nm} (empty symbols) rejections for AHA + 1 mM KCl + 1 mM $CaCl_2$ (10 bar, 25ºC, C_i = 2.0 – 2.6 mg C/L)).

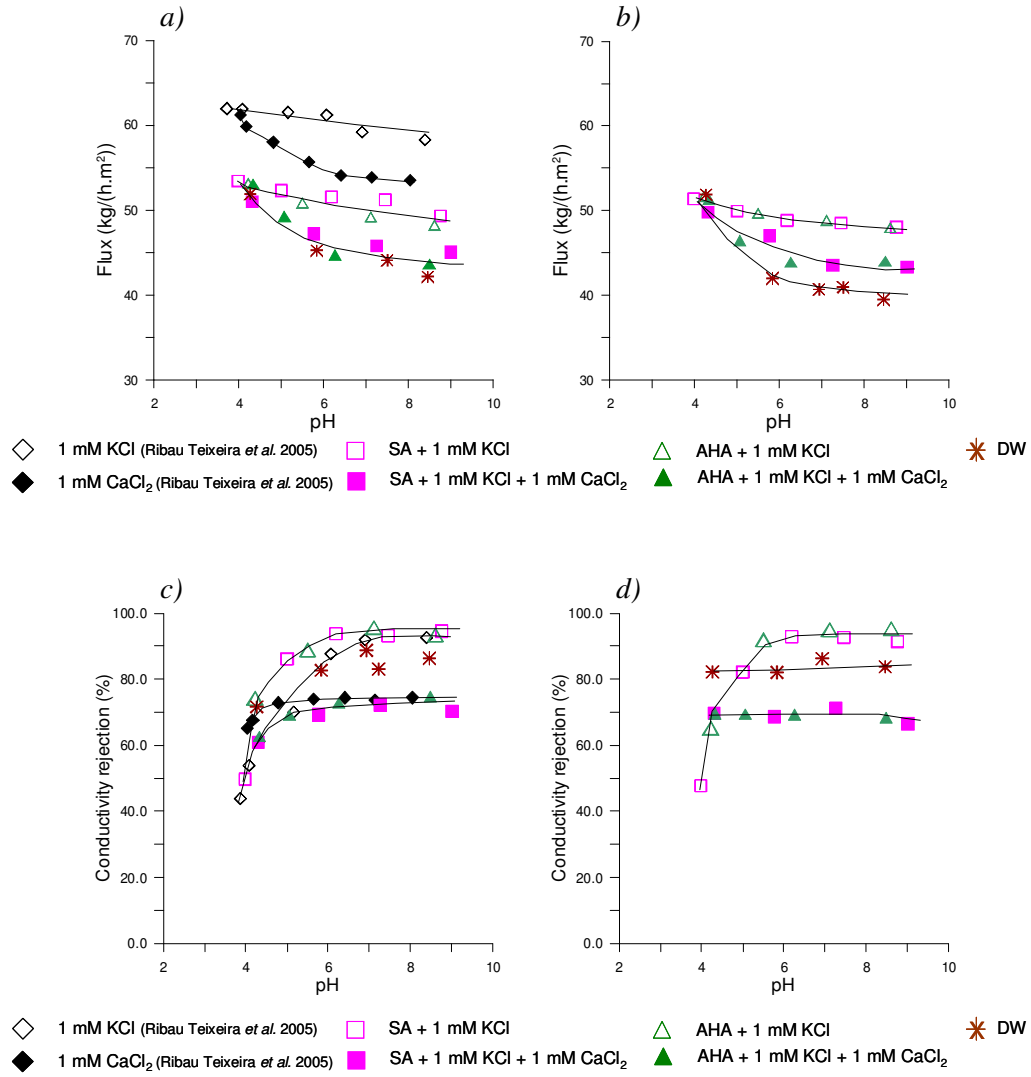

Figure 6.4 Variation of flux and conductivity rejection at different pH: **a)** and **c)** 0% water recovery rate, and **b)** and **d)** 90% water recovery rate (10 bar, 25ºC).

Flux results with DW (Figure 6.1a), SA (Figures 6.2a and 6.2d) and AHA (Figures 6.3a and 6.3d) show that there is no significant variation with recovery rate or running time. As far as the pH and calcium effects on flux are concerned, as previously observed with the electrolyte solution in the absence of NOM (Figures 6.4a and 6.4b), flux with these three NOM solutions (DW, and SA and AHA solutions) significantly decreases when passing from pH 4 to 6 and in the presence of $CaCl_2$. In this case, flux is higher at acidic pH (*ca.* 4.0-4.3), decreases until pH 6-7, above which a significant variation with pH is no longer observed (Figures 6.1a (DW), 6.2d (SA solution), 6.3d (AHA solution)). A much lower flux variation with pH was found in the absence of $CaCl_2$ (Figures 6.2a (DW), 6.3a (AHA solution), 6.4a and 6.4b (electrolyte without NOM)) similarly to what was obtained by Childress and Deshmukh (1998) and Childress and Elimelech (2000) with Suwannee River humic acid (2 mg/L of humics and 0.01 M NaCl). Yeh and Wang (2004) obtained no flux variation with pH for salicylic acid (20 mg/L as DOC and 0.015 M $NaClO_4$), when using microfiltration membranes. The presence of calcium ions is much more important for flux (flux reduction in the presence of calcium ions is twice the value obtained in the absence of calcium) than the type of NOM. In addition, calcium has the same effect regardless of the hyphophilicity and molecular weight of NOM (Figures 6.4a and 6.4b). Either with or without calcium the tendencies are very similar to those observed with no NOM in the feed stream, although in a lower range (Figure 6.4a).

Regarding the slat rejection, in the absence of calcium ions it presents no variation with the recovery rate but it increases with the pH for both SA (Figure 6.2b) and AHA (Figure 6.3b) solutions, matching the behaviour observed with the 1 mM KCl electrolyte (Figure 6.4c). The salt rejection in SA solution passes from *ca.* 50% at pH 4.0 to 85-87% at pH 5.0 and 93% at pH 6.2 to 8.8 (Figure 6.2b); the salt rejection in AHA solution varies from *ca.* 73% at pH 4.2

to 90-95% at pH above 5.5 (Figure 6.3b). In the presence of $CaCl_2$, the pH effect on salt rejection is much less significant, *i.e.* calcium is responsible for higher rejections at pH 4 – 4.3 and much lower rejections at the other pH values. At 0% recovery rate, rejections increase from 60% at pH 4.3 to 70% above pH 5.8 for both SA and AHA (Figure 6.4c) whereas for 90% recovery rate no variation was observed in both cases (Figure 6.4d). Both NOM model solutions fit the exact same trendlines for conductivity rejection either with or without calcium ions, which in addition are very close to the electrolyte solution trendlines (Figures 6.4c and 6.4d). DW has an intermediate behaviour between NOM model solutions and the electrolyte solutions.

For NOM parameters (UV_{254nm} and DOC), the behaviour is different especially with the recovery rate. DOC rejection increases with recovery rate and pH, while UV_{254nm} is almost completely rejected (90 – 100%) at all pH values and recovery rates (Figures 6.1c, 6.2c, 6.2f, 6.3c and 6.3f). DOC rejections are lower than UV_{254nm} rejections (Figures 6.1c, 6.3c and 6.3f). Such observation is not surprising since the UV absorbance at 254 nm is attributed mainly to aromatic/hydrophobic compounds, whereas DOC measures the concentration of carbon containing molecules dissolved in water (Schäfer *et al.* (2000), Seidel and Elimelech (2002)). A linear correlation ($R^2 = 0.998$) was found between the increase in DOC rejection and the bulk feed DOC concentration for SA at all pH values. This linearity and the mass balance calculations (data not shown) indicate no significant adsorption of the SA on the membrane surface. Other authors investigating SA demonstrated that it did not adsorb significantly on the tested membranes (Kimura *et al.* (2003)). AHA exhibits a relatively higher DOC rejection than SA due to the AHA higher molecular weight. Taking into account the feed SUVA values of SA (0.26 L/(mg.m)) and AHA (5.1 L/(mg.m)), and based on Edzwald and Van Benschoten (1990) classification, it is possible to conclude that this membrane removes both hydrophilic

and hydrophobic NOM, as already presented by other authors for NF membranes (Siddiqui *et al.* (2000), Kim and Yu (2004)). Consequently, NOM rejections for DW are high (especially for pH 8.5) (Figure 6.1c) despite the relatively hydrophilic nature and the low molecular weight of its organic matter (SUVA 0.92 L/(m.mg)). Such results demonstrate that this nanofiltration membrane is effective for reducing NOM from treated water, thus reducing the THM formation potential (EPA (1999)).

6.4 DISCUSSION

The shape of NOM curves with and without $CaCl_2$ is similar to that of KCl electrolyte with and without $CaCl_2$, respectively. Flux decreases in the presence of NOM and decreases further in the presence of both NOM and $CaCl_2$ (Figures 6.4a and 6.4b). The flux decrease and rejection increase are almost the same at 0% or 90% water recovery rate (Figure 6.4).

As a general observation, this membrane presents no significant flux decline over the running time with the two types of NOM surrogates studied and with DW. Two possible reasons could be addressed: first these are short-term experiments, and secondly minimal concentration polarisation occurs. Childress and Elimelech (2000) referred that the presence of humics was not believed to significantly affect the short-term performance of the membrane. Cho *et al.* (1999) concluded that minimal concentration polarisation occurred probably due to the low permeation rate and low feed DOC concentration, and apparently no adsorption occurred on their experiments. Elimelech and co-workers (Hong and Elimelech (1997), Seidel and Elimelech (2002)) demonstrated that NOM fouling was prevented in the runs performed at the lowest permeate flux, implying that their runs were performed near or below the critical flux.

Surprisingly, in the presence of NOM the lowest pH values showed a higher flux (more evident in the presence of $CaCl_2$). At low pH, NOM molecules are more protonated, the

molecules are less charged and tend to form more coiled structures. A greater fouling potential would be expected, so flux should be the lowest. However, as the pH increases, the membrane surface and pores become both more negatively charged due to the presence of anions (inorganic and/or NOM). As a result, the pore size of the membrane is reduced because of the repulsion between neighbour negatively-charged groups and adopts an extended conformation. In addition, the osmotic pressure near the membrane surface increases at high pH due to the high salt rejections, which decreases the net driving pressure. Together, these two mechanisms lead to a decrease in water flux and an increase in salt rejection with pH. In the presence of $CaCl_2$, the variation of flux between acid and basic pH is more evident (Figures 6.1a, 6.2d and 6.3d). At pH above 5.0 the membrane surface and pores are less negatively charged due to calcium adsorption (Ribau Teixeira *et al.* (2005), chapter 5), so the repulsion between the membrane and the negatively-charged functional groups of NOM and the co-ion exclusion effects are minimised. Besides matching the results obtained in a previous study using only electrolyte solutions (Ribau Teixeira *et al.* (2005), chapter 5), the same variations of flux and rejection with pH were obtained by Schäfer (2001), Kilduff *et al.* (2004) and Zhang *et al.* (2004). Schäfer (2001) referred that fouling conditions may be required to observe the expected effects of lower pH, lower fluxes. Kilduff *et al.* (2004) concluded that with a pH increase, the effective permeability increased, but the flux decreased as a result of increased osmotic effects resulting from increased solute rejection. Such behaviour is in contrast to looser membranes having low salt rejection, for which flux increased with pH as a result of the membrane permeability increase (Kilduff *et al.* (2004)). Zhang *et al.* (2004) in their experiments to remove pesticides with NF membranes concluded that the flux decline was not caused by the presence of pesticides or organic matter. Rather, the observed flux decline was attributed to the adsorption of ions on the membrane pores and subsequent pore narrowing.

The NOM rejection behaviour can be explained by two different mechanisms, size exclusion and electrostatic repulsion in accordance to Hong and Elimelech (1997). At low pH, humic macromolecules have a smaller macromolecular configuration due to reduced interchain electrostatic repulsion, and pass more easily through the membrane pores. Furthermore, at pH 4 – 4.3 (the membrane i.e.p.) the rejection is physically and chemically reduced. On one hand, the membrane pores are larger due to low membrane pore charge and, on the other hand, the low membrane surface charge results in reduced electrostatic repulsion between NOM and membrane surface. The adsorption of humic acids is more favourable at pH 4 – 4.3, since the membrane charge is zero or very low (it is close to membrane i.e.p.) and the humic macromolecules are more hydrophobic. At high pH values the sieving effects are stronger (narrower pores and larger molecules) and the repulsive forces between the membrane surface and the NOM could prevent the NOM from adhering onto the membrane surface, producing less fouling and improving the rejection (Figures 6.1c, 6.2c, 6.2f, 6.3c and 6.3f). Nilson and DiGiano (1996) concluded that the hydrophilic fraction had little influence on permeate flux, whereas the hydrophobic NOM fraction was responsible for the permeate flux decline. The hydrophilic fraction of NOM was less rejected than the hydrophobic fraction. Jucker and Clark (1994) demonstrated that humic substances were adsorbed onto hydrophobic membranes. Kimura *et al.* (2003) observed that the adsorption of hydrophobic compounds on the membrane occurred significantly, especially when the compounds were electrostatic neutral.

The membrane performance in the presence of NOM and calcium ions is influenced by several mechanisms (Hong and Elimelech (1997), Yoon *et al.* (1998)). Calcium forms complexes with the functional groups (carboxyl groups) of humics and, as a result, the

interchain repulsion of the humic macromolecules is reduced, and small and coiled humic macromolecules are formed. As investigated in a recent paper (Ribau Teixeira *et al.* (2005), chapter 5), calcium ions adsorb on the membrane surface and reduce its negative charge, so the interaction between the membrane and the NOM increases. Calcium may bridge between the two negatively charged functional groups of NOM macromolecules, and between the membrane and the negatively charged part (hydrophilic part) of humic molecules. These effects contribute to the formation of a dense fouling layer and to an increase of the membrane hydrophobicity. All these factors are responsible for the observed flux decline between solutions in the absence and in the presence of $CaCl_2$ (Figures 6.2a *vs.* 6.2d and 6.3a *vs.* 6.3d) and for the lower conductivity and DOC rejections in the presence of $CaCl_2$ (Figures 6.2b *vs.* 6.2e and 6.3b *vs.* 6.3e, and Figures 6.2c *vs.* 6.2f and 6.3c *vs.* 6.3f).

6.5 CONCLUSIONS

The water background inorganic matter (pH and calcium hardness ions) impacted the natural organic matter removal by a negatively charged nanofiltration membrane. The NF performance of water with low NOM content and moderate hardness was largely or even mostly influenced by the background pH and calcium hardness, rather than by the type of NOM. Both SA (hydrophilic, low molecular weight, SUVA 0.26 L/(m.mg)) and AHA (hydrophobic, high molecular weight, SUVA 5.10 L/(m.mg)) model solutions fitted the exact same trendlines for flux and salt rejection, either with or without calcium ions, but greatly modified by their presence. For all investigated NOM solutions flux presented low variation with recovery rate and running time, most probably due to the low DOC content and low permeation rate. It decreased with pH and particularly with $CaCl_2$. Alkaline pH showed higher conductivity and DOC rejections. The results were attributed to both physical and chemical aspects of NOM filtration. The pH increased the negative charge of the membrane,

responsible for pore narrowing and increased repulsions with the negatively-charged functional groups of NOM that inhibited the molecules from being adsorbed, besides increasing their hydrodynamic radii. In the presence of calcium, flux and rejection decreased further for it reduced the negative charge of the membrane and formed complexes with humics. DOC rejection increased in the order SA < DW < AHA, which was related to the size and hydrophobicity of the compounds. The results showed that this NF membrane was effective for reducing NOM from treated water, and consequently for reducing the THM formation potential.

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CHAPTER 7
MICROCYSTINS REMOVAL BY NANOFILTRATION MEMBRANES

ABSTRACT

Microcystins produced by cyanobacterial blooms have been extensively found in water reservoirs used for drinking water abstraction and many conventional water treatment technologies have been reported to be ineffective for removing them. Safe barriers against microcystins in drinking water are, therefore, needed. This paper investigates the removal of the most commonly found microcystin variants by a negatively charged nanofiltration (NF) membrane. Different electrolyte solutions (KCl and $CaCl_2$), pH (5-8.5) and types of natural organic matter (NOM) were used in order to study the influence of chemical feed characteristics on NF performance for mycrocistins removal. Three types of water samples were employed, ozonated water and decanted water (DW) from a Water Treatment Plant, and DW spiked with Aldrich humic acid and salicylic acid, due to the lack of hydrophobic, high molecular weight NOM in the selected natural waters. Microcystins revealed a strong membrane fouling ability for 150 μg/L as total microcystins, being this concentration one order of magnitude higher than those of natural occurring blooms. For 16 μg/L as total microcystins, the fouling behaviour was attenuated. All microcystin variants studied (MC-LR, MC-LY and MC-LF) were almost completely removed by NF (>97%). The NOM type and concentration, as well as the background inorganic matrix (pH and 1mM $CaCl_2$) showed no

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influence on the NF performance to remove microcystins. Microcystins concentrations in the NF permeate were always far below the drinking water guideline value of 1 µg/L MC-LR adopted by World Health Organisation, and usually below the quantification limit. Excellent water quality was also achieved in terms of turbidity (<0.13 NTU) and NOM content (DOC ≤1 mg C/L, UV_{254nm} <0.002 cm^{-1}).

7 MICROCYSTINS REMOVAL BY NANOFILTRATION MEMBRANES

7.1 INTRODUCTION

Cyanobacteria (blue green algae) may produce a wide range of toxins (cyanotoxins), as well as taste and odour compounds, as secondary metabolites under certain conditions of growth and environmental conditions. Depending on the cyanobacteria genera, these cyanotoxins may include the cyclic peptide hepatotoxins, such as nodularin and microcystins (MC), and the alkaloid neurotoxins like anatoxin-a (Carmichael (1994), Codd (1995)). Microcystins cause liver damage and are tumour promoters (Matsushima *et al.* (1992)). Their presence in water, even at low concentrations, is a matter of great concern due to the acute toxicity and sublethal toxicity of these toxins. In order to minimise public exposure, the World Health Organisation (WHO) adopted the drinking water guideline value of 1.0 μg/L for microcystin-LR, one of the most commonly occurring microcystin variants.

Microcystins produced by cyanobacterial blooms have been found worldwide in water reservoirs used for drinking water abstraction and many conventional water treatment technologies (coagulation (C)/ flocculation (F)/ sedimentation (S), filtration) have been reported to be ineffective for removing them (Hoffmann (1976), Chow *et al.* (1999), Hrudey *et al.* (1999)). Therefore, it is of great importance to investigate safe barriers against cyanotoxins in a wide range of natural waters, so that the public health risk may be reduced.

Cyanotoxins have been found to degrade in the presence of strongly oxidizing conditions, such as high levels of chlorine or ozone (Keijola *et al.* (1988), Rositano *et al.* (1998)). However, the effectiveness of these technologies depend upon the water quality, particularly the natural organic matter (NOM) content, pH and alkalinity (Tsuji *et al.* (1997), Rositano *et*

al. (2001)), and on the health implications of potential hazards associated with the by-products formed (Lawton and Robertson (1999)). Adsorption systems using granular or powdered activated carbon have been successfully employed to remove microcystins in both bench and full scale experiments (Falconer *et al.* (1989), Lambert *et al.* (1996), Pendleton *et al.* (2001), Cook and Newcombe (2002)). Nevertheless, their performance is also dependent on the NOM competitive adsorption (Donati *et al.* (1994), Lambert *et al.* (1996)).

An alternative to effectively remove cyanobacteria and cyanotoxins is membrane pressure-driven filtration. The membrane acts as a physical barrier, allowing water to pass while retaining the suspended solids and even dissolved materials, depending on the type of the membrane. In this particular application, microfiltration (MF) and ultrafiltration (UF) will be adequate for removing the cyanobacterial cells but not cyanotoxins, due to the large pore size and high molecular weight cut-off of these membranes. Since the molecular weight of microcystins is around 1000 Da, it is assumed that both nanofiltration (NF) and reverse osmosis (RO) membranes will successfully retain microcystins. As with other hazardous microcontaminates like pesticides, water organic and inorganic background matrixes affect the NF performance (Zhang *et al.* (2004)). Nevertheless, if a proper membrane and optimal operating conditions are used, NF should be a safe physical barrier against microcystins without the problem of potential health hazardous by-products formation. In addition, it will also remove multivalent ions, other small hazardous organic compounds (*e.g.* pesticides, endocrine disruptors) and NOM, major percursor of the disinfection by-products (DBPs), as well as viruses and microorganisms resistant to chemical oxidation (*Cryptosporidium*, *Giardia*) that might have escaped intact from previous water treatments. However, a membrane process produces a toxin enriched stream, which has to be safely disposed since the toxins are not destroyed by this treatment. This concentrate stream represents only 10-

20% of the original feed stream (Van der Bruggen *et al.* (2003)) and further treatment by oxidation processes (*e.g.* ozonation, wet air oxidation) should be feasible.

Few studies were found on membrane technology for removing cyanobacteria and/or cyanotoxins.

Concerning the cyanobacteria removal, a laboratory study with MF and UF in both dead-end and cross-flow modes showed high removal efficiency (> 98%) of *Microcystis aeruginosa* cells (Chow *et al.* (1997)). This study also examined the cell damage by measuring the leakage of chlorophyll *a* and microcystin-LR into the permeate. There were some cells damaged after filtration but no significant toxin increase in the permeate. In the UF experiments, the amount of microcystin was significantly lower on the permeate than in the feed, which suggested that this UF membrane might have rejection properties or adsorption ability for microcystins, since the membrane molecular cut-off (100 kDa) was much higher than the microcystins molecular weight (*ca.* 1000 Da). A pilot plant study using ceramic MF membranes was performed using raw water from lake Brugneto (Italy) to evaluate the membrane ability to remove particles, microorganisms, algae (including the cyanobacterium *Oscillatoria rubescens*) and disinfection by-products (Bottino *et al.* (2001)). Results indicated that despite its high content in raw water ($4.6x10^5$ cells/L), *Oscillatoria rubescens* was completely retained.

Hart and Stott (1993) evaluated the removal of microcystin by NF membranes, using a natural water spiked with 5 - 30 μg/L MC. Results showed concentrations below 1 μg/L in the permeate. Muntisov and Trimboli (1996) also showed that NF membranes removed microcystin-LR and nodularin from a river water spiked with 8 μg/L of these toxins.

Neumann and Weckesser (1998) tested three types of RO membranes at 25 – 35 bar to evaluate the removal of microcystin-LR and microcystin-RR (initial concentrations of 70 – 130 μg/L) from tap water and tap water containing 3000 mg/L NaCl (salt water). The average rejection, with a detection limit of 0.2 μg/L, varied between 96.7% and 99.9% in tap water, and 98.5 – 99.6% in salt water, so there was no statistical difference in rejection of the microcystins between the two waters. Vuori *et al.* (1997) evaluated the removal of nodularin from brackish water by RO. As the salt and toxin concentration increased in the raw water, traces of nodularin were detected in treated water although it remained below the limit of quantification.

The above mentioned studies indicate that membrane technology, particularly NF, is highly efficient for removing the microcystins present in water. Nonetheless, a comprehensive study of the impact of background organic (NOM) and inorganic matrixes on the NF performance for microcystins and NOM removal is still missing. During cyanobacterial blooms occurrence an increase in both NOM content and pH value is usually expected, in addition to the obvious turbidity and chlorophyll *a* increase. Besides its potential to form disinfection by-products when water is disinfected with chlorine (Blau *et al.* (1992), Pomes *et al.* (1999)) and its ability to support biofilm growth in the water distribution networks, NOM is a heterogeneous organic mixture of hydrophobic and hydrophilic compounds, considered one of the major cause of NF fouling during the filtration of surface waters (Nyström *et al.* (1995), Nilson and DiGiano (1996), Hong and Elimelech (1997), Cho *et al.* (1999)). NOM removal by NF was studied in a recent paper (Ribau Teixeira and Rosa (2005), chapter 6) addressing the chemical and physical aspects of NOM filtration and flux decline with negatively charged NF membranes and their relation with the feed water background inorganic matrix, *i.e.* water pH and calcium hardness.

The aim of the present study was to evaluate the NF performance for microcystins removal from moderately hard natural waters with different types of NOM (hydrophilic, low molecular weight and hydrophobic, high molecular weight NOM substances), hardness and pH values (*ca.* 5 – 8.5).

7.2 MATERIALS AND METHODS

7.2.1 MICROCYSTINS

Figure 7.1 depicts the general structure of microcystins. The main structural change in microcystins is the variability of L-amino acids 2 (designated as X) and 4 (Z) (Meriluoto (1997)). For microcystin-LR (MC-LR), leusine is in position X and arginine is in position Z. MC-LR contains two ionisable carboxyl groups and one ionisable amino group that are not part of peptide bonds that make up the cyclic peptide structure.

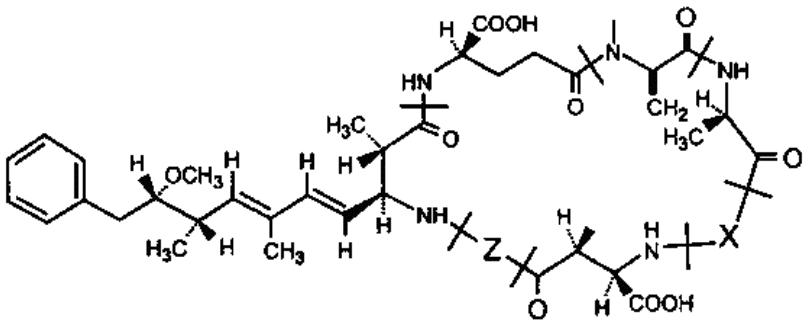

Figure 7.1 General structure of microcystins cyclo(-D-Ala[1]-L-X[2]-D-*erythro*-β-methylisoAsp[3]-L-Z[4]-Adda[5]-D-Glu[6]-*N*-methyledydroAla[7]): (1) D-Alanine, (3) D-*erythro*-β-methylaspartic acid, (5) Adda [(2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid], (6) D-glutamate and (7) *N*-methyldehydroalanine (Meriluoto (1997)).

Microcystins were extracted from a culture of *Microcystis aeruginosa* supplied by Pasteur Culture Collection (PCC7820) and maintained in laboratory.

After one and an half months of growth (corresponding approximately to the maximum of *Microcystis* growth obtained in the laboratory), the PCC7820 culture was centrifuged (6,000 x *g*, 10 min). The resultant pellet was resuspended, washed and centrifuged again. The supernatant was discarded between washes. This procedure was performed twice. The last centrifugation was at 10,000 x *g* during 10 min, the supernatant was discarded and the cells (pellets) were kept in the freezer (-20 ºC, in the dark) until use.

To prepare the microcystins stock solution, the cells were defrosted and resuspended in a small volume of methanol 75% (v/v). Microcystins were extracted from the cells at 4 ºC in the dark, during 18 h. After this period, this methanolic extract was centrifuged twice (10,000 x *g*, 10 min) and the pellet was discarded. The solution was concentrated by evaporation in a rotavapor (50-54 °C), dissolved in a small amount of methanol 75% (v/v) and analysed on HPLC-PDA to determine microcystin concentration. The necessary volume to produce the final solution for the NF experiments was then measured and evaporated again with N_2 at 50-54 ºC. The dry extract was then dissolved in the water to be used in the NF experiments.

After the NF experiments, microcystins were extracted from the aqueous sample using an isolute C18 solid phase extraction column, 1 g in a 6 mL reservoir, following the standard operation procedure developed by Meriluoto and Spoof (2005a). The cartridges were first conditioned with 10 mL methanol 75% followed by 10 mL of milli-Q water at a flowrate not exceeding 10 mL/min, without letting it dry during conditioning. The samples were then applied to the cartridge and the microcystins were eluted with 5 mL of methanol 90% containing 0.1% trifluoroacetic acid. The methanolic elute was evaporated at 50 ºC in a rotavapor, resuspended in 500 µL of 75% methanol, centrifuged for 10 min at 10,000 x *g* and

150 µL of supernadant were transferred to HPLC vials for analysis (Meriluoto and Spoof (2005a)).

The microcystin variants detected in these samples were MC-LR, microcystin-LY (MC-LY) and microcystin-LF (MC-LF) (Table 7.1).

Table 7.1 Characteristics of the microcystin variants identified in this study.

	MC-LR	MC-LY	MC-LF
Molecular weight	994	1001	985
Amino acids (X and Z)	Leucine, Arginine	Leucine, Tyrosine	Leucine, Phenylalanine
Net charge at pH 6-9	(-)	(2-)	(2-)
Hydrophobicity	Hydrophobic	Hydrophobic	Hydrophobic

7.2.2 NATURAL WATER SAMPLES

Ozonated water (OW) (after ozonation) and decanted water (DW) (after ozonation/ C/F/S) from Alcantarilha Water Treatment Plant (WTP), Algarve, Portugal, were the natural waters used in these experiments. Since 2000, this WTP supplies water to *ca.* half million people in southern Portugal (Algarve), and was designed to treat up to 3 m^3/s (*ca.* 1 million people by year 2020) of surface water from Funcho Dam reservoir (2 km^2 and 43.4 hm^3). OW and DW are moderately hard waters with the characteristics presented in Table 7.2. The lack of high molecular weight, hydrophobic NOM, in the selected natural waters (very low SUVA values in both OW and DW) was overcome by spiking DW with humic acids, as described below.

7.2.3 CHEMICALS AND NOM MODEL SUBSTANCES

Salicylic acid (SA) and Aldrich humic acid (AHA) were the NOM model substances used to spike DW (Table 7.2). The salicylic acid is a certified analytical grade from Merck (> 99.0% purity) with a low molecular weight (138.12 g/mol) and was used without any purification. AHA was purified through the repeated precipitation with HCl proposed by Hong and

Elimelech (1997) and already described in a previous paper (Ribau Teixeira and Rosa (2005), chapter 6). The molecular weight of purified AHA should be higher than 50 kDa since it was purified by a dialysis membrane with a molecular cut-off of 50 kDa.

Deionised water (DI) was used for the preparation of all stock solutions and for membrane performance experiments. Certified analytical grade potassium chloride (KCl) and calcium chloride ($CaCl_2$) salts were used. HCl was used for adjusting the solution pH.

Table 7.2 Characteristics of the studied water samples after spiking with microcystins (*ca.* 150 µg/L MC-LR eq.)

Water	pH	Conductivity (µS/cm)	Turbidity (NTU)	TOC (mg/L)	DOC (mg/L)	UV_{254nm} (1/cm)	SUVA (L/(m.mg))
1 mM KCl	5.8	97.4	-	-	1.82	-	-
1 mM KCl + 1 mM $CaCl_2$	6.0	240	-	-	1.89	-	-
OW	7.2	338	4.93	3.12	2.57	0.019	0.74
DW	7.1	338	1.78	2.51	2.27	0.012	0.53
DW+SA+AHA	7.0	342	4.31	-	3.75	0.095	2.53

SUVA: specific UV absorbance, defined as the UV absorbance expressed per meter of absorbance per unit concentration of DOC in mg/L.

7.2.4 MEMBRANES

The investigated NFT50 membrane is a thin film composite NF/RO membrane of polypiperazine amide on a polysulfone microporous support and a polyester support, from Alfa Laval, with an hydraulic permeability of 5.9 kg/(h·m^2·bar) at 25 ºC, a molecular cut-off of 150 Da and a pore radius of 0.43 nm (Ribau Teixeira *et al.* (2005b), chapter 5).

From previously published data on zeta potential measurements (Ribau Teixeira *et al.* (2005b), chapter 5), the membrane surface is slightly positive at pH 4 (1 mV), passes through an isoelectric point at pH 4.2 ± 0.2 and is negatively charged above this pH (until 8.3). The surface charge is about -10.3 mV at pH 6.9 with a background electrolyte of 1 mM KCl. In the presence of calcium divalent hardness cations (1 mM $CaCl_2$), the isoelectric point shifts

from 4.2 to 5-6 and the membrane is less negatively charged over the entire pH range (-2.6 mV at pH 7.3).

7.2.5 ANALYTICAL METHODS

Samples were analysed for pH (at 25ºC, using a Whatman WTW pH340 meter), conductivity (Crison GLP32 conductimeter), dissolved organic carbon (DOC) (Shimadzu TOC 5000A analyser, 50 ppb – 4000 ppm), UV_{245nm} absorbance (Spectronic Unicam UV300 UV/VIS spectrophotometer) and turbidity (HACH 2100N turbidity meter of high resolution, 0.001 NTU) using standard methods of analysis. The permeate fluxes were determined by weight (analytical balance Shimadzu, model BX 620S).

Microcystins were analysed by HPLC-PDA using a Dionex Summit system, which includes a high pressure gradient pump Dionex Summit, an autosampler Dionex ASI-100, a column oven Dionex STH-585 and a photo diode-array detector Dionex PDA-100. A C18 column was used (Merck Purospher STAR RP-18 endcapped, 3 μm particles, LiChroCART 55x4 mm). The mobile phase used a gradient of milli-Q water and acetonitrile, both with 0.05% (v/v) of trifluoracetic acid. Chromatograms were analysed between 180 – 900 nm, with a main detection at 238 nm for the typical microcystins spectra (Meriluoto and Spoof (2005b)).

7.2.6 NF PERMEATION EXPERIMENTS

The NF experiments were performed in a plate-and-frame unit, Lab-unit M20, from Danish Separation Systems (membrane area of 0.0360 m^2 up to 0.720 m^2; maximum pressure 80 bar; maximum flow 18 L/min and constant temperature maintained by an heat exchanger), where one (360 cm^2) or two pairs (720 cm^2) of membranes were tested. Therefore, for comparison

purposes, fluxes had to be made dimensionless and relative fluxes (%) were calculated (defined as the ratio of solution flux over the pure water flux).

The membranes were first compacted and were then stabilised with DI until achieving a steady permeate flux, at the pressure and crossflow velocity to be used in the experiments, 10 bar and 8 L/min respectively.

Two sets of trials were performed to evaluate the NF efficiency for microcystins removal from surface water.

In the first set of trials, microcystins were supplemented to a background electrolyte of 1 mM KCl and their removal was evaluated at different pH values (from 5.0 to 8.6), with or without 1 mM $CaCl_2$ (Table 7.2), to investigate the interactions between the cyanotoxins and the calcium ion hardness. These conditions were chosen since NFT50 membrane charge and performance are strongly influenced by the physical and chemical characteristics of the feed solution (Ribau Teixeira *et al.* (2005b), Ribau Teixeira and Rosa (2005), chapters 5 and 6 respectively). The feed water samples were spiked with 100 μg/L of MC-LR, 20 μg/L of MC-LY and 30 μg/L of MC-LF (*i.e.* 150 μg/L MC-LR eq.).

The second set of trials was performed to evaluate the microcystins efficiency removal from waters containing different types of NOM, at different pH values and water recovery rates. These experiments consisted of concentration runs, where it was intended to simulate the industrial NF operation at different water recovery rates, defined as the ratio between the permeate and the initial feed volumes. In the beginning of the concentration runs, the solutions were given a time to equilibrate after which a flux measurement and samples from

the feed and the permeate were taken to serve as baseline for flux and rejection at 0% water recovery rate. The permeate was then not recycled to the feed reservoir until a stipulated permeate volume was obtained. At this time, permeate was recycled to the feed reservoir during the stabilisation period, after which the flux was measured and feed and permeate samples were again collected and the run followed to the next recovery rate. All samples from the feed and permeate solutions, taken at different recovery rates, were analysed for microcystins, NOM (DOC and UV_{254nm}), turbidity and salt rejection (by conductivity measurements). Flux was continuously measured during the experiments. These experiments were performed with the waters listed in Table 7.2 spiked with 10 µg/L MC-LR and 6 µg/L MC-LY. The spiked solutions stayed overnight at room temperature before use. Two pH values were tested to cover the typical pH range of the ozonated and decanted waters from Alcantarilha WTP. Deionised water trials were also performed to serve as a baseline for the flux and to calculate the relative flux.

For both sets of trials, and between each NF run, membranes were washed until the pure water flux reached 90% of the initial value measured after compaction and the bulk conductivity was similar to that of DI. The temperature was maintained at 25 ºC during the experiments.

7.3 RESULTS AND DISCUSSION

Figures 7.2a and 7.2b present the relative flux and the conductivity rejection of KCl and KCl + $CaCl_2$ electrolytes spiked with 150 µg/L MC-LR eq., as a function of solution pH. For comparison purposes, similar data obtained with no microcystins spiking (Ribau Teixeira *et al.* (2005b), chapter 5) is also shown. Figures 7.2c and 7.2d depict the microcystins rejection with KCl and KCl + $CaCl_2$ electrolytes, respectively.

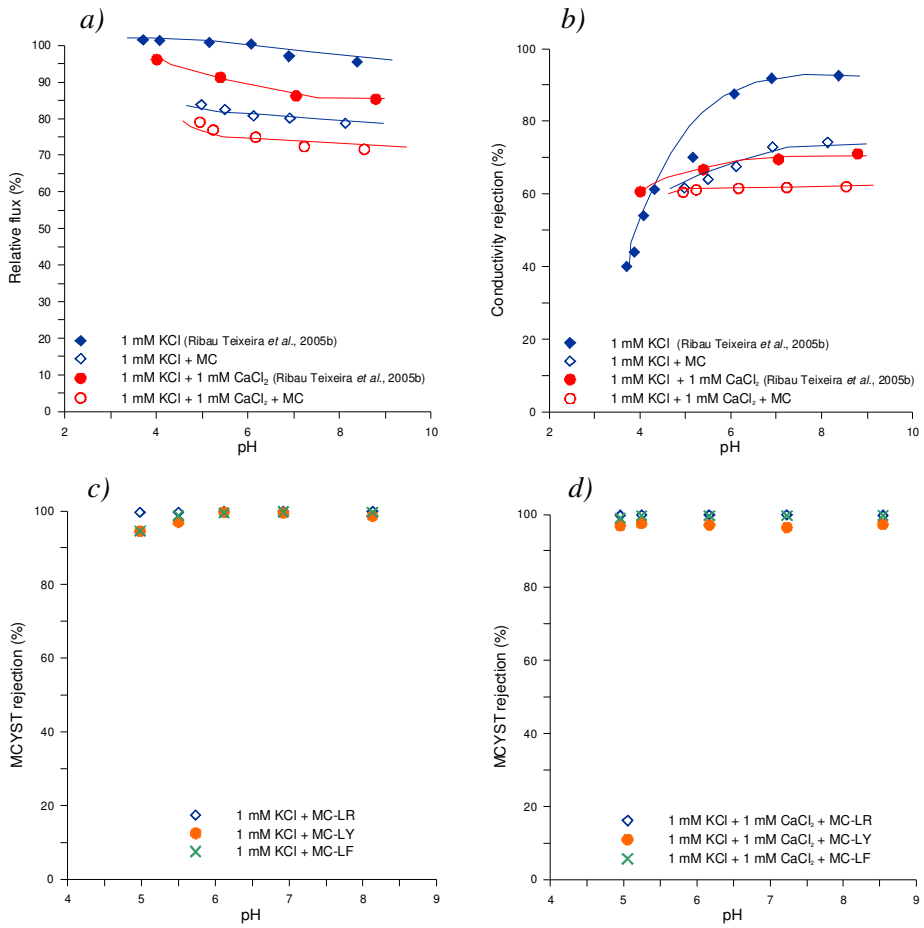

Figure 7.2 NF performance with the electrolyte solution spiked with 150 µg/L MC-LR eq. (100 µg/L MC-LR, 20 µg/L MC-LY and 30 µg/L MC-LF) at different pH: **a)** relative flux, **b)** conductivity rejection, and **c,d)** MC variants rejection (10 bar, 25 ºC).

In the presence of microcystins flux decreases approximately 16% for both KCl and $CaCl_2$ electrolytes. The observed flux decrease with pH was already found for these electrolytes without MC (Figure 7.2a). For conductivity rejection, there is also a decrease in the presence of MC for both electrolytes. Calcium is again responsible for the same flux and conductivity rejection decrease previously reported with no MC (Figures 7.2a and 7.2b). Microcystins rejections are very high for all identified MC variants, namely MC-LR, MC-LY and MC-LF, both in the presence or absence of $CaCl_2$ (Figures 7.2c and 7.2d).

The flux decrease and the conductivity rejection increase with pH are both attributed to the membrane negative charge and the osmotic pressure effects (Ribau Teixeira *et al.* (2005b),

chapter 5). As the pH increases, the membrane becomes more negatively charged and its pore size is reduced which causes a decrease in flux and a rejection increase. This effect is particularly important for KCl, for which the zeta potential varies significantly through the pores, whereas in the presence of $CaCl_2$ the zeta potential through pores is almost constant with pH. As the rejections increase with pH, the osmotic pressure near the membrane surface also increases, which results in a decrease in the net driving pressure and therefore leads to a decrease in water flux (Ribau Teixeira *et al.* (2005b), chapter 5). It seems that, in the presence of MC, these effects on flux and conductivity rejection are similar to those observed without MC but to a lower extent (Figures 7.2a and 7.2b).

MC-LR contains two ionisable carboxyl groups on D-glutamate and D-erythro-β-methylaspartic acid and one ionisable amino on arginine. Based on the pKa values of these groups, at the pH values used in this work (5 – 9) the dominant species is $(COO^-)_2(NH_2^+)$ and the net charge of MC-LR is negative, as a result of the dissociation of the carboxyl groups (Maagd *et al.* (1999), Cook and Newcombe (2002)). For MC-LY and MC-LF the net charge will be more negative than MC-LR as they contain the same two ionisable carboxyl groups on D-glutamate and D-erythro-β-methylaspartic acid and no more ionisable groups since the amino acids present in these microcystins (tyrosine and phenylalanine, respectively for MC-LY and MC-LF) are neutral. Lawton *et al.* (1998) referred that the MC net charge is negative but weakly charged.

Microcystins are very large compared to the membrane pore size (Table 7.1) and are highly rejected by the membrane, being usually below the quantification limit in the permeate. In addition, the identified MC variants are hydrophobic, and hydrophobic compounds adsorb onto membrane surfaces (Jucker and Clark (1994), Nilson and DiGiano (1996)). Therefore,

and despite the very low MC feed concentration, the significant flux decrease observed in the presence of MC may be due to the fouling or adsorption of the MC onto the membrane surface and/or to the osmotic pressure gradient developed. By mass balance calculations (Ribau Teixeira *et al.* (2005a)), the adsorption of microcystins in the presence of $CaCl_2$ is 49 – 57% of the total mass available, whereas 25 – 38% of the microcystins available adsorb in the absence of calcium. The existence of the fouling adsorption phenomenon agrees with the higher relative fluxes obtained (75% *vs.* 95%) when the MC feed concentration decreases by one order of magnitude (from 100 µg/L MC-LR + 20 µg/L MC-LY + 30 µg/L MC-LF *vs.* 10 µg/L MC-LR + 6 µg/L MC-LY, Figure 7.2a *vs.* Figure 7.3a). Similar studies with amino acids, peptides and proteins (since there are no similar studies with MC) have shown severe protein adsorption onto membranes (Kokubo *et al.* (1996), Burns and Zydney (1999), Atra *et al.* (2004), Groleau *et al.* (2004)). Burns and Zydney (1999) referred a permeability reduction of more than 40% due to the protein molecular weight higher than the membrane molecular cut-off. Pouliot *et al.* (1999) attributed the flux decrease of a peptide fraction from whey protein hydrolysate to the higher osmotic pressure gradient across the UF/NF membranes due to the ionic strength increase. Atra *et al.* (2004) obtained a permeate flux decrease of lactose from milk and whey proteins with the increase in the concentration factor related to the osmotic pressure increase.

The conductivity rejection decrease observed in the presence of microcystins (Figure 7.2b) may be explained by the interactions between the ionized groups on the membrane surface and the weakly charged MC and the inorganic ions. At pH above 5, the membrane exhibits a negative charge which causes Cl^- and MC co-ions repulsion. As a result, the repulsions between co-ions and the membrane surface produce a transmembrane flow of counterions which lead to an electric field towards the outside of the membrane. To maintain the

electroneutrality an electromigrative flux of the Cl^- co-ion will cross the membrane since it is more mobile and MC is too large. In fact, since the local ionic strength increases, the presence of MC in the mixture can reduce the membrane/co-ion repulsions. Higher ionic strength decreases the thickness of the double layer, for the shielding effect is stronger, leading to a decrease of the membrane-anions repulsion forces and finally to a lower rejection. Martin-Orue *et al.* (1998) and Grib *et al.* (2000) used the same mechanism to explain the separation of amino acids and peptides in salt solutions. Garem *et al.* (1997) reported that in a mixture of charged solutes every amino acids or inorganic salt is assumed to contribute individually to the Donnan equilibrium, as a function of its own respective concentrations on both sides of the membrane, and its own mobility. The content of inorganic ions in the mixture compared to the content of ionised amino acids may constitute an important parameter for optimisation of selectivity (Garem *et al.* (1997)). In the presence of $CaCl_2$, rejections decrease further (Figure 7.2b) for the membrane is less negatively charged, since $CaCl_2$ adsorbs onto the membrane surface (Ribau Teixeira *et al.* (2005b), chapter 5) and divalent Ca^{2+} ions bind to the negatively charged MC. Menon and Zydney (1999) also referred that the charge of the protein bovine serum albumin is significantly reduced in the presence of $CaCl_2$, compared to NaCl, due to the binding of Ca^{2+} to the negatively charged protein at pH above its isoelectric point. The reduction of the protein charge causes a significant decrease in protein rejections.

Microcystins rejections are quite constant and very high (97.0 – 99.9%) for all studied pH values (Figure 7.2c and 7.2d). In most cases, the lower rejections obtained (97%) are due to the MC quantification limit, *i.e.* they correspond to no quantification of a MC variant in the permeate.

The main mechanism responsible for MC rejection by the membrane must be size exclusion, due to the MC size (*ca.* 1000 Da, Table 7.1) compared to the membrane cut-off (150 Da) and the MC overall net charge (negative but weakly charged). However, other parameters apart from molecular size may affect rejection. Van der Bruggen *et al.* (1999) reported that polarity decreases rejection, which may be explained by electrostatic interactions directing the dipole towards the membrane. Bellona *et al.* (2004) referred, among other parameters that hydrophobic-hydrophobic interactions between the solute and the membrane are an important factor for the rejection of hydrophobic compounds (as the microcystins) and that steric hindrance may also contribute to rejection. The strong hydrophobic behaviour of MC, responsible for the above discussed flux decrease due to microcystins adsorption onto the membrane surface, may also contribute to the lower rejections obtained in few cases. Despite the short elapsed time in the experiments, MC may be slowly desorbing (and to a low extent) to the permeate.

The presence of $CaCl_2$ in this concentration seems not to influence the MC rejection by the membrane (Figure 7.3d), besides the already proved influence of the $CaCl_2$ on the membrane performance (Ribau Teixeira *et al.* (2005b), chapter 5).

Figure 7.3 shows the relative fluxes of DW, DW+SA+AHA and OW spiked with MC at two pH values (*ca.* 6 and *ca.* 8) and at different water recovery rates (0 – 90%). Table 7.3 presents the rejections of conductivity, turbidity, DOC, UV_{254nm}, MC-LR and MC-LY. Data relative to KCl + $CaCl_2$ electrolyte spiked with MC are also shown in Figure 7.3 and Table 7.3 for comparison purposes.

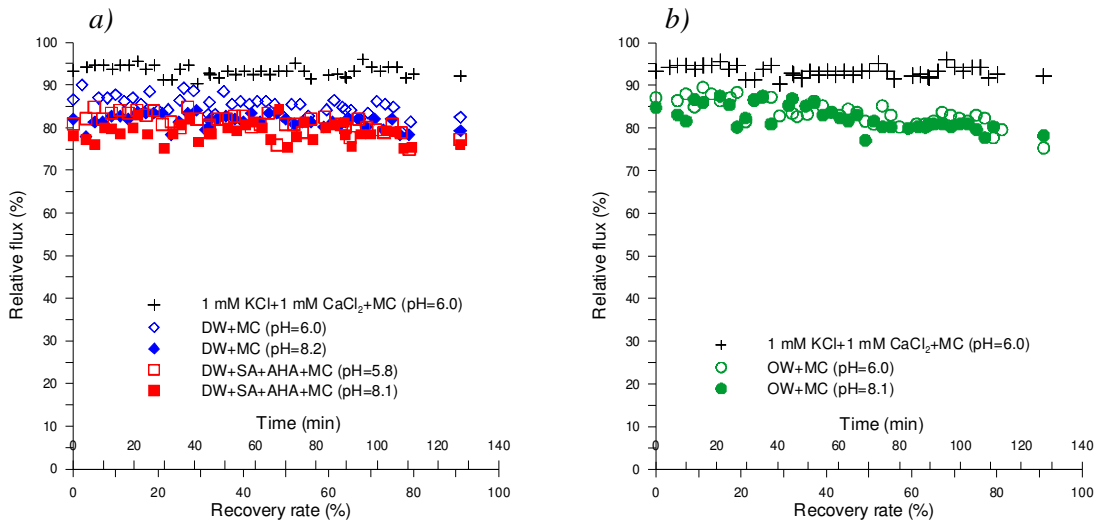

Figure 7.3 Relative fluxes of natural waters spiked with 16 µg/L MC-LR eq. (10 µg/L MC-LR and 6 µg/L MC-LY) at different water recovery rates and two pH values: **a)** DW and DW + SA + AHA, **b)** OW (10 bar, 25 ºC).

The results show the relatively low variation of flux with experimental time and recovery rate (Figure 7.3a) for all types of clarified water and pH values studied. Due to the progressive accumulation of particles near the membrane surface OW presents a slight flux decrease for higher water recovery rates (Figure 7.3b). In addition, fluxes are lower than those obtained with the electrolyte solution spiked with MC (1 mM KCl + 1 mM $CaCl_2$ + MC). This flux decrease is observed for all types of water and pH values studied (Figure 7.3) and must be due to both the organic (NOM) and the inorganic (calcium hardness) water matrixes. The results are attributed to both physical and chemical aspects of NOM filtration. NOM is considered one of the major causes of NF fouling during the filtration of surface waters (Nyström *et al.* (1995), Nilson and DiGiano (1996), Hong and Elimelech (1997)). In addition, calcium ions adsorb onto the membrane surface and reduce its negative charge, so the membrane permeability decreases (as reported previously for electrolyte solutions (Ribau Teixeira *et al.* (2005b), chapter 5)) and natural waters (Ribau Teixeira and Rosa (2005), chapter 6) and the membrane − NOM interaction increases. Calcium can also contribute to the increase of the membrane hydrophobicity by bridging two negatively charged functional groups of NOM

macromolecules and between the membrane, and the negatively charged part (hydrophilic part) of humic molecules (Ribau Teixeira and Rosa (2005), chapter 6). Nilson and DiGiano (1996) concluded that the hydrophilic fraction had little influence on the permeate flux and was less rejected than the hydrophobic fraction, whereas the hydrophobic NOM fraction was responsible for the permeate flux decline. Kimura *et al.* (2003) observed a significant adsorption of hydrophobic compounds onto the membrane, especially when the compounds were electrostatic neutral. According to Edzwald and Van Benschoten (1990) classification based on SUVA values, the organic matter of the water samples studied is hydrophilic, has low molecular weight, and DOC is largely composed of non-humic substances. NOM hydrophilicity decreases from DW to OW and finally to DW+SA+AHA (Table 7.2), as well as the flux (Figure 7.3). There is no significant flux variation in the studied pH range, as already obtained with this membrane and operating conditions in similar experiments with the same natural water samples but with no MC spiking (Ribau Teixeira and Rosa (2005), chapter 6).

As far as the conductivity rejections are concerned (Table 7.3), there is no significant variation with pH (in this 6 – 8 pH range), recovery rate and type of water. The same behaviour was observed in similar experiments with NOM model solutions without MC (Ribau Teixeira and Rosa (2005), chapter 6) and with MC model solutions without NOM (Figure 7.3b). As already mentioned, at these pH values (above 5 – 6), the membrane has a slight negative and constant charge, the anions are rejected and with them the cations.

Turbidity rejections decrease from DW+SA+AHA to OW and DW (Table 7.3). This is related with the influent turbidity of the water samples (OW as the highest turbidity and DW the lowest, Table 7.2), and with the initial adsorption of the OW turbidity particles onto the

membrane surface. In fact, the influent OW turbidity was 4.93 NTU (no particle removal takes place in ozonation) and for DW+SA+AHA it was 4.31 NTU, this turbidity coming largely from the AHA amendment. After the NF stabilisation time, the turbidity of the first measurement after the stabilisation period at 0% recovery rate was 2.90 NTU for OW and 4.01 NTU for DW+SA+AHA. This means that turbidity particles present in OW adsorb faster onto the membrane surface than those from DW+SA+AHA. There is also a slight decrease of turbidity rejection with recovery rate attributed to the turbidity adsorption since the OW feed turbidity decreases with recovery rate. Nevertheless, permeate turbidity is always below 0.13 NTU (Table 7.4), much lower than the Portuguese standard of 1 NTU for drinking water.

Concerning the NOM parameters, DOC and UV_{254nm} absorbance, rejections do not significantly vary with the pH nor with type of water, due to the similar DOC content of the waters (Table 7.2) and the high rejections of UV_{254nm} (~100%). DOC rejections are lower than UV_{254nm} rejections since the UV absorbance at 254 nm is mainly due to the adsorption by aromatic/hydrophobic compounds, whereas DOC measures the dissolved concentration of carbon containing molecules (Schäfer *et al.* (2000)). DOC rejections increase with recovery rate as the feed DOC concentrations increase (Ribau Teixeira and Rosa (2005), chapter 6). Although there are no national standards for UV_{254nm} and TOC or DOC in drinking water, these are capital parameters due to their relation to the trihalomethane (and other disinfection by-products) formation potential (THMFP) in the finished water. The results of permeate DOC, UV_{254nm} and SUVA quality show very low values (≤1 mg C/L, <0.002 cm^{-1} and 0 – 0.25 L/(m.mg), respectively, Table 7.4) from which minimal THMFP may be expected (EPA (1999)), and also for the fact that hydrophilic DOC has lower potential to form THM than hydrophobic DOC (Galapate *et al.* (2001)).

Table 7.3 Conductivity, turbidity, DOC, UV_{254nm} and microcystins variants removal efficiency (%) for the different types of water samples and pH values studied, at 0%, 64% and 90% recovery rates.

Parameters	Conductivity	Turbidity	DOC	UV_{254nm}	MC-LR	MC-LY
	0% / 64% / 90%	0% / 64% / 90%	0% / 64% / 90%	0% / 64% / 90%	0% / 64% / 90%	0% / 64% / 90%
1 mM KCl + 1 mM $CaCl_2$ + MC						
pH = 6.0	73.0 / 75.4 / 76.4	-	65.4 / 66.0 / 71.5	-	>98.6 / >99.4 / 98.8	97.3 / 96.6 / 97.2
DW + MC						
pH = 6.0	80.0 / 80.8 / 80.4	87.0 / 84.6 / 82.9	82.6 / 89.9 / 94.9	100 / 100 / 100	>97.9 / 98.8 / 98.1	>98.6 / >98.4 / >98.9
pH = 8.2	85.1 / 84.3 / 84.3	89.4 / 88.9 / 87.4	70.9 / 86.8 / 91.5	100 / 100 / 100	>98.4 / >98.9 / 98.8	>97.6 / >98.8 / >99.4
DW+AHA+SA + MC						
pH = 5.8	82.1 / 82.0 / 81.6	97.7 / 97.5 / 97.6	81.2 / 88.2 / 92.2	100 / 100 / 100	>98.1 / >98.8 / >99.4	>97.6 / >98.4 / >98.9
pH = 8.1	84.1 / 83.8 / 83.5	97.6 / 96.3 / 93.5	79.6 / 86.2 / 93.3	100 / 100 / 100	>98.5 / >99.1/ >99.3	>98.1 / >98.8 / >99.5
OW + MC						
pH = 6.0	79.3 / 80.7 / 79.3	96.8 / 87.7 / 85.3	61.6 / 80.1 / 89.7	100 / 97.1 / 96.3	>98.3 / >98.8 / >99.1	94.2 / 98.6 / 97.5
pH = 8.1	84.2 / 83.3 / 83.4	95.1 / 85.5 / 85.9	72.9 / 82.3 / 90.7	100 / 100 / 100	>98.4 / >98.7 / >98.5	>98.9 / >98.9 / >99.5

Table 7.4 Conductivity, turbidity, DOC, UV_{254nm} and microcystins variants permeate quality for the different types of water samples and pH values studied, at 0%, 64% and 90% recovery rates.

Parameters	Conductivity (µS/cm)	Turbidity (NTU)	DOC (mg C/L)	UV_{254nm} (cm^{-1})	MC-LR (µg/L)	MC-LY (µg MC-LR eq./L)
	0% / 64% / 90%	0% / 64% / 90%	0% / 64% / 90%	0% / 64% / 90%	0% / 64% / 90%	0% / 64% / 90%
1 mM KCl + 1 mM $CaCl_2$ + MC						
pH = 6.0	62.1 / 65.7 / 67.1	-	0.67 / 0.90 / 0.64	-	<0.11 / <0.10 / 0.23	0.18 / 0.38 / 0.44
DW + MC						
pH = 6.0	55.0 / 82.7 / 118.5	0.08 / 0.06 / 0.06	0.61 / 0.58 / 0.62	0 / 0 / 0	<0.13 / 0.16 / 0.25	<0.13 / <0.14 / <0.13
pH = 8.2	38.9 / 66.3 / 95.5	0.08 / 0.07 / 0.06	0.64 / 0.53 / 0.57	0 / 0 / 0	<0.13 / <0.13 / 0.20	<0.13 / <0.12 / <0.12
DW+AHA+SA + MC						
pH = 5.8	47.9 / 76.3 / 110.7	0.09 / 0.11 / 0.09	1.00 / 0.89 / 0.80	0 / 0 / 0	<0.12 / <0.13 / <0.12	0.17 / <0.14 / <0.13
pH = 8.1	41.6 / 67.2 / 98.1	0.08 / 0.08 / 0.09	1.24 / 1.01 / 0.88	0 / 0 / 0	<0.13 / <0.12 / 0.14	<0.13 / <0.12 / <0.12
OW + MC						
pH = 6.0	56.7 / 82.7 / 125.4	0.09 / 0.09 / 0.08	1.27 / 0.81 / 0.80	0 / 0.001 / 0.002	<0.12 / <0.12 / <0.14	0.20 / 0.18 / 0.27
pH = 8.1	40.7 / 68.1 / 97.1	0.13 / 0.12 / 0.10	0.95 / 0.73 / 0.53	0 / 0 / 0	<0.12 / <0.12 / 0.24	<0.12 / <0.12 / < 0.14

Microcystins rejections are very high for both MC-LR, and MC-LY variants (which are usually not quantified in the permeate), and no significant variation with recovery rate and pH is observed. The high rejections are mainly related with the MC size compared to the membrane pore size, as explained earlier. The possibility of slow desorption of MC to the permeate is more evident in this set of trials, since the few cases of toxin quantification in the permeate occur, mainly, for the higher water recovery rates, *i.e.* for the longer experimental time and higher influent concentrations of the hydrophobic MC. Another important observation from these results is that the type and the NOM concentrations studied do not influence the rejection of MC (compare Figure 7.2d and Table 7.3). In turn, the presence of MC does not change the rejection of NOM (Ribau Teixeira and Rosa (2005), chapter 6). Microcystins concentrations obtained in the NF permeate (Table 7.4) are always far below the WHO drinking water guideline value of 1 μg/L for MC-LR. In fact, in most cases they are below the quantification limit.

7.4 CONCLUSIONS

This study demonstrated that NF membranes are an effective barrier against microcystins in drinking water. NF removed all the microcystin variants present in water (MC-LR, MC-LY and MC-LF) regardless of the variations in feed water quality.

These hepatotoxic cyclic peptides revealed a strong membrane fouling ability for total concentration of 150 μg/L (as MC-LR eq.) which although low are still, at least, one order of magnitude higher than what could be expected in natural waters. For 16 μg/L of total microcystins, the fouling behaviour was largely attenuated. The high MC rejections obtained were mainly related to size exclusion effects, based on the high MC size compared to the membrane pore size, and on the MC overall net charge (negative but weakly charged). The

presence of $CaCl_2$ and NOM, in the studied range, seemed to have no influence on the MC rejection by the membrane.

A relatively low variation of flux was found with the experimental time and recovery rate for all types of clarified water and pH values studied. Fluxes of natural waters spiked with MC were lower than those obtained with the electrolyte solutions spiked with MC. These results were attributed to both the organic and the inorganic water background matrixes, *i.e.* NOM and calcium cations, which reduced the negative charge of the membrane and complexed with humics. DOC and UV_{254nm} rejections did not significantly vary with the pH nor with the type of water, due to the similar DOC content of the waters and the very high (~100%) UV_{254nm} rejections. Permeate DOC, UV_{254nm} and SUVA showed very low values (≦1 mg C/L, < 0.002 1/cm and 0 – 0.25 L/(m.mg), respectively) from which minimal THMFP may be expected. Microcystins concentrations in the NF permeate were always far below the WHO drinking water guideline value of 1 μg/L for MC-LR, being usually below the quantification limit.

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CHAPTER 8
NEUROTOXIC AND HEPATOTOXIC CYANOTOXINS REMOVAL BY NANOFILTRATION MEMBRANES

ABSTRACT

This study investigates the influence of chemical feed characteristics on NF performance for cyanotoxins removal, namely the neurotoxic anatoxin-a (low molecular weight alkaloid, positively charged) and the hepatotoxic microcystins (cyclic peptides of *ca.* 1000 g/mol, negatively charged). Experiments with electrolyte solutions of mono (KCl) and divalent ($CaCl_2$) cations in acid and neutral-alkaline conditions showed that the anatoxin-a removal was governed by electrostatic interactions and steric hindrance, whereas for microcystins, at *ca.* 10 μg/L, the latter was the main mechanism. Decanted water spiked with Aldrich humic acid and salicylic acid was used to study the hydrophobic, high molecular weight and the hydrophilic low molecular weight NOM fractions. Microcystins in the NF permeate were always below the quantification limit, hence far below the drinking water guideline of 1 μg/L MC-LR adopted by the World Health Organisation. Anatoxin-a was almost completely removed from this moderately hard water by NF (94%), regardless of the presence of background organics (NOM and competitive toxin), the water recovery rate (0-90%) and the pH value (4 or 7). In turn, fluxes were significantly impacted by background organics (NOM and microcystin) and, especially, inorganics (pH and calcium).

8 NEUROTOXIC AND HEPATOTOXIC CYANOTOXINS REMOVAL BY NANOFILTRATION MEMBRANES

8.1 INTRODUCTION

Cyanobacteria (blue green algae) may produce a wide range of toxins, as well as taste and odour compounds, as secondary metabolites under certain conditions of growth. Depending on the genera, these toxins may include alkaloid neurotoxins, such as anatoxin-a (Carmichael (1994)), and cyclic peptide hepatotoxins, such as microcystins and nodularin (Codd (1995)).

Anatoxin-a (ATX-a) is a low molecular weight (166 g/mol) alkaloid, a secondary amine 2-acetyl-9-azabicyclo(4-2-1)non-2-ene (Figure 8.1). It mimics the neurotransmitter acetylcholine and can induce muscle twitching and cramping, followed by fatigue and paralysis. If respiratory muscles are affected, ATX-a may cause convulsions and death by suffocation (Carmichael (1994)). It is a potent acute neurotoxin of significant concern with regard to chronic toxicity (Sivonnen and Jones (1999)).

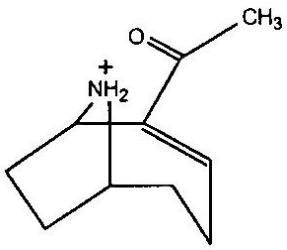

Figure 8.1 The chemical structure of anatoxin-a (Sivonnen and Jones (1999)).

Microcystins cause liver damage and are tumour promoters (Matsushima *et al.* (1992)). As depicted in Figure 8.2, the main structural change in microcystins variants is the variability of L-amino acids 2 (designated as X) and 4 (Z) (Meriluoto (1997)). For microcystin-LR (MC-LR), leusine is in position X and arginine is in position Z. MC-LR contains two ionisable carboxyl groups and one ionisable amino group which are not part of the peptide bonds that

make up the cyclic peptide structure. Microcystins molecular weight varies between 909 – 1115 g/mol, being 994 g/mol for MC-LR.

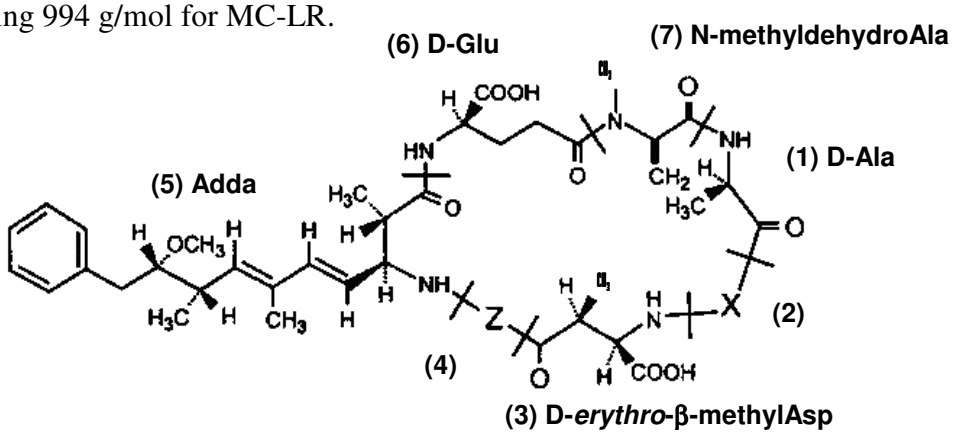

Figure 8.2 General structure of microcystins (Meriluoto (1997)).

The presence of toxins in water, even at low concentrations, is a matter of great concern due to their acute toxicity and sublethal toxicity. Hence, the risk associated with the presence of these toxins in drinking water supplies has become a matter of major concern. To minimise public exposure, the World Health Organisation (WHO) has set a drinking water guideline value of 1.0 μg/L for MC-LR, one of the most commonly occurring cyanotoxins. For anatoxin-a there is still no guideline value adopted by WHO, but the guideline value of 3 μg/L ATX-a is already used in New Zealand (Ministry of Health (2002)).

ATX-a and the several microcystins variants, including MC-LR, have been extensively found in water reservoirs used for drinking water abstraction, and many conventional water treatment technologies (coagulation (C)/ flocculation (F)/ sedimentation (S) and filtration) have been reported to be ineffective for removing them (Hoffmann (1976), Himberg *et al.* (1989), Chow *et al.* (1999), Hrudey *et al.* (1999)). Therefore, to reduce the public health risk it is of great importance to investigate safe barriers against cyanotoxins in a wide range of natural waters. One promising treatment process to effectively remove cyanobacteria and cyanotoxins, with no problems of potential health hazardous by-products formation, is membrane pressure-driven filtration, since it acts as a physical barrier. Microfiltration (MF)

and ultrafiltration (UF) will be adequate for removing the cyanobacterial cells but not the cyanotoxins, due to the large pore size and high cut-off of these membranes. Nanofiltration (NF) will successfully retain microcystins but anatoxin-a removal will depend on the membrane charge and cut-off, as well as on the operating conditions. Besides the process hydrodynamics, both the organic and inorganic water background matrixes impact the NF membrane performance (Zhang *et al.* (2004), Ribau Teixeira and Rosa (2005a), chapter 6). Consequently, a proper membrane and optimal operating conditions should be used to guarantee that both low and high molecular weight cyanotoxins (anatoxin-a and microcystins) will be removed.

Despite its high potential, few studies were found in the literature on membrane technology for removing cyanobacteria and cyanotoxins, none of them studied the anatoxin-a removal. Chow *et al.* (1997) obtained a high removal efficiency (>98%) of *Microcystis aeruginosa* cells by MF and UF in both dead-end and cross-flow laboratory modes. There were some cells damaged after filtration but no significant toxin increase in the permeate. Bottino's *et al.* (2001)) pilot plant study evaluated the ability of a ceramic MF membrane to remove particles, microorganisms, algae and disinfection by-products from lake Brugneto (Italy) raw water. Results indicated that, despite its high content in the raw water ($4.6x10^5$/L), the cyanobacterium *Oscillatoria rubescens* was completely retained. Hart and Stott (1993) NF studies with natural waters spiked with 5 – 30 μg/L of microcystins showed microcystins concentrations below 1 μg/L in the permeate. Muntisov and Trimboli (1996) also demonstrated that MC-LR and nodularin were removed by NF membranes from a river water spiked with 8 μg/L of such toxins. Vuori *et al.* (1997) investigated the removal of nodularin from brackish water by RO. As the salt and toxin concentration increased in the raw water, traces of nodularin were detected in the treated water, still it remained below the limit of

quantification. Neumann and Weckesser (1998) evaluated the removal of MC-LR and MC-RR (initial concentrations of 70 – 130 μg/L) from tap water and tap water containing 3000 mg/L NaCl (salt water) by three types of RO membranes (25 – 35 bar). Average rejection varied between 96.7% and 99.9% in tap water, and 98.5% and 99.6% in salt water.

The performance of a negatively charged NF membrane on microcystins removal was recently evaluated, addressing the effects of feed water background organic and inorganic matrixes, particularly NOM and calcium (Ribau Teixeira and Rosa (2005b), chapter 7). Results showed that this NF membrane is a very effective barrier against all microcystin variants present in the raw water (MC-LR, MC-LY and MC-LF), regardless of the variations in feed water quality. Microcystins revealed a strong membrane fouling ability for concentrations of 150 μg/L MC-LR eq., but for 16 μg/L of total microcystin (similar to what may be expected in natural waters) the fouling behaviour was largely attenuated. The high microcystins rejections obtained (above 97%) were related to size exclusion effects, based on the high microcystin size compared to the membrane pore size, and on the microcystins overall net charge (negative but weakly charged). Microcystin concentration values obtained in the NF permeate (<0.23 μg/L MC-LR and usually below the quantification limit) were always far below the WHO drinking water guideline value of 1 μg/L MC-LR. The presence of $CaCl_2$ and NOM, in the studied range, seemed to have no influence on the microcystins rejection by this membrane.

From those results, the NF removal of low molecular weight toxin, such as anatoxin-a, for which steric hindrance might not be the major removal mechanism should be investigated. Competitive aspects between anatoxin-a and other cyanotoxins (microcystins), or background organic (NOM) and inorganic matrixes, must also be studied. Therefore, the aim of the

present study is to evaluate the NF performance for anatoxin-a and microcystin removal from natural waters, in the presence of NOM and $CaCl_2$, at acid and alkaline conditions.

8.2 MATERIALS AND METHODS

8.2.1 CYANOTOXINS

Anatoxin-a used in this study is a pure reagent kindly supplied by G.A. Codd and J. Metcalf (University of Dundee, UK) within the TOXIC European Project, "Barriers against cyanotoxins in drinking water".

Microcystins were extracted from a culture of *Microcystis aeruginosa* supplied by Pasteur Culture Collection (PCC7820) and maintained in laboratory. The experimental procedures to extract microcystins from cultures, to prepare the microcystins stock solution and solutions for NF experiments were already described in Ribau Teixeira and Rosa (2005b) (chapter 7), which followed the standard operation procedure developed by Meriluoto and Spoof (2005b).

8.2.2 NATURAL WATER SAMPLES

Decanted water (DW) (after ozonation, C/F/S) from Tavira Water Treatment Plant (WTP), Algarve, Portugal, was the natural water used in these experiments. This WTP supplied water to *ca.* 730,000 people in southern Portugal (Algarve) in 2004, and was designed to treat up to 2.2 m^3/s of surface water from Odeleite and Beliche Dams reservoirs (165 hm^3). It is a moderately hard water (95 - 112 mg $CaCO_3/l$) with the characteristics presented in Table 8.1. The lack of high molecular weight, hydrophobic NOM in the selected natural water (very low SUVA values, ensured by water pre-ozonation) was overcome by spiking DW with humic acids, as described below.

8.2.3 CHEMICALS AND NOM MODEL SUBSTANCES

Salicylic acid (SA) and Aldrich humic acid (AHA) were the NOM model substances used to spike the DW (Table 8.1). The salicylic acid is a certified analytical grade from Merck (>99.0% purity) with a low molecular weight (138.12 g/mol) and was used without any purification. AHA was purified through a repeated precipitation with HCl as described by Hong and Elimelech (1997) and already presented in Ribau Teixeira and Rosa (2005a) (chapter 6). The molecular weight of purified AHA should be higher than 50 kDa, since it was purified by a dialysis membrane with a cut-off of 50 kDa.

Deionised water (DI) was used for the preparation of all stock solutions. Certified analytical grade potassium chloride (KCl) and calcium chloride ($CaCl_2$) salts were used. HCl and KOH were used for adjusting the solution pH.

Table 8.1 Characteristics of the studied water samples after spiking with cyanotoxins (10 µg/L ATX-a and MC-LR each).

Water type	pH	Conductivity (µS/cm)	Turbidity (NTU)	DOC (mg/L)	UV_{254nm} (1/cm)	SUVA (L/(m.mg))
1 mM KCl	5.8	100	-	1.80	-	-
1 mM KCl + 1 mM $CaCl_2$	6.0	217	-	1.89	-	-
DW	7.0	185	0.30	2.60 *	-	-
DW+SA+AHA	6.9	224	3.35	7.50	0.23	3.07

SUVA: specific UV absorbance, defined as the UV absorbance expressed per meter of absorbance per unit concentration of DOC in mg/L. (*) TOC value.

8.2.4 MEMBRANES

The investigated NFT50 membrane is a thin film composite NF/RO membrane of polypiperazine amide on a polysulfone microporous support and a polyester support, from Alfa Laval, with an hydraulic permeability of 5.9 kg/(h·m^2·bar) at 25 ºC, a cut-off of 150 g/mol and a pore radius of 0.43 nm (Ribau Teixeira *et al.* (2005), chapter 5).

From previously published data on zeta potential measurements (Ribau Teixeira *et al.* (2005), chapter 5), the membrane surface is slightly positive at pH 4 (1 mV), passes through an isoelectric point (i.e.p.) at pH 4.2 ± 0.2 and is negatively charged above this pH (until 8.3). The surface charge is about -10.3 mV at pH 6.9 with a background electrolyte of 1 mM KCl. In the presence of calcium divalent hardness cations (1 mM $CaCl_2$), the i.e.p. shifts from 4.2 to 5-6 and the membrane is less negatively charged over the entire pH range (-2.6 mV at pH = 7.3).

8.2.5 ANALYTICAL METHODS

Samples were analysed for pH (at 25ºC, using a Whatman WTW pH340 meter), conductivity (Crison GLP32 conductimeter), dissolved organic carbon (DOC) (Shimadzu TOC 5000A analyser, 50 ppb – 4000 ppm), UV_{245nm} absorbance (Beckamn DU-640B UV/VIS spectrophotometer) and turbidity (HACH 2100N turbidity meter of high resolution, 0.001 NTU) using standard methods of analysis. The permeate fluxes were determined by weight (analytical balance Shimadzu, model BX 620S).

ATX-a was first extracted from the aqueous samples using an isolute C18 solid phase extraction column, 1 g in a 6 mL reservoir, following the interim standard operation procedure developed for ATX-a by Metcalf and Codd (2005b). The cartridges were first conditioned with 10 mL methanol 100% followed by 10 mL of milli-Q water, without letting it dry during conditioning. The samples were then applied to the cartridge and the ATX-a was eluted with 5 mL methanol 100% containing 0.1% of trifluoroacetic acid. The methanolic elute was evaporated at 50-54 ºC in a rotavapor, resuspended in 500 µL milli-Q water, centrifuged at 10,000 x *g* during 10 min, and transferred to HPLC vials for analysis (Metcalf and Codd (2005b)).

The extraction of microcystins from the water samples was already described in Ribau Teixeira and Rosa (2005b) (chapter 7), and followed the standard operation procedure developed by Meriluoto and Spoof (2005b).

Anatoxin-a and microcystins were analysed by HPLC-PDA using a Dionex Summit system, which includes a high pressure gradient pump (Dionex Summit), an autosampler (Dionex ASI-100), a column oven (Dionex STH-585) and a photo diode-array detector (Dionex PDA-100). A C18 column was used (Merck Purospher STAR RP-18 endcapped, 3 μm particles, LiChroCART 55x4 mm). The mobile phase used a gradient of milli-Q water and acetonitrile, both with 0.05% (v/v) of trifluoracetic acid. Chromatograms were analysed between 180 - 900 nm, with a main detection at 230 nm for the typical anatoxin-a spectra (Metcalf and Codd (2005a)) and at 238 nm for microcystins (Meriluoto and Spoof (2005a)).

8.2.6 NF PERMEATION EXPERIMENTS

The experiments were carried out with a M20 plate and frame unit from Danish Separation Systems (membrane area of 0.0360 m^2 up to 0.720 m^2; maximum pressure 80 bar; maximum flow 18 L/min, and constant temperature maintained by an heat exchanger). In this study the membrane area tested was 0.0720 m^2. The membranes were first compacted and were then stabilised with DI until achieving a steady permeate flux, at the pressure and crossflow velocity to be used in the experiments, 10 bar and 8 L/min respectively.

Two types of trials were performed to evaluate the NF efficiency for cyanotoxins removal from surface waters.

In the first set of trials, the experiments were performed with a background electrolyte of 1 mM KCl in DI spiked with 10 μg/L ATX-a. The ATX-a removal was evaluated at two pH values (*ca.* 4 and 8) with and without 1 mM $CaCl_2$, to investigate the toxin removal mechanisms. These conditions were chosen since NFT50 charge and performance are strongly influenced by the physical and chemical characteristics of the feed solution (Ribau Teixeira *et al.* (2005), chapter 5, and Ribau Teixeira and Rosa (2005a), chapter 6).

The second set of trials was designed to evaluate the anatoxin-a removal efficiency from water samples containing NOM and microcystin, at different water recovery rates, and to study the competitive effects between NOM and cyanotoxins. The first experiment used 1 mM KCl+1 mM $CaCl_2$ in DI spiked with 10 μg/L of ATX-a. DW amended with SA, AHA and ATX-a (10 μg/L) was used in the second experiment. The last experiment was performed with DW+SA+AHA spiked with both ATX-a and MC-LR (10 μg/L each), at two pH values (*ca.* 4 and 7). The spiked solutions stayed overnight at room temperature before use. All these experiments consisted of concentration runs, for it was intended to simulate the industrial NF operation at different water recovery rates, defined as the ratio between the permeate and the initial feed volumes. In the beginning of the concentration runs, the solutions were given a time to equilibrate, after which a flux measurement and samples from the feed and the permeate were taken to serve as baseline for flux and rejection at 0% water recovery rate. Permeate was then not recycled to the feed reservoir until a stipulated permeate volume was obtained. At this time, the permeate was recycled to the feed reservoir during the stabilisation period, after which the flux was measured, and feed and permeate samples were again collected, and the run followed to the next recovery rate. All samples from the feed and permeate solutions, taken at the different recovery rates, were analysed for ATX-a,

microcystins, NOM (DOC and UV_{254nm}), turbidity and salt rejection (by conductivity measurements). Flux was continuously measured during the experiments.

For both sets of trials, between each NF run, membranes were washed until the pure water flux reached 90% of the initial value measured after compaction, and the bulk conductivity was similar to that of DI. The temperature was maintained at 25 ºC during the experiments.

8.3 RESULTS AND DISCUSSION

Figure 8.3 shows the flux variation and the removal efficiencies of conductivity and ATX-a at acid and basic pH, with and without $CaCl_2$.

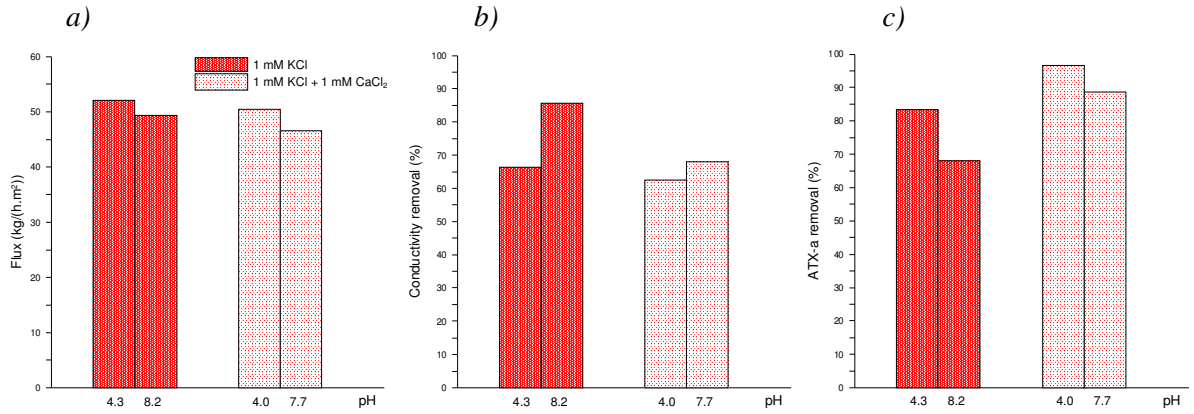

Figure 8.3 NF performance with the electrolyte solutions spiked with 10 µg/L ATX-a at two pH values: **a)** flux, and removal efficiencies of **b)** conductivity and **c)** ATX-a (10 bar, 25 ºC).

Figure 8.3a shows the flux decrease with pH already found for these electrolytes without ATX-a spiking (Ribau Teixeira *et al.* (2005), chapter 5). In the absence of calcium ions, the flux is also higher and the flux decrease with the pH is lower, as well as the conductivity rejections (especially significant at neutral to basic pH, Figure 8.3b).

Such results of flux decrease and conductivity rejection increase with pH are both attributed to the membrane negative charge and to the osmotic pressure effects (Ribau Teixeira *et al.*

(2005), chapter 5). As the pH increases, the membrane surface and pores become both more negatively charged due to the presence of anions, and the membrane pore size is reduced by the repulsion between neighbouring negatively-charged groups (Childress and Elimelech (2000), Schaep and Vandecasteele (2001)). Such effect is particularly important for KCl, since the zeta potential varies significantly through the pores, whereas in the presence of $CaCl_2$ the zeta potential through the pores is almost constant with pH (Ribau Teixeira *et al.* (2005), chapter 5). As the rejections increase with pH, the osmotic pressure near the membrane surface also increases which results in a decrease in the net driving pressure and leads to a water flux decrease.

ATX-a is quite close to the membrane cut-off (defined for 91% solute removal), namely 166 g/mol for ATX-a *vs.* 150 g/mol for membrane cut-off. Based on the sieving curve obtained earlier for this membrane (Ribau Teixeira *et al.* (2005) at pH ~ 6 and 1 mM KCL in DI) one could expect an ATX-a removal efficiency of 95%. However, ATX-a is a secondary amine and its net charge is positive (*p*Ka 9.36, Koskinen and Rapoport (1985)). Data in Figure 8.3c show the important role of membrane−ATX-a electrostatic interactions on ATX-a removal. At basic pH without $CaCl_2$, membrane surface is negatively charged and a membrane−ATX-a electrostatic attraction takes place. This electrostatic attraction overcomes the membrane pore narrowing phenomenon developed under these conditions and leads to a rejection decrease. As the pH decreases, the membrane surface charge becomes less negatively charged (it is close to membrane i.e.p. with 1 mM KCl as background electrolyte), the electrostatic attraction decreases and the ATX-a rejection increases. It is also possible that ATX-a slightly changes the membrane charge, due to adsorption onto the membrane surface, as referred by Garem *et al.* (1997) for amino acids, since there are no studies with ATX-a. They concluded that the counterionic amino acid (1 g/L) may be able to neutralise the membrane charge and

consequently may affect its selectivity. In fact, a previous study (Ribau Teixeira *et al.* (2005), chapter 5) showed that the membrane i.e.p. shifted towards basic pH in the presence of divalent cations such as Ca^{2+} (the i.e.p. shifts from 4.2±0.2 to 5-6 in the presence of $CaCl_2$, Ribau Teixeira *et al.* (2005)). Therefore, there is the possibility that ATX-a increases the membrane i.e.p., so the membrane is slightly positive at the acid pH tested, and the rejection is again higher than at basic pH. The same happens to a greater extent with $CaCl_2$ at acid pH, as the membrane is positively charged at pH 4.3. Rejection increases at this pH due to the repulsive forces between the positively charged membrane and ATX-a, reducing ATX-a adsorption onto the membrane surface. At pH 7.7, the membrane is negatively charged and rejection decreases since ATX-a is attracted to the membrane surface. However, probably due to the low ATX-a concentration used in the experiments (10 μg/L), the effect of ATX-a on membrane charge should be to a low extent, as it does not change the variation of flux and conductivity rejection with the pH obtained in earlier experiments with NOM (negatively charged) or microcystins (weakly negatively charged). Comparing rejections with and without $CaCl_2$, in the presence of $CaCl_2$ rejections are higher for both acid and especially basic pH due to Ca^{2+} ions ability to partially neutralise the membrane negative charge (Ribau Teixeira *et al.* (2005), chapter 5). In addition, ATX-a size also plays a role in the rejection, since rejections are high and the pH affects (narrows) the membrane pore size, as already referred. In the presence of Ca^{2+} and acid pH, rejection is close to what could be expected from the membrane sieving curve. Similar results were obtained by Timmer *et al.* (1998) and Grib *et al.* (2000) with amino acids, and Zhang *et al.* (2004) with the pesticides atrazine and simazine.

Figure 8.4 shows the fluxes of DW+SA+AHA+ATX-a with and without MC-LR, at two pH values and at different water recovery rates, as well as the rejections for conductivity, turbidity, DOC, ATX-a and MC-LR.

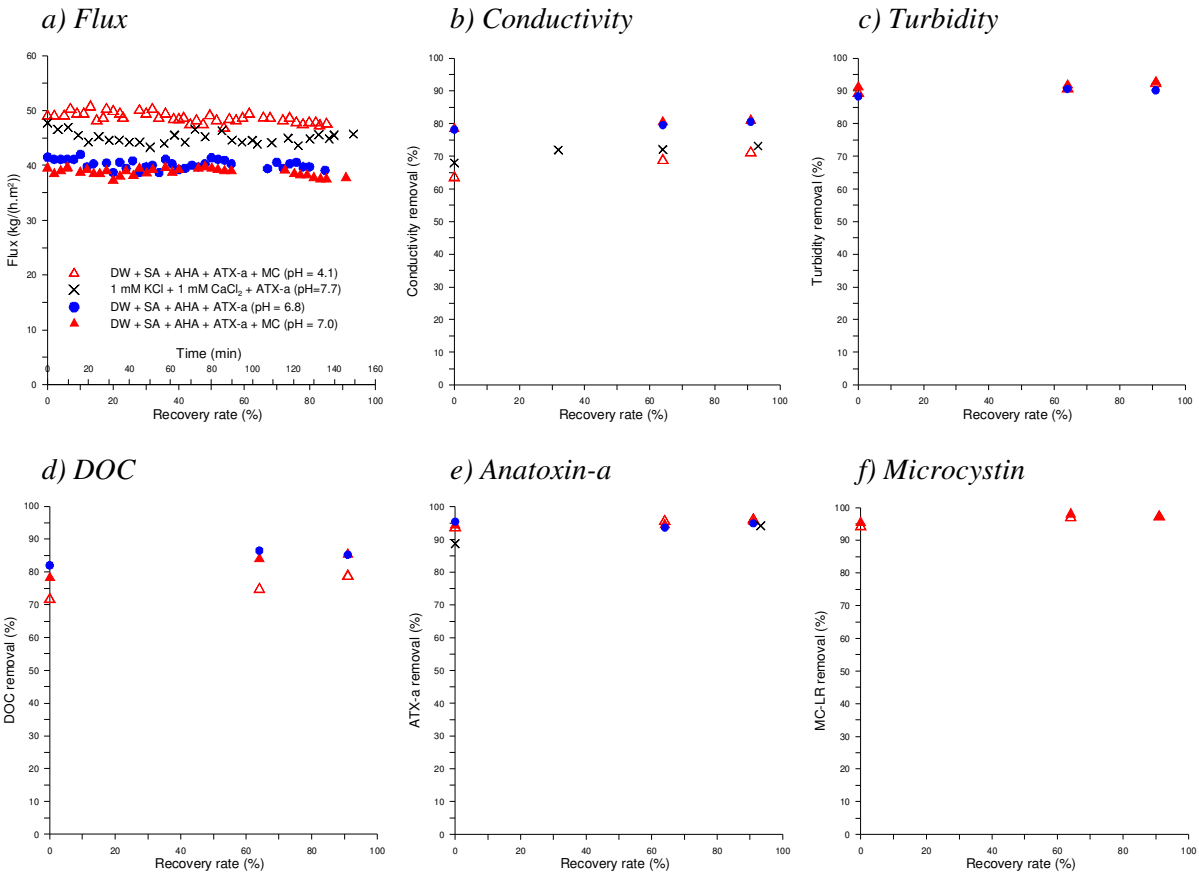

Figure 8.4 NF performance with the natural waters spiked with SA, AHA, ATX-a and MC-LR at different water recovery rates and two pH values: **a)** fluxes and removal efficiencies of **b)** conductivity, **c)** turbidity, **d)** DOC, **e)** ATX-a and **f)** MC-LR (10 bar, 25 ºC, initial concentrations of ATX-a and MC-LR are 10 µg/L each).

Flux shows low variation with experimental time and recovery rate (Figure 8.4a) for this type of clarified water, and for all the situations analysed. Furthermore, experimental data clearly show, once again, the important role of pH on the membrane fluxes (Figure 8.4a) and salt rejections (Figure 8.4b). In neutral to alkaline conditions (*ca.* 7-8), lower fluxes where achieved with this background matrix ($CaCl_2$ and NOM) than the obtained with the electrolyte solution spiked with ATX-a without NOM (KCl+$CaCl_2$) (Figure 8.4a). The results are attributed to both physical and chemical aspects of NOM filtration and calcium hardness. NOM is considered one of the major causes of NF fouling during the filtration of surface water (Nyström *et al.* (1995), Nilson and DiGiano (1996), Hong and Elimelech (1997)).

Calcium ions contribute to the increase of membrane–NOM hydrophobic interactions. On one hand, they adsorb onto the membrane surface and reduce its negative charge (Ribau Teixeira *et al.* (2005), chapter 5). On the other hand, calcium ions can bridge between two negatively charged functional groups of NOM macromolecules, decreasing their charge and increasing their size (*i.e.* increasing NOM hydrophobicity). They are also able to bridge between the membrane and the negatively charged part (hydrophilic part) of humic molecules (Hong and Elimelech (1997), Yoon *et al.* (1998), Ribau Teixeira and Rosa (2005a), chapter 6). The increase in membrane hydrophobicity and membrane solute hydrophobic interactions leads to a permeate flux decline, as well as higher rejections (Nilson and DiGiano (1996), Kimura *et al.* (2003)). According to Edzwald and Van Benschoten (1990) classification, the organic matter of DW+SA+AHA (3.07 L/(m.mg), Table 8.1) is between hydrophilic with low molecular weight (DOC composed by non-humic substances), and hydrophobic with high molecular high (DOC composed by humic substances). Therefore, the flux variation of DW of moderate NOM content and hardness spiked with ATX-a and MC-LR is influenced by the calcium hardness and NOM characteristics. As expected from previous studies (Ribau Teixeira and Rosa (2005b), chapter 7), the flux further decreases in the presence of MC-LR, due to the progressive accumulation of this highly rejected cyclic peptide near the membrane surface.

Permeation of DW spiked with SA, AHA, ATX-a and MC-LR at pH 4 yields much higher fluxes (*ca.* 25% higher), and lower salt rejections (65 – 70% *vs. ca.* 80%) than those obtained at neutral pH. Such results were already obtained in previous studies with NOM but without cyanotoxins (Ribau Teixeira and Rosa (2005a), chapter 6), due to the same mechanisms of membrane (surface and pore) charge and osmotic pressure effects.

As far as membrane ability for water clarification is concerned, turbidity rejections do not significantly vary with pH and recovery rate and they are always above 90% (Figure 8.4c). Concerning the NOM parameters (UV_{254nm} and DOC), the UV_{254nm} rejections are not shown as they are always very high (~100%), regardless of the water pH, the water recovery rate and the presence of cyanotoxins. In turn, as expected, DOC rejections are greatly affected by pH. In acid conditions, NOM passes more easily through the membrane pores, whereas in neutral-alkaline conditions NOM permeation through the membrane pores is more sterically and chemically hindered. At low pH values, both membrane and NOM charges are less negative, hence the membrane pores are larger, the macromolecular configuration smaller and the membrane–NOM electrostatic repulsion is reduced. At high pH values, the sieving effects are stronger (narrow pores and larger molecules) and the repulsive forces between the membrane surface and the NOM could prevent the NOM from adhering onto the membrane surface, producing less fouling and improving the rejection (Figure 8.4d).

In relation to the main aim of this study, both the low molecular weight (ATX-a) and the high molecular weight (MC-LR) cyanotoxins are highly rejected (above 94%), and these rejections do not vary neither with the water recovery rate nor with the water pH. For ATX-a the presence of NOM and MC-LR eliminates the pH effect observed in Figure 8.3c, where calcium shows its ability to attenuate the pH effect. These results may be attributed to the opposite charge between ATX-a and NOM that may reduce the overall net charge of the ATX-a, therefore decreasing the membrane–ATX-a attraction and increasing ATX-a rejection. Moreover, as found for pesticides (Zhang *et al.* (2004)), ATX-a can associate with the NOM functional groups and form macromolecular complexes, which increase the steric hindrances and enhance rejection. MC-LR rejection does not vary with pH, and in all cases, MC-LR concentration in the permeate is below the quantification limit (Table 8.2). The high

removal efficiencies obtained agree with those found in a previous study (Ribau Teixeira and Rosa (2005b), chapter 7), and are related with the MC-LR size compared to the membrane pore size.

One important observation from these results is that the presence of different cyanotoxins (in this case ATX-a and MC-LR) does not significantly change the NF performance. Unlike the widely used adsorption systems, where competition between adsorbates reduces the overall performance for the target contaminants, no negative effects were found between ATX-a, MC-LR and NOM and NF can reach low residuals for all parameters (Table 8.2). Permeate turbidity is always below 0.17 NTU, much lower than the Portuguese standard of 1 NTU for drinking water. There are no national standards for UV_{254nm} and DOC in drinking water. However, these are very important parameters due to their relation to the trihalomethane (and other disinfection by-products) formation potential (THMFP) in the finished water. The permeate shows very low values of DOC (not exceeding 1.5 mg C/L at neutral pH) and no UV_{254nm} absorbance, from which one may expect minimal THMFP (EPA (1999)). For ATX-a there is no standard nor guideline value and the highest residual obtained is 1.3 μg/L at 90% water recovery rate (Table 8.2, for which bulk concentration was 27 μg/L), lower than the New Zealand's drinking water guideline value. MC-LR could never be quantified in the permeate.

Table 8.2 Conductivity, turbidity, DOC, UV_{254nm}, anatoxin-a and microcystins concentrations in the NF permeate for the different types of water and pH values, at 0%, 64% and 90% water recovery rates.

	1 mM KCl + 1 mM $CaCl_2$ + ATX-a pH = 7.7	*DW + SA + AHA + ATX-a* pH = 6.8	*DW + SA + AHA + ATX-a + MC-LR* pH = 4.1	*DW + SA + AHA + ATX-a + MC-LR* pH = 7.0
	0% / 64% / 90%	0% / 64% / 90%	0% / 64% / 90%	0% / 64% / 90%
Conductivity (µS/cm)	67.1 / 83.4 / 99.5	35.6 / 52.7 / 63.9	80.8 / 87.1 / 106.0	31.1 / 47.5 / 62.0
Turbidity (NTU)	-	0.14 / 0.08 / 0.11	0.14 / 0.15 / 0.17	0.09 / 0.09 / 0.12
DOC (mg/L)	-	0.85/ 1.06/ 1.51	1.51 / 1.49 / 2.05	1.31 / 1.01 / 1.23
UV_{254nm} (1/cm)	-	No absorbance	No absorbance	No absorbance
ATX-a (µg/L)	0.89 / - / 0.66	0.89 / 1.27 / 1.33	0.79 / 0.97 / 1.02	0.74 / 1.22 / 1.25
MC-LR (µg/L)	-	-	<0.29 / <0.29 / <0.29	<0.36 / <0.36 / <0.58

8.4 CONCLUSIONS

This study demonstrates that NF membranes are an effective barrier against anatoxin-a and microcystins in drinking water. Anatoxin-a and especially microcystins were almost completely removed, regardless of the variations in feed water quality (NOM and competitive toxin), the water recovery rate and the pH values. In turn, fluxes were significantly impacted by background organics (NOM and microcystin) and, especially, inorganics (pH and calcium).

For this negatively charged NF membrane with a cut-off of 150 g/mol, the main mechanisms involved in anatoxin-a rejections (a 166 g/mol alkaloid, positively charged) were electrostatic interactions and steric hindrance, whereas for microcystins rejection (*ca.* 1000 g/mol cyclic peptide with weak negative charge), steric hindrance was the main mechanism.

At acid pH, solutions without NOM content showed an ATX-a rejection increase due to the repulsive forces developed between the positively charged membrane and ATX-a, reducing ATX-a adsorption onto the membrane surface. At pH 7.7, the membrane was negatively

charged and rejection decreased, since ATX-a was attracted to the membrane surface. In the presence of $CaCl_2$, rejections were higher for both acid and especially basic pH, due to the Ca^{2+} ions ability to partially neutralise the membrane negative charge.

For ATX-a the presence of calcium ions, NOM and MC-LR eliminated the pH effect. These results may be attributed to both physical and chemical aspects of NOM filtration and calcium hardness, since calcium partially neutralises the membrane negative charge and the opposite charge between ATX-a and NOM could reduce the overall net charge of the ATX-a. Hence, membrane−ATX-a attraction decreases and ATX-a rejection increases. In addition, ATX-a could associate with the NOM functional groups and form macromolecular complexes, which increased the steric hindrances and enhanced rejection.

Unlike the widely used adsorption systems, where competition between adsorbates reduces the overall performance for the target contaminants, no negative effects were found between ATX-a, MC-LR and NOM and NF can reach low residuals for all parameters. The permeate turbidity and DOC showed very low values (≤ 0.17 NTU, ≤ 1.5 mg C/L at neutral pH, respectively) and no UV_{254nm} absorbance, from which one may expect minimal THMFP. Anatoxin-a concentrations in the NF permeate were always below 1.3 µg/L, much lower than the New Zealand's drinking water guideline value of 3 µg/L. Microcystins in the NF permeate was always below the quantification limit, hence far below the WHO drinking water guideline value of 1 µg/L for MC-LR.

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CHAPTER 9

INTEGRATION OF DISSOLVED GAS FLOTATION AND NANOFILTRATION FOR *M. AERUGINOSA* AND ASSOCIATED MICROCYSTINS REMOVAL

ABSTRACT

The removal of *Microcystis aeruginosa* and associated microcystins was investigated by a dissolved gas flotation (preceded by coagulation/flocculation) – nanofiltration sequence. The experiments were conducted with a freshwater spiked with *M. aeruginosa* cell aggregates to simulate a naturally occurring bloom. Two types of gases were used in the flotation pre-treatment, air (DAF) and a mixture of CO_2/air. Very good results in terms of nanofiltration (NF) fluxes, overall removal efficiencies and final water quality were achieved with both sequences. However, the CO_2/air mixture presented no benefit to the overall sequence, both in terms of toxin release to water during flotation and lower natural organic matter removal by NF, which was due to an overall negative effect of the acid pH. NF was able to completely remove cyanobacteria (100% removal efficiency of chl_a) and microcystins (always under the quantification limit), regardless of the pre-treatment used and the water recovery rate (up to 84%). Therefore, DAF-NF sequence is a safe barrier against *M. aeruginosa* and microcystins

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in drinking water. In addition, it ensures an excellent control of particles, disinfection by-products formation, and other micropollutants that may be present in raw water.

9 INTEGRATION OF DISSOLVED GAS FLOTATION AND NANOFILTRATION FOR *M. AERUGINOSA* AND ASSOCIATED MICROCYSTINS REMOVAL

9.1 INTRODUCTION

Cyanobacteria (blue-green algae) have been identified worldwide, posing a significant risk to water supplies when they occur in reservoirs, lakes and rivers used as water sources, due to their ability to produce toxins – as well as taste and odour compounds – as secondary metabolites under particular conditions of growth. These cyanotoxins include hepatotoxic cyclic peptides, neurotoxic alkaloids (*e.g.* anatoxin-a) and dermatotoxins, amongst which the hepatotoxic microcystins (and, in particular, the microcystin-LR variant) are the most commonly occurring cyanotoxins in water sources (Sivonnen and Jones (1999), Codd (2000)). As a result of the increasing concern with their health implications, the World Health Organisation (WHO) established a drinking water guideline value of 1.0 μg/L for microcystin-LR (MC-LR) Bartran. Toxins may occur within the cells (intracellular or cell-bound toxins), or be released from cells to water (extracellular or dissolved toxins) under certain conditions of growth and/or external (environmental) stress factors responsible for cell lysis.

To definitely improve the overall drinking water treatment effectiveness and economics, cyanobacterial cells should be removed without causing cell damaging (*e.g.* coagulation / flocculation, flotation), although such procedure will not avoid the need for further treatments addressing the removal of dissolved toxins, taste and odour compounds (*e.g.* oxidation, activated carbon, membrane technology). Actually, when seeking complete algae removal, the most promising solution for the treatment of algal-rich waters may lie in a combination of conventional and membrane processes (Mouchet and Bonnélye (1998)).

Therefore, dissolved air flotation (DAF) and nanofiltration (NF) were first studied separately, and for each technology the key operating conditions were optimised for removing, respectively, cyanobacterial cells (Ribau Teixeira and Rosa (2005a), Ribau Teixeira and Rosa (2005b), chapters 2 and 4 respectively, and chapter 3), and cyanotoxins and natural organic matter (NOM) (Ribau Teixeira *et al.* (2005), Ribau Teixeira and Rosa (2005c), Ribau Teixeira and Rosa (2005d), chapters 5, 6, 7 respectively, and chapter 8). The results showed that both technologies accomplished their aims, regardless of the influent concentration and type of cells (single cells, colonies and filaments), cyanotoxins (several microcystin variants and anatoxin-a) and water organic matrix (hydrophilic, low molecular weight to hydrophobic, high molecular weight NOM). Results from coagulation /flocculation /DAF experiments using tap water and raw water from a Water Treatment Plant spiked with *Microcystis aeruginosa* cells and cell aggregates, and *Planktothrix rubescens* filaments showed that flotation needs particle destabilisation (Edzwald (1995), Ribau Teixeira and Rosa (2005a)). Cell and cell-bound toxin removal, measured by chlorophyll *a* and intracellular microcystin-LR, was high for both types of waters (> 89%). No release of MC-LR was obtained for all the studied cyanobacterial morphologies, with the operating conditions tested (Ribau Teixeira and Rosa (2005a)). Nanofiltration experiments with electrolyte solutions of mono (KCl) and divalent ($CaCl_2$) cations in acid and neutral-alkaline conditions showed that the anatoxin-a removal was governed by electrostatic interactions and steric hindrance, whereas for microcystins (at *ca.* 10 μg/L) the latter was the main mechanism. Microcystins in the NF permeate were always below the quantification limit (far below the WHO drinking water guideline) and anatoxin-a was almost completely removed (94%), regardless of the presence of background organics (NOM and competitive toxin), the water recovery rate (0-90%) and the pH value (4 or 7). In turn, fluxes were significantly impacted by background organics

(NOM and microcystin) and, especially, inorganics (pH and calcium) (Ribau Teixeira and Rosa (2005d) and chapter 8).

The objective of the present study is to investigate the overall performance of the dissolved gas flotation – NF integrated sequence, as a safe barrier against cyanobacteria and cyanotoxins in drinking water. The importance of studying these two technologies together (in sequence) lies in the two mechanisms of cyanotoxin release that impair the drinking water quality: the natural active toxin release that occurs in water reservoirs through cell lysis, and the induced toxin release that may occur during the water treatment process, as a result of mechanical and/or chemical stress factors, that influence the cyanobacterial cell stability (Jurczak *et al.* (2005), Schmidt *et al.* (2002)).

The proposed sequence (DAF – NF) intends to minimise the induced cyanotoxins release, while safely removing the dissolved toxins existing in raw freshwaters. DAF is used to profit from the natural flotation ability of cyanobacteria for their removal without cell lysis. A CO_2/air mixture was also investigated to evaluate the advantages from the pH decrease (produced by CO_2 dissolution) in the flotation and NF processes. The change in the pH (in the direction of acidification) may sometimes allow savings on coagulant demand for particle destabilisation and effective coagulation (Mouchet and Bonnélye (1998)). As far as NF is concerned, while a negative effect of acid pH was previously observed on rejections, a positive effect was found on flux (Ribau Teixeira *et al.* (2005), Ribau Teixeira and Rosa (2005c)). The NF objective is to remove the cyanotoxins present in water (by natural and/or induced cell release) to a safe level for human consumption. The referred sequence is applied to the Alcantarilha Water Treatment Plant (WTP) case-study.

9.2 MATERIAL AND METHODS

9.2.1 CYANOBACTERIAL CELLS AND CYANOTOXINS

Microcystis aeruginosa supplied by Pasteur Culture Collection (PCC 7820) was grown in laboratory, according to the enclosed instructions. Aggregates of these cultures were produced by medium manipulation as described in Chapter 4. Cultured *M. aeruginosa* cell aggregates were used in this study, since no cyanobacterial blooms occurred in the Funcho dam reservoir (section 9.2.2) during the period of these experiments, though they were expected to occur. The cell aggregates used in the treatment experiments were all provided from the same culture, and were harvested within a 24 h period.

These PCC 7820 aggregates produced four microcystin variants, MC-LR, MC-LY, MC-LW and MC-LF. As detailed in a previous paper (Ribau Teixeira and Rosa (2005d)), microcystins are cyclic hepatotoxic peptides. The main structural change in microcystins is the variability of L-amino acids 2 (designated as X) and 4 (Z) (Meriluoto (1997)). For MC-LR, leusine is in position X and arginine is in position Z. Microcystins are hydrophobic compounds, with a net charge, at pH 6-9, slightly negative, and a molecular weight varying between 909 – 1115 g/mol (994 g/mol for MC-LR).

9.2.2 NATURAL WATER SAMPLES

The Funcho dam reservoir was chosen for it is one of the most important drinking water sources in western Algarve, southern Portugal (*ca*. 2 km^2 of surface area and a volume capacity of 43.4 hm^3) and has a record of toxic cyanobacterial blooms occurrence (Rosa *et al.* (2005)). This reservoir has been used for water abstraction to Alcantarilha WTP (run by Águas do Algarve, SA, an affiliate of Águas de Portugal, SGPE, SA) since January 2000. Besides the strong seasonal water demand (*ca.* 650,000 people during summer and 180,000

during winter), this WTP has to face important seasonal variations in raw water quality. It was designed to treat up to 3 m^3/s by a conventional treatment of pre-ozonation, coagulation /flocculation /sedimentation, rapid sand filtration and chlorination.

The treatment experiments were performed with raw water (RW) from Alcantarilha WTP, spiked with *M. aeruginosa* cell aggregates until a specific concentration of chlorophyll *a* (chl_a) was achieved, namely Alert Level 2 established by Bartram *et al.* (1999) for potentially toxic cyanobacterial bloom (Table 9.1). Alert Level 2 corresponds to the worst scenario (cyanobacterial biomass 100,000 cells per mL or 50 μg/L chl_a) and describes a toxic bloom with high biomass, and possibly also localised scum (Bartram *et al.* (1999)).

As depicted in Table 9.1, the water used in the experiments is a moderately hard water with moderate organic matter content (EPA (1999)). According to Edzwald and Van Benschoten (1990) classification based on SUVA values, the organic matter is hydrophilic, has low molecular weight, and DOC is largely composed by non-humic substances. Table 9.1 also shows that MC-LR accounts for 77% of total microcystins, the other three variants (MC-LY, MC-LW, MC-LF) having similar contributions. In addition, *ca.* half of the toxins are in the dissolved form.

Table 9.1 Characteristics of the water used in the experiments (confidence interval for the mean value with $\alpha = 95\%$, nº of samples = 4).

Parameters	RW + PCC 7820 aggregates	Parameters	RW + PCC 7820 aggregates (μg MC-LR eq./L)
		Extra-MC-LR	8.20 ± 1.11
pH	7.5 ± 0.3	Intra-MC-LR	16.96 ± 1.26
Conductivity (μS/cm)	358 ± 10	Extra-MC-LY	0.61 ± 0.05
Turbidity (NTU)	7.40 ± 0.09	Intra-MC-LY	1.81 ± 0.69
DOC (mg C/L)	4.00 ± 0.65	Extra-MC-LW	0.66 ± 0.10
UV_{254nm} (1/cm)	0.042 ± 0.001	Intra-MC-LW	1.69 ± 0.11
SUVA (L/(m.mg))	1.07 ± 0.15	Extra-MC-LF	1.06 ± 0.14
Chl_a (μg/L)	52.6 ± 5.1	Intra-MC-LF	1.82 ± 0.52

9.2.3 MEMBRANES

The investigated NFT50 membrane is a thin film composite NF/RO membrane of polypiperazine amide on a polysulfone microporous support and a polyester support, from Alfa Laval, with an hydraulic permeability of 5.9 kg/(h·m^2·bar) at 25 ºC, a molecular cut-off of 150 g/mol and a pore radius of 0.43 nm (Ribau Teixeira *et al.* (2005)).

From previously published data on zeta potential measurements (Ribau Teixeira *et al.* (2005)), the membrane surface is slightly positive at pH 4 (1 mV), passes through an isoelectric point at pH 4.2 ± 0.2 and is negatively charged above this pH (until 8.3). The surface charge is about -10.3 mV at pH 6.9 with a background electrolyte of 1 mM KCl. In the presence of calcium hardness cations (1 mM $CaCl_2$), the isoelectric point shifts from 4.2 to 5-6 and the membrane is less negatively charged over the entire pH range (-2.6 mV at pH 7.3).

9.2.4 ANALYTICAL METHODS

Samples were analysed for pH (at 25ºC, using a Whatman WTW pH340), conductivity (Crison GLP32 conductimeter), DOC (Shimadzu TOC 5000A analyser, 50 ppb – 4000 ppm), chl_a and UV_{245nm} absorbance (Spectronic Unicam UV300 UV/VIS spectrophotometer), and turbidity (HACH 2100N turbidity meter of high resolution, 0.001 NTU) using standard methods for analysis of water (Clesceri *et al.* (1998)). In the NF experiments, the permeate fluxes were determined by weight (analytical balance Shimadzu, model BX 620S).

After extraction, samples were also analysed for extra and intracellular microcystin by HPLC/PDA, according to the standard operating procedures presented in Meriluoto and Codd (2005) with the deviations as described below. For the extraction of intracellular microcystins

(intra-MC), samples were filtered through a Whatman GF/F glass microfiber, were put in 20 mL methanol 75% (v/v) and stayed during 18-24h in the freezer (-18 ºC, in the dark). After this period, the filters were washed with a small volume of methanol 75% and centrifuged (6,000 x *g*, 10 min). The supernatant was collected and evaporated in a rotavapor (50°C), the residue was resuspended in 500 µL methanol 75% and centrifuged again during 10 min, at 10,000 x *g*. The supernatant was then transferred to a vial, either analysed immediately on HPLC-PDA or remained in the freezer (-18 ºC, in the dark) until the analysis. The extracellular microcystins (extra-MC) were concentrated from the filtered water samples using an isolute C18 solid phase extraction column (1 g in a 6 mL reservoir). The cartridges were first conditioned with 10 mL methanol (75% v/v) followed by 10 mL milli-Q water at a flowrate not exceeding 10 mL/min, without letting them dry during conditioning. The samples were then applied to the cartridge and the microcystins were eluted with 5 mL methanol (90% v/v) containing 0.1% trifluoroacetic acid. The methanolic elute was evaporated at 50ºC in a rotavapor, resuspended in 500 µL methanol (75% v/v), centrifuged for 10 min at 10,000 x *g*, and 150 µL of supernatant were transferred to HPLC vials for analysis. All the four microcystin variants detected in the samples (MC-LR, MC-LY, MC-LW and MC-LF) were quantified as MC-LR equivalent, *i.e.* µg MC-LR eq./L..

A Dionex Summit HPLC-PDA system was used, which includes a high pressure gradient pump (Dionex Summit), an autosampler (Dionex ASI-100), a column oven (Dionex STH-585) and a photo diode-array detector (Dionex PDA-100). A C18 column was used (Merck Purospher STAR RP-18 endcapped, 3 µm particles, LiChroCART 55x4 mm). The mobile phase used a gradient of milli-Q water and acetonitrile, both with 0.05% (v/v) of trifluoracetic acid. Chromatograms were analysed between 180 – 900 nm, with a main detection at 238 nm for the typical microcystins spectra (Meriluoto and Spoof (2005)).

9.2.5 FLOTATION EXPERIMENTS

The dissolved gas flotation experiments were performed in a laboratory-made flotation cell adapted from De Pinho *et al.* (2000). This *apparatus* has a 2 L pressure chamber and a 3 L calibrated cylinder. A paddle for the coagulation/flocculation experiments was installed inside the calibrated cylinder. The experimental procedure followed Eckenfelder (2000) and has been detailed in Ribau Teixeira and Rosa (2005b). In these experiments, the calibrated cylinder was partially filled with raw water spiked with aggregates of *M. aeruginosa*, while the pressure chamber contained raw water. This procedure simulates the pressurised recycle mode of operation, used to avoid subjecting the cyanobacterial cell aggregates to shearing stresses which may be responsible for cell lysis and subsequent toxin release to water.

In C/F/DAF and C/F/DCO_2F experiments, rapid and slow mixing modes were performed. The operating conditions were: a) coagulation at 380 s^{-1} for 2 min using 8 mgAl_2O_3/L of WAC (aluminium polyhydroxichlorosulphate) with a relative basicity of 60-70% (Elf Atochem, stock solution with 850 mgAl_2O_3/L), b) flocculation at 70 s^{-1} for 8 min, and c) DAF or DCO_2F for 8 min with an applied recycle ratio of 0.08 and a relative pressure of 5 bar. The CO_2/air mixture was prepared by mixing 3 bar of air with the raw water in the pressure chamber, and the CO_2 was then applied until the pressure chamber manometer indicated 5 bar.

These operating conditions were optimised in earlier experiments (Ribau Teixeira and Rosa (2005b)). For DAF and DCO_2F experiments a correction factor for dilution (1 + R/Q) was used when computing all analytical parameters in the clarified water.

In order to further understand some of the results obtained, two additional sets of experiments were conducted using tap water (TW). For the first set, DAF experiments were performed

with *M. aeruginosa* cell aggregates at two pH values (5.6 and 7.7). For the second set, DAF and DCO_2F experiments were made with *M. aeruginosa* cells.

9.2.6 NANOFILTRATION EXPERIMENTS

The NF experiments were performed in a plate-and-frame unit, Lab-unit M20, from the Danish Separation Systems (membrane area of 0.0360 m^2 up to 0.720 m^2; maximum pressure 80 bar; maximum flow 18 L/min and constant temperature maintained by an heat exchanger). In this study, a membrane surface area of 0.0720 m^2 was used.

The membranes were first compacted and were then stabilised with deionised water until a steady permeate flux was achieved at the pressure and crossflow velocity planned for the experiments, 10 bar and 8 L/min, respectively.

The NF experiments were performed after C/F/DAF or C/F/DCO_2F, meaning that the treated water from C/F/DAF or C/F/DCO_2F experiments was used as NF feed water, as illustrated in Figure 9.1. Therefore, two sets of trials were made, both in duplicate.

These trials consisted on concentration runs, in order to simulate the industrial NF operation at different water recovery rates (defined as the permeate volume over the initial feed volume). The experimental procedure has already been presented in Ribau Teixeira and Rosa (2005c). Between each consecutive NF run, membranes were washed until the pure water flux reached 90% of the initial value, measured after compaction, and the bulk conductivity was similar to that of deionised water. The temperature was maintained at 25ºC during the experiments.

At the beginning, the water was given a time (*ca.* 10 minutes) to equilibrate with the membrane surface. After reaching a steady permeate flux, this value was recorded, and samples of feed water and permeate were taken to serve as baseline for flux and rejection at 0% of water recovery rate. Samples from the feed and permeate solutions were also taken whenever a stipulated water recovery rate was achieved. These samples were analysed for extra and intra-cellular microcystin variants, NOM (DOC and UV_{254nm}), turbidity, chl_a, pH and salt rejection (by conductivity measurements). Flux was measured throughout the experiments.

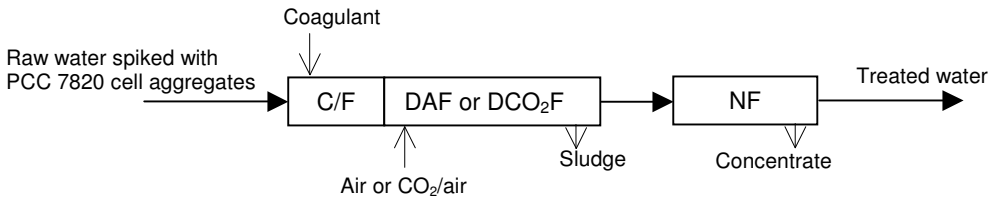

Figure 9.1 Linear diagram of the treatment sequence studied.

9.3 RESULTS AND DISCUSSION

9.3.1 FLOTATION

Results from C/F/flotation experiments using raw water spiked with *M. aeruginosa* cell aggregates are shown in Figure 9.2. Turbidity, chl_a and intra-MC removal efficiencies are high, being relatively low (~20%) for DOC and UV_{254nm}, either with DAF or DCO_2F. Unexpectedly, DAF seems to be more efficient than DCO_2F. The pH decrease promoted by CO_2 dissolution (DCO_2F influent water pH is 5.8 while for DAF it is 7.5) would be expected to benefit C/F, due to the increase of the positive charges that neutralise the negative charges of the compounds present in water. In fact, in the coagulation of *S. quadricaula* suspension with 2.96 mM Al(III) and Fe(III), Widrig *et al.* (1996) reported an increase of the overall C/F/sedimentation removal efficiencies at pH 5 compared to pH 8. However, Edzwald (1995) referred an improvement in flotation when the pH increased to values near the isoelectric

point (*ca.* pH 8) of the freshly precipitated aluminium hydroxide particles in pure water (10 NTU of initial turbidity). Accordingly, the results obtained may be related to the isoelectric points of the compounds present in water (Edzwald (1995)) and/or to the type of the coagulant used (pre-polymerised), although further investigation is required.

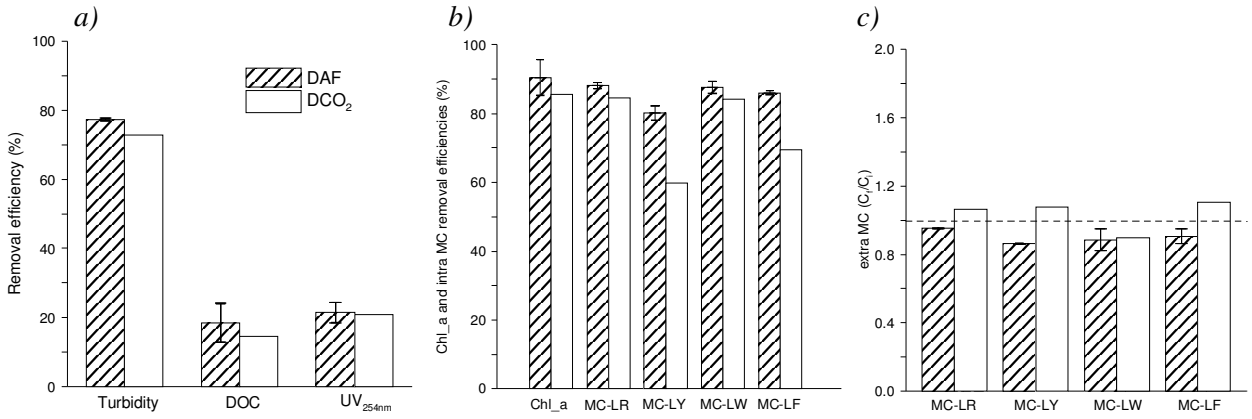

Figure 9.2 C/F/DAF and C/F/DCO_2F results using raw water spiked with *M. aeruginosa* cell aggregates.

Extracellular microcystins results (Figure 9.2c) indicate a toxin release into water (observed by the increase in the final concentrations of MC-LR, MC-LY and MC-LF, expressed by the C_f/C_i ratio above 1) when DCO_2F is used, while with DAF no toxin increase in the clarified water was found. The removal efficiency of extra-MC by DAF is very low (~4.7%), as already obtained in previous works (Ribau Teixeira and Rosa (2005b)). Besides decreasing the water pH, the CO_2/air mixture also produces larger and a higher amount of gas bubbles (due to the high solubility of CO_2 in water), which might decrease the gas bubble particle attachment opportunities, while increasing the shearing stresses eventually responsible for cell lysis. To better understand this result, DCO_2F and DAF experiments were performed with *M. aeruginosa* aggregates and cells spiked in tap water. In the DAF experiments, two pH values were studied, namely 7.7 (without changing the pH) and 5.6 (approximately the pH of the DCO_2F influent water) (Figure 9.3). These experiments intended to investigate if the release

of microcystins to water is related with pH and coagulant dose, or with the type of cell aggregates.

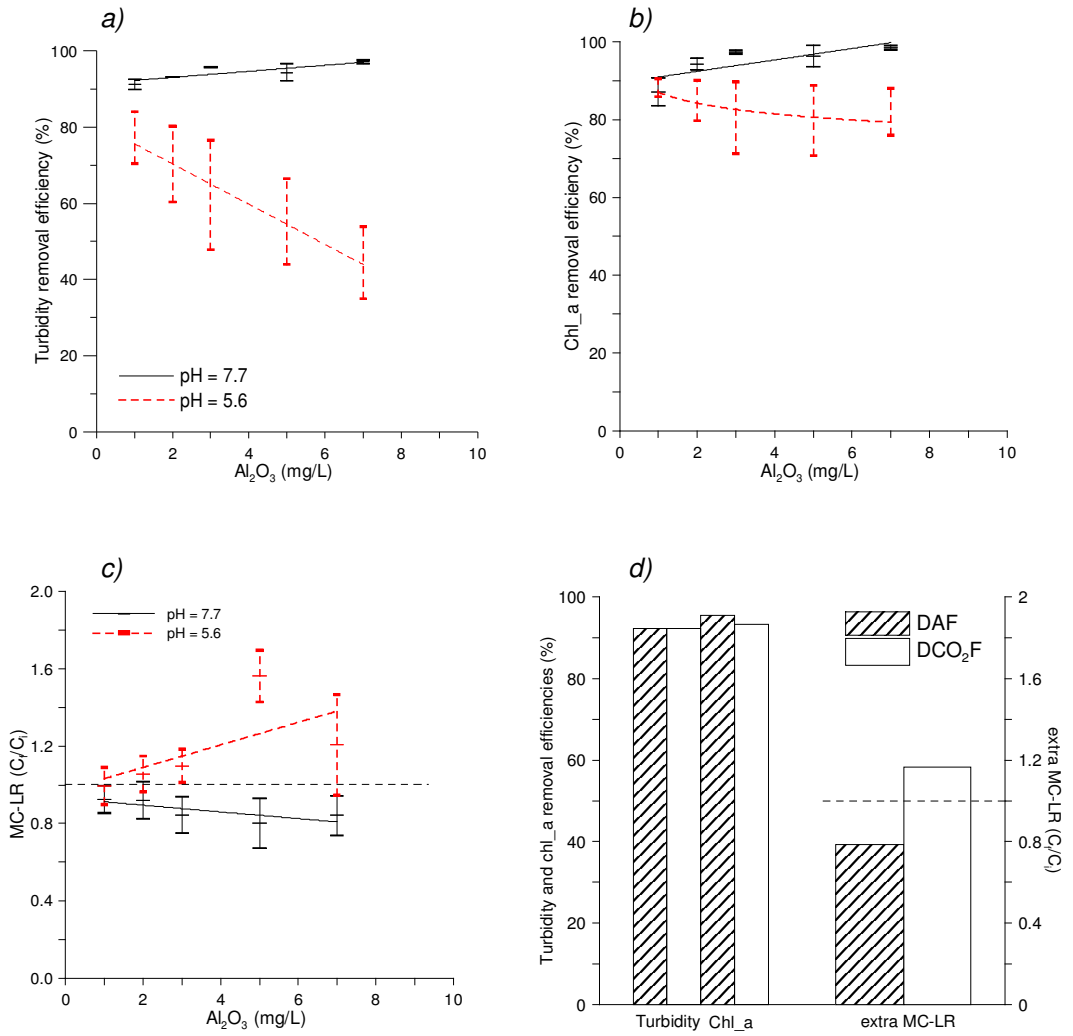

Figure 9.3 a, b, c) C/F/DAF results with *M. aeruginosa* cell aggregates in tap water, and **d)** Comparison between DAF and DCO_2F for *M. aeruginosa* cells with 2 mg Al_2O_3/L of WAC added to the tap water.

Results in Figure 9.3 show the great impact of water pH on DAF performance. At neutral pH (7.7), turbidity and chl_a removal efficiencies increase with the coagulant dose from 1 to 7 mg Al_2O_3/L, whereas, under acidic conditions (pH 5.6), a negative effect of coagulant overdosing is found from coagulant doses as low as 2 mg Al_2O_3/L. In addition, data show a release of MC to the water at pH 5.6 for all coagulant doses added to the water, while for pH

7.7 no release of MC is observed (Figure 9.3c). For *M. aeruginosa* cells (Figure 9.3d) in tap water, results show again a release of MC when CO_2 is used (pH ~5.6). Such results evidence the negative effect of acid pH on the coagulation / flocculation and *M. aeruginosa* stability, since for both cells and cell aggregates spiked in RW or in tap water, the results lead to the same conclusions. The same results were also referred by Velzeboer *et al.* (1995) when using aluminium sulphate under acidic conditions. Nonetheless, a more in depth investigation is recommended, *e.g.* through zeta potential measurements of the flocculated suspensions and/or the floated sludges produced under different pH conditions.

9.3.2 NANOFILTRATION

Results displayed in Figure 9.4 show no significant NF flux decline with the running time nor with the water recovery rate, for both DAF and DCO_2F pre-treatments. Nevertheless, a slight flux decrease is observed using DCO_2F for recovery rate higher than 50%, above which fluxes with both pre-treatments become very close. As already discussed in Ribau Teixeira and Rosa (2005c), minimal concentration polarisation was guaranteed by the low permeation rates (a 10 bar transmembrane pressure was used), coupled with the good feed hydrodynamic conditions (Reynolds number of 965 (Ribau Teixeira *et al.* (2005)), and the low influent DOC concentration of low SUVA values, *i.e.*, low foulant behaviour. According to the literature, minimal concentration polarisation is mainly related to operation under the critical flux. Apparently, no adsorption occurred on Cho *et al.* (1999) experiments, so they concluded that the experiments were performed below the critical flux. Hong and Elimelech (1997) and Seidel and Elimelech (2002) demonstrated the same: NOM fouling was prevented in the runs conducted at the lowest permeate flux, implying that their runs were performed near or below the critical flux.

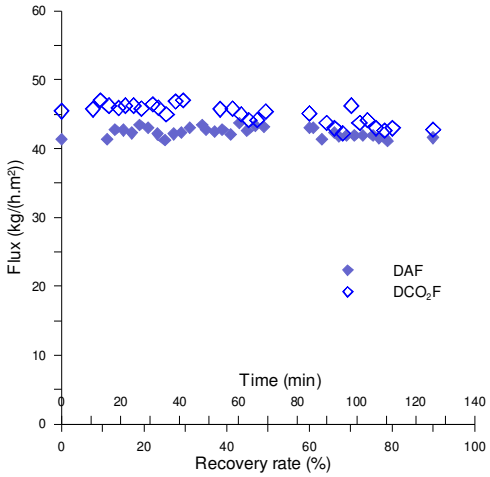

Figure 9.4 NF flux at different water recovery rates after C/F/DAF and C/F/DCO_2F pre-treatments.

As expected, due to a pH effect, flux is slightly higher using DCO_2F than DAF, at least for recovery rates below 50%. As already found in previous studies with the same membrane (Ribau Teixeira *et al.* (2005), Ribau Teixeira and Rosa (2005c)), flux decreases with the pH increase, as a result of the increase in the negative charge of the membrane surface and pores, which leads to a reduction of the membrane pore size. In addition, the osmotic pressure near the membrane surface must be slightly higher at high pH due to the (slightly) higher salt rejection (Figure 9.5a), which decreases the net driving pressure.

While similar removal efficiencies are achieved by DAF and DCO_2F pre-treatments, significantly higher efficiencies of dissolved organics are found for DAF over DCO_2F (Figure 9.5). Particularly for DOC substances such differences are, once again, attributed to the pH effect (pH 7.5 for DAF and pH 5.8 for DCO_2F). When DAF pre-treatment is used, the membrane is highly negatively charged due to the higher solution pH. Besides reducing the membrane pore size (as explained above), high pH values prevent NOM from adhering onto the membrane surface, producing less fouling and improving the rejection, due to the repulsive forces developed between NOM and the membrane surface (Ribau Teixeira *et al.* (2005), Ribau Teixeira and Rosa (2005c)).

The apparently poor NF removal efficiency of turbidity achieved for all water recovery rates (*ca.* 70%) is related with the very low NF influent turbidity (0.64 NTU), high quality water being always produced (Table 9.2).

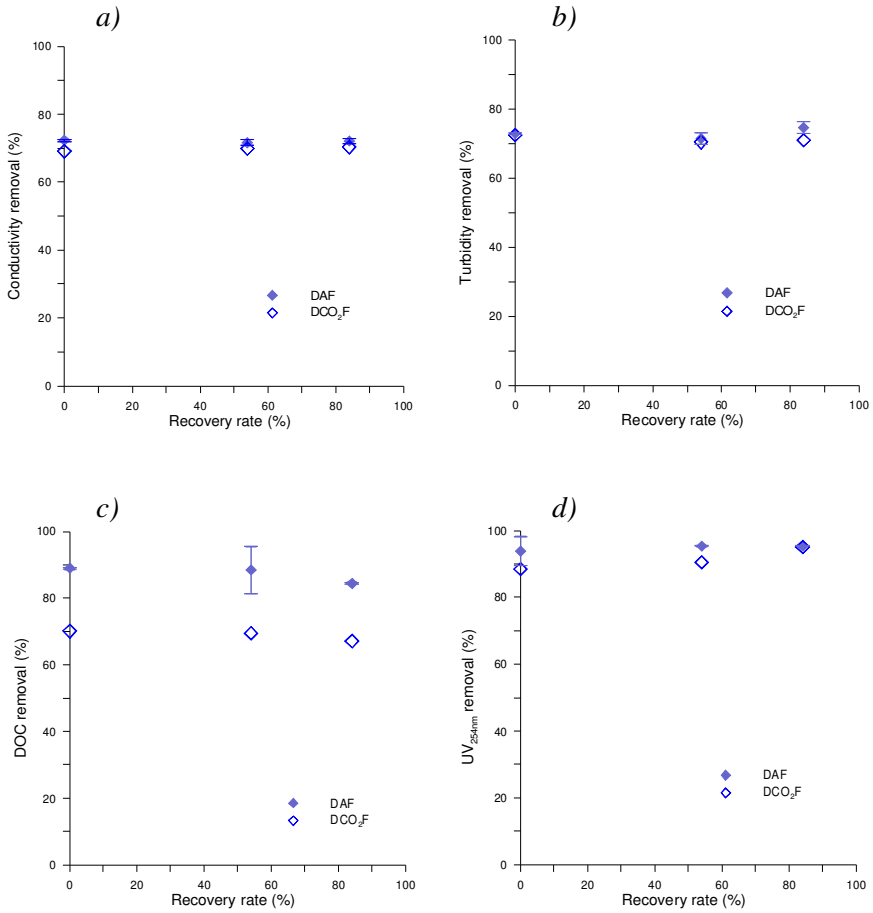

Figure 9.5 NF removal efficiency after DAF and DCO_2F: **a)** conductivity, **b)** turbidity, **c)** DOC, and **d)** UV_{254nm} results.

A most important observation from these results is the low variation of flux and rejection with the water recovery rate. In fact, high fluxes and removal efficiencies are obtained for high water recovery rates (Figures 9.4 and 9.5, respectively). These results are quite similar to those obtained in earlier experiments using water with three different types of NOM spiked with MC, at two pH values (*ca.* 6 and 8) (Ribau Teixeira and Rosa (2005d)).

Moreover, NF is able to completely remove both the cyanobacteria (100% removal efficiency of chl_a, Table 9.2) and the associated microcystins (all variants could never be quantified in the NF permeate, Table 9.2), regardless of the water recovery rate (up to 84%) and the pre-treatment used (even when some microcystins are released during the C/F/DCO_2F process, Figure 9.2c).

Such results were already obtained with model solutions (chapter 8). Those results showed that neither the type and NOM concentrations studied influenced the MC rejection, nor the presence of MC affected the rejection of NOM. They also indicated that the high MC rejections obtained are mainly related to size exclusion effects, based on the high MC size (994 to 1001 g/mol depending on the variant) and on the MC overall net charge (negative, but weakly charged).

Table 9.2 presents the overall removal efficiencies for the C/F/DAF + NF and C/F/DCO_2F + NF processes. The quality of the final water produced by the two studied processes, as well as of the treated water from Alcantarilha WTP (results from one single day, during the period when these experiments took place) is also presented in Table 9.2.

Comparing the overall removal efficiencies of the two treatment sequences, they are quite similar (Table 9.2) except for DOC. DOC removal efficiency by C/F/DAF + NF sequence is higher than by C/F/DCO_2F + NF, due to the already discussed pH effect on the NF separation ability. Consequently, the treated water results demonstrate that the two sequences reach similar water qualities except for DOC, for which a superior water quality is achieved by C/F/DAF + NF.

Table 9.2 Influent and treated water quality, and removal efficiencies (%) achieved by C/F/DAF+NF and C/F/DCO_2F+NF for 84 % of water recovery rate; influent and treated water quality from Alcantarilha WTP.

		C/F/DAF + NF		C/F/DCO_2F + NF		Alcantarilha WTP	
	Influent [(1)]	Effluent	Overall removal (%)	Effluent	Overall removal (%)	Influent	Effluent
pH	7.5 ± 0.3	7.5	-	7.4	-	7.2	7.2
Conductivity (µS/cm)	358 ± 10	277.5	62.6	287.0	61.3	338	-
Turbidity (NTU)	7.40 ± 0.09	0.15	98.0	0.17	97.7	1.46	0.12
DOC (mg C/L)	4.00 ± 0.65	1.10	72.5	2.01	49.6	3.22	2.80
UV_{254nm} (1/cm)	0.042 ± 0.001	0.003	92.9	0.003	92.9	0.040	0.009
SUVA (L/(m.mg)	1.05	0.27	-	0.15	-	1.24	0.32
Chl_a (µg/L)	52.6 ± 5.1	0	100	0	100	-	-
Extra-MC-LR (µg/L)	10.53	<0.052	>99.4	<0.052	>99.4	-	-
Intra-MC-LR (µg/L)	22.28	<0.052	>99.7	<0.052	99.7	-	-

[(1)] Values from Table 9.1.

For turbidity, the treated water is far below the national standard of 1 NTU for both sequences. Although there are no national standards for UV_{254nm} and DOC in drinking water, these are very important parameters due to their relation with the trihalomethane (and other disinfection by-products) formation potential in the finished water. UV_{254nm}, DOC and SUVA values in the treated water are very low and much lower than the values obtained in Alcantarilha WTP final water (despite of the higher influent concentration), particularly for C/F/DAF + NF sequence (1.1 mg C/L, 0.003 1/cm and 0.27 L/(m.mg)), respectively, Table 9.2). Therefore, minimal THMFP may be expected (EPA (1999)) – besides the very low DOC concentration, the hydrophilic DOC (very low SUVA values) has a lower potential to form THM than hydrophobic DOC (Galapate *et al.* (2001)). In addition to this control of disinfection by-products formation, these processes integrating NF ensure an excellent control of particles, as well as of other micropollutants (above *ca.* 200 g/mol, *e.g.* anatoxin-a (chapter 8) that may be present in raw water or be released during treatment.

The extra- and intra-MC of the treated water is under the limit of quantification (< 0.052 µg/L, Table 9.2), *i.e.* far below the WHO guideline value for drinking water, 1 µg/L for MC-LR. According to the Alert Levels (Bartram *et al.* (1999)), the studied scenario corresponds to a toxic bloom with high biomass, so it represents one of worst scenarios that a water treatment plant might have to face. Therefore, the C/F/DAF + NF sequence is a safe barrier against *M. aeruginosa* and the associated microcystin variants (MC-LR, MC-LY, MC-LW and MC-LF) in drinking water, even when high concentrations of biomass and toxins are present in the raw water (Table 9.2).

9.4 CONCLUSION

This work investigated the ability of the C/F/flotation + NF sequence for removing cyanobacteria and the associated microcystin variants from natural waters (moderately hard freshwater with hydrophilic, low molecular weight NOM) containing a toxic *M. aeruginosa* bloom with high biomass (53 µg/L chl_a). Dissolved air flotation and dissolved CO_2/air flotation were tested, and a negatively charged, tight, commercial NF membrane was used.

Good results in terms of NF fluxes, overall removal efficiencies and final water quality were achieved with both C/F/DAF + NF and C/F/DCO_2F + NF sequences. However, C/F/DAF + NF presented a superior performance over C/F/CO_2F + NF in NOM removal, due to the negative effect of acid pH on NF separation efficiency. This study also demonstrated the negative influence of the acid pH on *M. aeruginosa* cells and cell aggregates stability, since there was toxin release to the water when the pH decreased to *ca.* 5.6, using both pre-treatments studied, and different feed water background matrixes (raw water and tap water). Results also showed slightly higher NF fluxes for DCO_2F than for DAF pre-treated water (due to the pore narrowing effect promoted by the pH increase). Although no significant NF flux

decline was observed with the running time nor with the recovery rate for both NF pre-treatments, a slight decrease was observed using DCO_2F for water recovery rates higher than 50%, above which both processes yielded similar fluxes. Therefore, the CO_2/air gas mixture studied in the flotation process presented no benefit to the overall sequence.

As far as the final water quality is concerned, C/F/DAF + NF guaranteed a full removal of the cyanobacterial biomass (100% removal of chl_a) and the associated microcystins. Microcystin concentrations in the treated water were always under the quantification limit, *i.e.* far below the WHO guideline value of 1 µg/L for MC-LR in drinking water. Therefore, C/F/DAF + NF sequence is a safe barrier against *M. aeruginosa* and the associated microcystins variants (MC-LR, MC-LY, MC-LW and MC-LF) in drinking water, even when high concentrations are present in raw water and NF water recovery rates as high as 84% are used. In addition, it ensures an excellent control of particles (turbidity), and disinfection by-products formation (very low values of DOC, UV_{254nm} and SUVA were achieved, much below those of Alcantarilha WTP final water), as well as other micropollutants (above *ca.* 200 g/mol, *e.g.* anatoxin-a) that might be present in the water.

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CHAPTER 10
CONCLUSIONS

ABSTRACT

In this chapter, the most relevant conclusions obtained in the work are summarised and discussed together.

10 CONCLUSIONS

Following the structure of this thesis, the conclusions are divided in two major parts: the study of the cyanobacteria removal by flotation and the study of cyanotoxins removal by nanofiltration. The most relevant conclusions obtained in the preceding sections are summarised and discussed together.

10.1 FLOTATION

The first part of this research focused on the removal of cyanobacteria by dissolved air flotation. Different approaches were followed: i) the evaluation of the most relevant operating conditions, with the coagulant type and doses normally used in similar full-scale treatment for the most commonly found cyanobacteria (*Microcystis aeruginosa*), and its comparison with the conventional treatment by coagulation (C)/ flocculation (F)/ sedimentation (S); ii) the evaluation of the C/F /dissolved air flotation (DAF) performance with different types of water background natural organic matter (NOM); and iii) the study of the removal of different types of cyanobacteria morphology, namely unicellular (spherical), colonies and multicellular filaments.

The results emphasises the importance of the C/F pre-treatment on the particle destabilisation and cyanobacteria size increase required for cell-bubble contact enhancement and subsequent formation of flocs of strength and size adequate for effective DAF treatment.

The best set of C/F/DAF operating conditions indicated that destabilised particles (low to non charged, hydrophobic particles), strong and small size flocs, and a minimum recycle are needed for effective DAF treatment, namely low recycle ratios (8%), low coagulant doses (2-

3 mg Al_2O_3/L of WAC), slow coagulation (380 s^{-1}), strong but shorter flocculation (8 min at 70 s^{-1}) yielded very high chlorophyll *a* removals (93 – 98%) with no toxin release to water. Although both treatment processes, C/F/S and C/F/DAF, can efficiently remove *M. aeruginosa* cells with no toxin release to water, under the operating conditions tested, C/F/DAF process presents better removal efficiencies and lower residuals than C/F/S.

Furthermore, it becomes clear that the pre-polymerised coagulant of high basicity (WAC) performed better than alum, for both clarification processes (sedimentation and DAF). The pre-polymerised coagulant achieved higher removal efficiencies, lower pH decrease, lower residuals and lower optimal dose. It also showed a superior ability to cope with the influent cell concentration increase. Pre-polymerisation of the metal salt coagulant enhances the charge interaction mechanism of colloid destabilisation as a consequence of slowing down of the hydrolysis of the metal salt. For both coagulants, cell removal efficiency increased with the influent concentration, although higher doses are required to reach the same residuals.

At this point, the question raised was whether the NOM present in natural waters would influence the overall removal or increase the coagulant demand that might damage the *M. aeruginosa* cells. Experiments with different water background NOM spiked with *M. aeruginosa* cultured cells (worst treatment scenario) demonstrated an important NOM influence on the removal, for both C/F/S and C/F/DAF processes. In the presence of NOM higher coagulant dose is necessary to destabilise all the particles present in water and to achieve the same residuals obtained in the experiments without NOM, but no release of microcystins was observed. Compared to C/F/S, C/F/DAF showed better removal of *M. aeruginosa* cells (higher than 90%) and lower residuals with lower coagulant dose. Pre-ozonated water containing *M. aeruginosa* cells showed higher removal efficiencies than the

respective raw water, and lower coagulant demand. In fact, the preozonation oxidises the adsorbed organics to more polar forms, changes the configuration of the adsorbed organics, and oxidises the organics to form carboxylic acid functional groups, which benefits the coagulation and flocculation processes and increases the overall removal.

The different basic cyanobacterial morphology was then investigated. Cultured *M. aeruginosa* cells aggregates were grown in laboratory by medium manipulation. Cell aggregation did not significantly influence the removal efficiency of *M. aeruginosa* by C/F/DAF process. The observed increase in removal efficiency could be related with the increase in influent cyanobacterial mass concentration when aggregates were present. However, cell aggregation was important for DAF with no coagulant addition.

It was also possible to demonstrate that filamentous cyanobacteria, such as *Planktothrix rubescens*, can be efficiently removed by the C/F/DAF process. C/F/DAF was as much or even more efficient for removing these cyanobacteria than *M. aeruginosa* cells or even cell aggregates. Both DAF and C/F/DAF processes showed high removal efficiencies of chl_a (88-93%) and intra MC-LR (86-93%). In addition, with the tested operating conditions, no release of MC-LR was observed. Actually, there was a significant removal of extra MC-LR (34-48%). This high removal of extra MC-LR may be attributed to its adsorption onto *P. rubescens* filaments and/or DOC substances, since DOC concentrations were very high. As these high DOC concentrations are due to the medium contribution during the spiking, such high removal efficiencies of extra MC-LR will not be expected in natural waters.

This research pointed out DAF as a real alternative to conventional treatment by sedimentation to remove cyanobacteria (low density particles of negative surface charge)

from natural waters. Furthermore, it contributed to the understanding of DAF separation mechanism of cyanobacteria from natural waters namely: i) the importance of the coagulation / flocculation prior to DAF, ii) the influence of the raw water quality characteristics, iii) the cyanobacterial morphology and iv) the key C/F/DAF operating conditions for efficient removal of intact cyanobacterial biomass.

10.2 NANOFILTRATION

The study of the removal of cyanotoxins (microcystins and anatoxin-a) by nanofiltration down to a safe level for human consumption constituted the second part of this research program. Firstly, the effects of the pH on the membrane charge and performance, as well as the water background matrix, namely hardness ions and NOM, were analysed. Cyanotoxins were then added to both treated and natural waters, in order to investigate the separation mechanisms and optimise the NF performance. Finally, competitive aspects between NOM and two types of cyanotoxins (microcystins and anatoxin-a) were evaluated.

The results showed the importance of divalent hardness salts ($CaCl_2$ and $MgSO_4$) on the NF performance with natural waters. Streaming potential measurements along surface and through pores made with several electrolyte solutions at different pH fully explain the NF performance with pH. The maximum flux and minimum retention were obtained at the membranes i.e.p. (4.2±0.2), *i.e.* uncharged membranes. With the pH increase, the membrane negative charge increased, the flux decreased and the retention increased. For divalent hardness ions ($CaCl_2$ and $MgSO_4$), the membrane was less negatively charged and the flux decreased further. The shape of the salt retention curves corresponded to the shape of the membrane charge curves, *e.g.* for $CaCl_2$ electrolyte and pH above i.e.p., when the membrane was less negatively charged, the electrolyte retention presented the lowest values. Membrane

pore narrowing, electroviscous effect and osmotic pressure mechanisms explain these results. When divalent salt concentration increased, retention decreased, due to a higher ionic strength of the more concentrated solutions and to a less negative membrane charge.

Additionally, the NF performance of water with low NOM content and moderate hardness was largely or even mostly influenced by the background pH and calcium hardness, rather than by the type of NOM. Both hydrophilic, low molecular weight (SUVA 0.26 L/(m.mg)) and hydrophobic, high molecular weight (SUVA 5.10 L/(m.mg)) model solutions fitted the exact same trendlines for flux and salt rejection, either with or without calcium ion, but greatly modified by its presence. For all investigated NOM solutions, flux presented low variation with recovery rate and running time, probably due to the low DOC content and low permeation rate. Results showed that flux decreased with increasing pH, particularly in the presence of Ca^{2+}. Alkaline pH showed the highest conductivity and DOC rejections. These results were attributed to both physical and chemical aspects of NOM filtration. The pH increased the negative charge of the membrane (responsible for pore narrowing) and the negative charge of NOM functional groups, inhibiting these molecules from being adsorbed, besides increasing their higher hydrodynamic radii. In the presence of calcium ion, the decrease in flux and increase rejection was lower, because calcium reduces the negative charge of the membrane and forms complexes with humics. The results showed that this NF membrane is effective for reducing NOM in the treated water, and consequently for reducing the THMFP.

NF also demonstrated to be an effective barrier against microcystins (a cyclic peptide of *ca.* 1000 g/mol, negatively charged hepatotoxin) in drinking water. NF removed all the

microcystin variants present in the water (MC-LR, MC-LY and MC-LF) regardless of the variations in the feed water quality.

Results revealed a strong membrane fouling for total concentration of 150 μg/L MC-LR eq. which is, at least, one order of magnitude higher than what might be expected in natural waters. For 16 μg/L of total microcystins, the fouling behaviour was largely attenuated. The high microcystin rejections obtained (above 97%) were mainly related to size exclusion effects, based on the high microcystin size compared to the membrane pore size, and on the microcystin overall net charge (negative but weakly charged). The flux decrease and conductivity increase with pH was already found without microcystins, being smaller in the presence of microcystins. The presence of $CaCl_2$ and NOM, in the studied range, seemed to have no influence on the microcystin rejection by the membrane. Microcystins concentrations in the NF permeate were always far below the WHO guideline value of 1 μg/L MC-LR for drinking water, being usually below the quantification limit.

A relatively low variation of the flux was found with the experimental time and recovery rate for all types of clarified waters and pH values studied.

Fluxes of natural waters spiked with microcystin were lower than those obtained with the electrolyte solutions spiked with microcystin. These results were attributed to both the organic and the inorganic water background matrixes, *i.e.* NOM and calcium cations, which reduced the negative charge of the membrane and complexed with humics. Permeate DOC, UV_{254nm} and SUVA showed very low values (≤ 1 mg C/L, < 0.002 1/cm and 0 – 0.25 L/(m.mg), respectively) from which minimal THMFP are expected.

Results demonstrated that this NF membrane is also able to effectively retain anatoxin-a, a low molecular weight (166 g/mol) alkaloid, positively charged neurotoxin. Experiments with both anatoxin-a and microcystins showed that anatoxin-a and especially microcystins (>95%) were almost completely removed, regardless of the variations in the feed water quality (NOM and competitive toxin), the water recovery rate and the pH values.

The high rejections obtained for anatoxin-a (above 94%) were related with both steric hindrance and charge effects due to the electrostatic interactions. Therefore, the rejection of anatoxin-a spiked in electrolyte solutions with no NOM or hardness cations varied with the solution pH, since the membrane charge is also pH dependent: higher rejections were obtained at acid pH (electrostatic repulsion) and lower rejections at alkaline pH (electrostatic attraction). In the presence of $CaCl_2$, rejections were higher for both acid and especially basic pH, due to the Ca^{2+} ions ability to partially neutralise the membrane negative charge. For anatoxin-a, the presence of calcium ions, NOM and microcystins eliminated the pH effect. These results may be attributed to both physical and chemical aspects of NOM filtration and calcium hardness, since calcium partially neutralises the membrane negative charge and the opposite charge between ATX-a and NOM could reduce the overall net charge of the ATX-a. Hence, membrane−ATX-a attraction decreases and ATX-a rejection increases. In addition, ATX-a could associate with the NOM functional groups and form macromolecular complexes, which increased the steric hindrances and enhanced rejection.

Flux results with anatoxin-a were similar to the results obtained only with microcystins: at acid pH the fluxes were higher than at basic pH; fluxes with natural water spiked with anatoxin-a were lower than those obtained with the electrolyte solutions spiked with anatoxin-a. These results are attributed to membrane negative charge, osmotic pressure effects, organic

and the inorganic water background matrixes, *i.e.* NOM and calcium cations (which reduce the negative charge of the membrane and complexed with humics).

Unlike the widely used adsorption systems for microcontaminants removal from drinking water, where competition between adsorbates reduces the overall performance for the target contaminants, no negative effects were found between ATX-a, MC-LR and NOM, and NF is able to reach low residuals for all parameters: ≤ 0.17 NTU for turbidity, ≤ 1.5 mg C/L for DOC, and no UV_{254nm} absorbance from which minimal THMFP are expected. Anatoxin-a concentrations in the NF permeate are always below 1.3 µg/L, far below the New Zealand's guideline value of 3 µg/L, and microcystins are usually under the quantification limit and always far below the WHO guideline value of 1 µg/L MC-LR for drinking water.

This research contributed to the understanding of the cyanotoxins separation mechanisms by NF membranes, including the impact of the water background organic and inorganic matrixes effect on the NF performance. Moreover, it demonstrated the ability of NF as a safe barrier against cyanotoxins in drinking water, while facing variations in influent cyanotoxins concentration and type (from the high molecular weight, negatively charged microcystins to the small, positively charged anatoxina-a), and background water matrixes (NOM and hardness).

The main objective of this research was to create a safe sequence for treating algae-rich natural waters, containing both cyanobacteria and cyanotoxins. Therefore, the integration of the two studied technologies (C/F/DAF and NF) was investigated.

Good results in terms of NF fluxes, overall removal efficiencies and final water quality were achieved with both C/F/DAF + NF and C/F/DCO_2F + NF sequences. However, C/F/DAF + NF presented a superior performance over C/F/CO_2F + NF in NOM removal, due to the negative effect of acid pH on NF separation efficiency. The negative influence of the acid pH on *M. aeruginosa* cells and cell aggregates stability was also demonstrated, since there was toxin release to the water when the pH decreased to *ca.* 5.6, using both pre-treatments studied, and different feed water background matrixes (raw water and tap water). Results also showed slightly higher NF fluxes for DCO_2F than for DAF pre-treated water (due to the pore narrowing effect promoted by the pH increase). Although no significant NF flux decline was observed with the running time nor with the recovery rate for both NF pre-treatments, a slight decrease was observed using DCO_2F for water recovery rates higher than 50%, above which both processes yielded similar fluxes. Therefore, the CO_2/air gas mixture studied in the flotation process presented no benefit to the overall sequence.

As far as the final water quality is concerned, C/F/DAF + NF guaranteed a full removal of the cyanobacterial biomass (100% removal of chl_a) and the associated microcystins. Microcystin concentrations in the treated water were always under the quantification limit, *i.e.* far below the WHO guideline value of 1 μg/L for MC-LR in drinking water. Therefore, C/F/DAF + NF sequence is a safe barrier against *M. aeruginosa* and the associated microcystins variants (MC-LR, MC-LY, MC-LW and MC-LF) in drinking water, even when high concentrations are present in raw water and NF water recovery rates as high as 84% are used. In addition, it ensures an excellent control of particles (turbidity), and disinfection by-products formation (very low values of DOC, UV_{254nm} and SUVA were achieved, much below those of Alcantarilha WTP final water), as well as other micropollutants (above *ca.* 200 g/mol, *e.g.* anatoxin-a) that might be present in the water.

10.3 SUGESTIONS FOR FUTURE RESEARCH

Despite the contribution of this thesis for the understanding of the removal mechanisms of cyanobacteria and cyanotoxins by dissolved air flotation and nanofiltration, there are still some important aspects that should be investigated in the future, *e.g.*:

- In DAF technology:
  - to analyse the mechanisms of the cell aggregates removal;
  - to investigate the reason for the high extracellular MC-LR removal efficiencies obtained with *Planktothrix rubescens*;
  - to study the effect of the low pH values on the cyanobacterial integrity and separation mechanisms, with recourse to measurements of particle charge and size;
  - to study the removal efficiency of naturally occurring blooms with different types of cyanobacterial morphologies;
- In NF technology:
  - to study the NF performance with other cyanotoxins of increasing concern, *e.g.* *Cylindrospermopsin*, saxitoxins;
  - to evaluate the effect of long-term experiments (fouling experiments at high recovery rates) in the membrane performance at a pilot scale (using this membrane but in spiral-wound modules, compact and economic modules widely used in drinking water treatment, which would require the optimisation of the hydrodynamic conditions);
- In the overall sequence:
  - to evaluate the C/F/DAF + NF performance in the presence of a naturally occurring cyanobacterial bloom at laboratory and full scales;
  - to estimate the capital and exploitation costs of the proposed sequence based on pilot plant experiments.