CHAPTER 6

EVALUATION OF CYANOBACTERIAL CELLS REMOVAL AND LYSIS BY ULTRAFILTRATION

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ABSTRACT

The aim of this study was to evaluate the ultrafiltration (UF) performance for removing *Microcystis aeruginosa* cells under different growth ages (1, 2, 3 and 4 months). Especial attention was given to cell damaging and subsequent release of microcystins to permeate. Experiments were performed with a hollow-fibre cellulose acetate membrane (100 kDa). UF achieved an absolute removal of *M. aeruginosa* single cells, yielding a permeate free of chlorophyll-a and with a turbidity below 0.1 NTU. Cell lysis occurred at all cell growth phases, but greater damage was observed for older cultures. However, the permeate quality was never deteriorated and its microcystins concentration was always identical or lower than the dissolved concentration on the feed water. The hydrophilic UF membrane presented low microcystins adsorption, but microcystins rejection increased in the presence of algogenic organic matter (AOM). The type rather than the overall concentration of salts and organics ruled the membrane fouling, 1-month suspension (polysaccharide-rich AOM with scaling multivalent ions) presenting higher fouling potential than the 3-month suspension (protein-rich AOM with much lower content of multivalent ions).

6.1 INTRODUCTION

Cyanobacterial blooms seasonally challenge drinking water treatment due to the massive input of cells and also the release of algogenic organic matter (AOM) into the water, causing poor settling, filter clogging, tastes and odours, disinfectant consumption and production of disinfection by-products (Paralkar and Edzwald, 1996; Her *et al.*, 2004; Chen and Yeh, 2006; Ma *et al.*, 2006). Nevertheless, the greatest concern is the ability for several strains of cyanobacteria to produce potent toxins as secondary metabolites, cyanotoxins, including cyclic peptide hepatotoxins (*e.g.*, microcystins) and/or the alkaloid neurotoxins (*e.g.*, anatoxin-a). Microcystins are the most frequently occurring cyanotoxins, derived from the genera *Microcystis*, *Anabaena*, *Planktothrix*, *Nostoc* and *Anabaenopsis*, and may be liver damaging and tumour promoters (Sivonen and Jones, 1999). The World Health Organization (WHO) derived a drinking water provisional guideline value of 1 µg/L for daily exposure to the microcystin-LR, one of the most toxic and frequent microcystin variant.

Cyanotoxins may occur both within the cells (cell-bound or intracellular) or dissolved in water (extracellular), a feature that strongly influences the removal efficiency throughout the water treatment train. In general, if properly operated, conventional treatment (coagulation, flocculation, sedimentation and sand filtration) is able to control to some extent the cyanobacterial cells, but is ineffective in the removal of extracellular cyanotoxins (Falconer *et al.*, 1989; Himberg *et al.*, 1989; Donati *et al.*, 1994). Toxin release into water may be due to natural toxin release (natural cell lysis or active release) and/or induced toxin release (resulting from cell damage in the treatment processes caused by mechanical and/or chemical stress) (Pietsch *et al.*, 2002; Schmidt *et al.*, 2002). The optimal treatment strategy is to remove intact cyanobacterial cells (Drikas *et al.*, 2001), thereby retaining the majority of metabolites

inside the cells. While such strategy will not avoid further treatments for dissolved organics control, it will improve the overall process effectiveness and economics.

Literature is not consensual with respect to the removal effectiveness of algal cells through conventional treatment, the most controversial subjects being the cell lysis and the AOM effect. Some studies refer the occurrence of cell lysis and release of intracellular material (Himberg *et al.*, 1989; Lam *et al.*, 1995; Hrudey *et al.*, 1999; Pietsch *et al.*, 2002), while others reported no effect on the cell integrity (Kenefick *et al.*, 1993; Chow *et al.*, 1998, 1999; Drikas *et al.*, 2001; Ribau Teixeira and Rosa, 2006, 2007). There is also some controversy about the use of a preoxidation step in algal-rich waters, since its effects are dependent on the preoxidant type and dosage (Chen and Yeh, 2006). Besides the problem of by-products formation, oxidation processes may lyse cyanobacterial cells (Lam *et al.*, 1995; Paralkar and Edzwald, 1996; Ma and Liu, 2002; Pietsch *et al.*, 2002; Chen and Yeh, 2006; Ma *et al.*, 2006; Daly *et al.*, 2007), and soluble toxin destruction is not always ensured.

Several authors have referred membrane filtration as an attractive technology to reach high removal efficiencies of algae (Chow *et al.*, 1997; Mouchet and Bonnélye, 1998; Hrudey *et al.*, 1999; Pietsch *et al.*, 2002; Ribau Teixeira and Rosa, 2005; Gijsbertsen-Abrahamse *et al.*, 2006), applied either alone or following conventional clarification or DAF, depending on the raw water quality (Mouchet and Bonnélye, 1998). UF is a low pressure process, ensuring effective disinfection. UF removal efficiencies are not as greatly affected by the raw water quality parameters as the conventional treatment efficiencies, and a complete removal of the cyanobacterial cells may be expected by UF, given the relative size of the cells and the UF pores (usually two orders of magnitude lower) (Chow *et al.*, 1997).

However and to my knowledge, only two studies were carried out on the removal of cyanobacterial cells by ultrafiltration. Chow *et al.* (1997) used flat-sheet membranes, operated under dead-end (DE) and cross-flow (CF) modes to study the effect of membrane filtration on the *Microcystis aeruginosa* cells. Their results showed that 99-100% of chlorophyll-a was removed, a small portion of cells were damaged by the treatment (*ca.* 2-5% under DE operation and 4-10% under CF operation), but there was no increase of toxin in the permeate. Gijsbertsen-Abrahamse *et al.* (2006) used a hollow-fibre membrane, operated under DE to determine the removal efficiency of cell-bound cyanotoxins from *Planktothrix* strains. Microscopic measurement indicated slight damage of one third of the *P. agardhii* filaments, but the damages were not considered substantial and corresponded to a maximum of 2% of cell-bound microcystin release detected in the permeate. Further studies were still recommended by the same authors before these results can be generalised, especially in what concerns the cell sensitivity to shear stress and the toxin release into water.

In this context, the aim of this study is to evaluate the UF performance for removing laboratory grown *M. aeruginosa* cells under different growth ages (using hydrophilic hollow-fibre, 100 kDa cutoff membrane in CF mode of operation). Particular attention is given to cell damaging and subsequent release of microcystins to the permeate, and also to membrane fouling by AOM. *M. aeruginosa* is one of the most commonly occurring cyanobacteria and grows in laboratory as single cells, which is an advantage for this study. Besides they better represent the size of algae that is more prone to escape from a conventional clarification process, *M. aeruginosa* single cells may be used as surrogate to assess the removal efficiency of particles of problematic size range (3-10 μm), like *Giardia* cysts and *Cryptosporidium* oocysts.

6.2 MATERIALS AND METHODS

6.2.1 Cyanobacterial Culture

M. aeruginosa culture (Pasteur Culture Collection, PCC 7820) was grown in the laboratory, in BG11 medium, at 23-24ºC, under a light regimen of 12h fluorescent light, 12 h dark. Cultures were harvested at different growth ages in order to access distinct lysis vulnerabilities. This strain of *M. aeruginosa* produces four microcystin variants (MC-LR, -LY, -LW, -LF), and the overall concentration was quantified in μg/L MC-LR equivalent concentration. The studied microcystins are cyclic heptapeptides, relatively hydrophobic, with a molecular weight between 985 and 1024 g/mol and have a net negative charge (-1 for MC-LR, -2 for the other microcystins) at neutral pH.

6.2.2 UF Feed Waters

Trials were carried out with deionised water (DI) spiked with a predetermined volume of the *M. aeruginosa* culture to obtain a chlorophyll-a (chl-a) concentration *ca.* 20 μg/L (Table 6.1). This specific concentration is less than half the WHO Alert Level 2 established by Bartram *et al.* (1999) for a potentially toxic cyanobacterial bloom (cyanobacterial biomass 100,000 cells/mL or 50 μg/L chl-a) and aims to simulate a weak bloom and/or a strong bloom after preliminary treatment. The cultures were harvested at different growth ages (1, 2, 3 and 4 month - M). To avoid osmotic shock, which may result in cyanobacterial lysis, before spiking DI was amended with potassium chloride until the water conductivity reached *ca.* 260 μS/cm. The pH was also corrected to 7±0.3 with KOH and H_2SO_4. Microcystins were extracted from *M. aeruginosa* culture, following the procedure of Meriluoto and Spoof described by Campinas and Rosa (2006).

Table 6.1- Characteristics of *M. aeruginosa* suspensions used in the UF experiments.

Suspensions	UF Experiment	pH	EC (µS/cm)	Turbidity (NTU)	Chl-a (µg/L)	TOC (mg C/L)	MC-LR_{eq} (µg/L)	
							Extra	Intra
1M	Constant TMP	7.3	301	4.5	17.4	3.3	9.7	--
	Constant Flow	7.2	310	5.3	28.0	2.2	8.9	--
2M	Constant Flow	6.7	293	2.5	20.6	--	0.6	1.5
3M	Constant TMP	6.7	256	2.8	17.4	2.1	1.4	--
	Constant Flow	7.3	294	3.4	20.0	3.4	2.9	--
4M	Constant Flow	6.7	301	2.8	19.2	--	0.5	2.1

M: months; TMP: transmembrane pressure; EC: electric conductivity; Chl-a: chlorophyll-a

Model solutions of microcystins (DI with 12.3-13.7 µg/L MC-LR_{eq}, 2.5 mM background ionic strength (1 mM IS of KCl and 1.5 mM IS of $CaCl_2$) and pH 7±0.3) were used in preliminary UF runs performed to assess the microcystin adsorption to the UF membrane.

6.2.3 Membrane

An Aquasource cellulose acetate hollow-fibre (inside-out configuration) membrane was used. This UF hydrophilic membrane has a molecular weight cut-off (MWCO) of 100 kDa and a hydraulic permeability of 250 L/(h.m^2.bar) (manufacturer data). The module has 16 fibres, 1.1 m length and 0.93 mm internal diameter, with a total surface area of 0.05 m^2. The manufacturer recommends a maximum UF pressure of 1.5 bar and a maximum backflushing pressure of 2.5 bar.

6.2.4 Ultrafiltration Experiments

The membranes were first compacted with DI until achieving a steady permeate flux, at the pressure and cross-flow velocity to be used in the experiments. Two types of UF experiments were performed: (1) constant flow runs and (2) concentration runs at constant transmembrane

pressure (TMP). A schematic diagram of the UF lab-scale unit is provided in Figure 6.1 (dotted lines represent the adaptations made for constant flow runs).

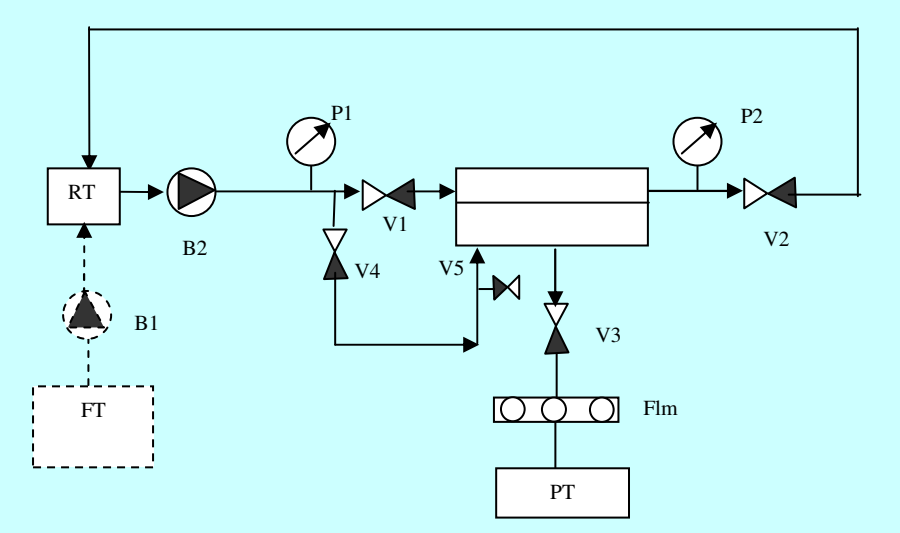

Figure 6.1 – Schematic diagram of laboratory UF installation (FT: Feed tank; RT: Recirculating tank; PT: Permeate Tank; Flm: Flowmeter; P: Manometers; V1, V4, V5: Valves for backwashing; V2: Concentrate valve; V3: Permeate valve; B1: Peristaltic pump; B2: Positive displacement pump).

The first type of runs (1) were performed at constant permeate flow of 3.5 L/h and an initial transmembrane pressure (TMP) of 0.65 bar. A feed glass tank (FT) fed the raw water to the loop recirculating tank (RT) through a multihead peristaltic pump at a constant flow of 3.5 L/h to balance the output flow of permeate. The positive displacement pump provided the necessary pressure and recirculation at a cross-flow velocity of 0.5 m/s (a variable frequency drive allowed the concentrate velocity in the hollow-fibres to be adjusted). The concentrate was conducted to the RT and blended with the additional feed water from FT, and the permeate was continuously collected in a beaker until a 1 L sample was obtained. By that time, RT was sampled (200 mL) to characterise the UF feed water. Filtration cycles lasted 60 minutes, this is, four samples of permeate (three samples of 1 L each (0-17 min., 17-34 min., 34-51 min.) and the last one with *ca.* 0.5 L (51-60 min.)) were always collected.

Individual concentrations were determined, and whenever necessary for data interpretation, cycle-averaged values (0-60 min.) were computed. After each filtration run the membranes were washed with a 5 mg/L (as Cl_2) sodium hypochlorite solution to inhibit the biological activity on the UF system, and flushed with deionised water.

Concentration runs (2) were performed to evaluate the effect of the initial concentration on the membrane performance. These experiments were performed at a constant TMP of 0.6 bar, with the permeate being discarded and the concentrate being recycled to the feed tank, thereby continuously increasing the water recovery rate (WRR, defined as the ratio between the permeate and the initial feed volumes). In these set of runs, FT and RT were the same and the peristaltic pump was not used. Permeate was discarded and when the defined WRR were achieved (0%, 66% and 90%) permeate was recycled to the feed tank during five minutes, after which samples of feed and permeate were taken (250 mL).

Flux was continuously measured during the experiments and normalised fluxes (defined as the ratio of solution flux over the pure water flux) were calculated. All flux values were corrected to a constant temperature (20 ºC), using the equation derived by Crozes *et al.* (1997), which is based on the variation of water viscosity with temperature:

$$J_{20}=J_T\, e^{-0.0239\,(T-20)}$$

where J_{20} is the flux (L/min.m^2) at 20ºC, T is the temperature (ºC) and J_T (L/min.m^2) is the flux at temperature T.

6.2.5 Analytical Methods

Samples were analysed for pH (at 20ºC, using a WTW 340 pH meter), electric conductivity (EC) (at 25ºC, using a Crison GLP 32 conductimeter), total organic carbon (TOC) (measured

as non-purgable organic carbon in a high temperature combustion Shimadzu TOC 5000A analyser), turbidity (HACH 2100N turbidimeter of high resolution, 0.001 NTU) and chlorophyll-a, using standard methods for analysis of water. For chlorophyll-a, volumes of 100-200 mL (feed samples) or 1 L (permeate samples) were used and the optical densities (665 nm and 750 nm) were analysed on a UV-VIS spectrophotometer (Beckman DU 640B).
In the first experiments (1 M, 3 M) only dissolved microcystins were determined in the feed samples (200 mL), whereas in the last experiments (2 M, 4 M) both cell-bound and dissolved microcystins were analysed (100 mL and 200 mL, respectively). As for permeate samples, in all runs only extracellular microcystins were analysed (1000 mL in constant flow experiments and 200 mL in concentration runs).

Microcystins analysis followed the standard operation procedures developed by Meriluoto and Spoof (2005 a, b, c) with some adaptations. Extracellular microcystins were first isolated from cell-bound microcystins by sample filtration through a Whatman GF/C glass microfibre filter. The dissolved fraction was then concentrated by solid phase extraction in isolute C18 cartridges and the microcystins were eluted with 5 mL methanol (90%) containing 0.1% (v/v) trifluoracetic acid (further details in Ribau Teixeira and Rosa (2006)). For intracellular microcystins, the filters were frozen-thawed twice and kept in methanol (75% v/v) during 18 – 24 h at 4ºC. The methanolic extracts (extra and cell-bound) were rotary evaporated (50ºC), resuspended in 500 μL methanol (75%), centrifuged (10000 x g, 10 min) and 150 μL of the supernatant were transferred to HPLC vials for analysis. Microcystins were analysed by high performance liquid chromatography with photodiode-array detection (HPLC-PDA), using a Dionex Summit System.

6.3 RESULTS AND DISCUSSION

6.3.1 Microcystins Rejection by UF

Preliminary experiments with model single-solute solutions of microcystins were designed to evaluate if the UF cellulose acetate membrane was able to reject microcystins and if microcystins exhibited a fouling behaviour. Low-molar mass solutes may cause drastic flux reductions during UF due to adsorption, and the solute concentration and membrane material have an important influence on the membrane fouling potential (Jönsson and Jönsson, 1995; Jönsson *et al.*, 1997). Microcystins are relatively hydrophobic compounds with a molar mass (909 −1115 Da) much below the MWCO of the hydrophilic membrane (100 kDa), making rejection through sieving not an option, but probably through adsorption.

Figure 6.2 presents the normalised flux (Figure 6.2, left) and the microcystins rejection (Figure 6.2, centre) as a function of WRR during a concentration run and the cycle-averaged concentration of microcystins on the feed and permeate during a constant flow UF cycle (Figure 6.2, right) (error bars represents the standard deviations).

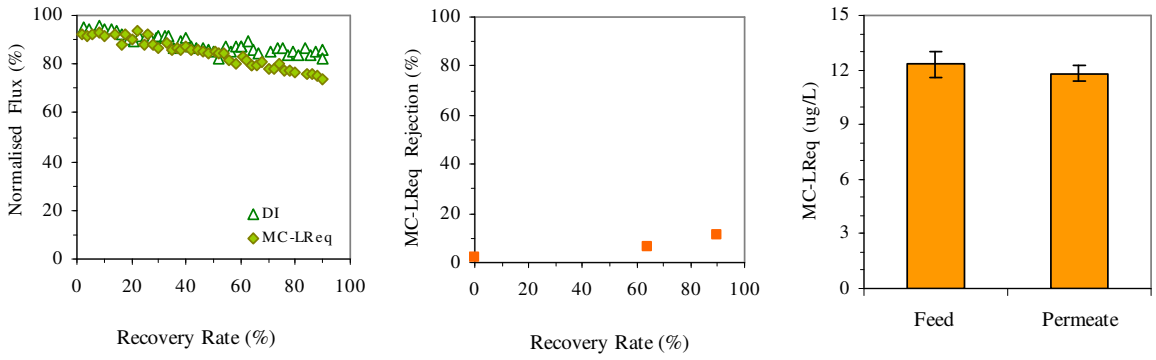

Figure 6.2 – Normalised flux (left) and dissolved microcystins rejection (centre) during UF concentration runs, and cycle-averaged concentration of microcystins on the feed and permeate during constant flow runs (right) with single-solute model solution.

Normalised flux is very similar in the presence and in the absence of microcystins for WRR up to 60%, corresponding to 52 min of operation (Figure 6.2, left). Above 60%, microcystins seem to slightly promote the flux decline (*ca.* 10% maximum), probably due to increased microcystins adsorption onto the membrane surface. In fact, the initially negligible (2%) microcystins rejection slightly increased up to 11% for 90% WRR and 110 min of operation (Figure 6.2, centre). The microcystins foulant behaviour was also observed by Ribau Teixeira and Rosa (2005) with a hydrophilic NF membrane – a strong membrane fouling with a feed concentration of 150 μg/L MC-LR_{eq} and a largely attenuated fouling with 16 μg/L MC-LR_{eq} feed water.

In the experiment performed at constant flow, no significant pressure changes were observed during the run and these data are therefore not shown. Concerning the water quality, it is possible to observe in Figure 6.2 (right) that the microcystins concentration in the feed and in the permeate is quite similar (4% average rejection), indicating a low adsorption under the studied conditions. These results are not far from those obtained by Lee and Walker (2006) with a cellulose acetate flat-sheet UF membrane (0.9% of rejection). Higher rejections were reported by others for hydrophobic membranes. Using polyethersulphone membranes, Lee and Walker (2006) obtained 67-78% adsorption and Gijsbertsen-Abrahamse *et al.* (2006) referred a 53% rejection. The polysulphone membrane used by Chow *et al.* (1997) exhibited rejection properties for microcystins, particularly in the CF run (~ 23% of rejection).

6.3.2 Characterisation of Cultures Growth Phases

This study involved a first characterisation of *M. aeruginosa* cultures harvested at different growth ages, in order to tackle the main objective of this paper, which was to evaluate the

cyanobacterial cells retention and integrity during ultrafiltration and the effect of cell age (cell vulnerability and AOM content) onto the UF performance.

Cyanotoxins are produced at all stages of cyanobacterial growth and generally remain in the cell (intracellular) until age or stress driven cell lysis causes their release into the surrounding water (extracellular) (Sivonen and Jones, 1999). During the growth phases there are some strong changes in the cyanobacterial biomass, in the total cyanotoxins content, in the relation between intra- and extracellular cyanotoxins, and in the AOM production. In general, intracellular microcystins are produced during the exponential growth phase. Further generation happens during the stationary phase and a sharp rise of dissolved microcystins occurs during decaying algae growth phase (Pietsch *et al.*, 2002). Pietsch *et al.* (2002) found that a *M. aeruginosa* sample was characterised by a 0.05 and 1 extra/intra MC-LR ratio, for the exponential and for the stationary phases, respectively.

Pivokonsky *et al.* (2006) studies also showed that the amount of AOM produced by *M. aeruginosa* culture increased with ageing and the proportion of proteinic organic matter also increased, reaching about 31% of DOC in the stationary phase. A significantly greater portion of proteins were in the IOM (intracellular organic matter) fraction, while the EOM (extracellular organic matter) fraction was mainly composed of organics with no proteic character (*e.g.*, polysaccharides). The organic matter content and the contribution of IOM to the total AOM concentration both increased during the stationary phase due to autolytic processes. To understand the growth phases of the *M. aeruginosa* cultures used in this study, five cultures of different ages (1 month to 6 months old) were characterised in terms of chlorophyll-a content, turbidity, total extra- and intracellular microcystins concentrations and extra/intra MC-LR_{eq} ratio (Figure 6.3).

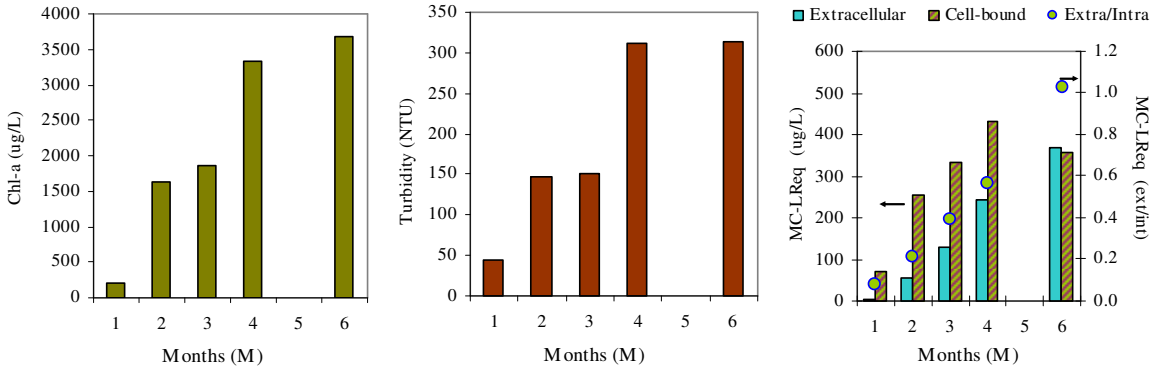

Figure 6.3 - Characterisation of *M. aeruginosa* cultures growth phases.

Figure 6.3 shows that all parameters have three increasing stages: at 1 M, chlorophyll-a was 211 µg/L, increasing to 1622-1857 µg/L at 2-3 M, and finally to 3331-3687 µg/L at 4-6 M; turbidity had the same pattern, increasing from 45 NTU (1 M) to 147-150 NTU (2-3 M) and afterwards to 312-314 NTU (4-6 M); total microcystins content also augmented from 74.3 µg/L MC-LR_{eq} (1 M) to 308-461 µg/L (2-3 M) and finally to 673-725 µg/L (4-6 M). For all samples, the ratio between extra- and intracellular microcystins increased with ageing and presented the minimum value of 0.08 for the culture 1 M old and the maximum value of 1 for the culture 6 M old. These results indicate that the cultures used in the UF experiments were in three different growth phases; 1 M culture corresponded to exponential phase, 2 M and 3 M cultures to late exponential phase, and 4 M culture to stationary phase.

6.3.3 Retention of Cyanobacterial Cells

In this study, chlorophyll-a was used to assess the overall removal efficiency of cyanobacterial cells. Since it was observed a relation between chlorophyll-a and turbidity for *M. aeruginosa* laboratory grown cultures (10-11 µg/L chl-a ≈ 1 NTU), turbidity results were also used for such purpose. Figure 6.4 presents turbidity and chl-a feed profiles (Figure 6.4, left) and cycle-averaged concentrations on the feed and permeate (Figure 6.4, right) during

constant flow UF cycles performed with *M. aeruginosa* cells (2 M and 4 M). The error bars represented in the figure are the standard deviations.

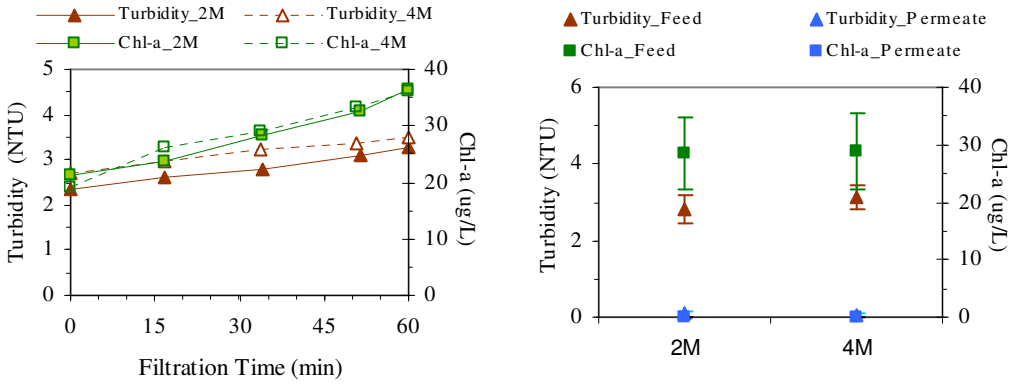

Figure 6.4 - Turbidity and chlorophyll-a feed profiles (left) and cycle-averaged concentrations (right) on the feed and permeate during constant flow runs with *M. aeruginosa* cells of 2 M and 4 M.

Although during the filtration cycle of both 2 M and 4 M cells, turbidity and chl-a concentration have increased in the feed, respectively from 2.4-2.7 NTU to 3.3-3.5 NTU and 19-21 µg/L to 36 µg/L, the quality of the permeate was always high. Turbidity was kept below 0.1 NTU (except for one sample) (> 97% rejection) and chlorophyll-a was never detected in the permeate (complete rejection). Similar results were obtained in the experiments performed with 1 M and 3 M cells (data not shown). The results indicate an absolute removal of *M. aeruginosa* cells, as expected, since they are 400-600 times larger than the membrane pores (single cells diameter is about 4 - 6 µm and the membrane MWCO is 100 kDa, which corresponds to an average pore diameter *ca.* 0.01 µm (Chow *et al.*, 1997)).

Ultrafiltration results of cell retention are comparable and usually substantial better than the removal achieved by a conventional treatment of coagulation/flocculation, sedimentation or DAF (C/F/S or C/F/DAF) and filtration (F). Vlaski *et al.* (1996) obtained algae removal

efficiencies of 90% for C/F/DAF/F and 95% for C/F/S/F, whereas Ribau Teixeira and Rosa (2006, 2007) reported *M. aeruginosa* cells retentions of 69-94% using C/F/S and 77-99% using C/F/DAF.

As single cells are the lowest size cyanobacteria (and must therefore be the most difficult to retain by a solid-liquid separation) and may be used as *Giardia* and *Cryptosporidium* surrogates (Vlaski *et al.*, 1996), these results confirm that UF is a safe barrier against cyanobacteria and protozoa, as reported in the literature (Jacangelo *et al.*, 1995; Ottoson *et al.*, 2006). This is a very important feature due to the chemical resistance to oxidation exhibited by the *Giardia* cysts and the *Cryptosporidium* oocysts.

6.3.4 Cell Lysis and Microcystins Release to Water

Another objective of this study was the analysis of cyanobacterial cells integrity during ultrafiltration, since the shear stresses developed at the membrane surface or from pumping may cause cell damage, with subsequent release of intracellular cyanotoxins and IOM into the permeate. UF constant flow runs were performed with *M. aeruginosa* cells.

Cell lysis was not directly measured, but rather indirectly assessed since it was intended to evaluate the effect of cell lysis on the permeate quality and not the cell lysis phenomenon itself. Given the previous results of no significant rejection of extracellular microcystin by the UF cellulose acetate membrane, it was initially decided to indirectly assess cell lysis through the evolution of the dissolved microcystin content in the feed during the filtration cycle.

Results from the first set of experiments performed with cultures one month (1 M) and three months (3 M) old are shown in Figure 6.5. It was obtained a cycle-averaged microcystins

rejection of 33% with 1 M cells and 65% with 3 M cells. The most probable explanation for the microcystins rejection exhibited by both 1 M and 3 M cultures is AOM-microcystins interaction, which confers rejection properties to the hydrophilic membrane.

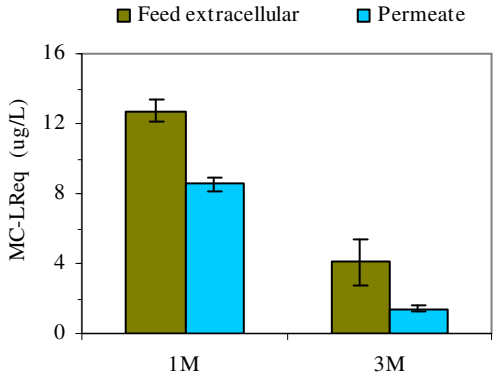

Figure 6.5 - Cycle-averaged concentration of microcystins on the feed and permeate during constant flow runs with *M. aeruginosa* cells (1M and 3M). The error bars represents standard deviations.

One remark has to be made relative to the higher concentration of microcystins in 1 M culture compared to 3 M, which is not a real situation since older cultures have in general higher microcystins content. This is explained by the larger spiking volume used for 1 M, since the main purpose was to obtain initial identical chlorophyll-a concentration.

Due to the existence of AOM-driven microcystins adsorption, cell lysis evaluation based only on the time evolution of feed and permeate dissolved microcystins concentration is not valid, rather it underestimates the cell lysis phenomenon. As cell lysis and microcystins adsorption may happen simultaneously and may not be differentiated, their net contribution was computed from mass-balance derived for dissolved microcystins.

Mass-balance equation considered three inputs (+) and three outputs (-): (+) the initial mass in RT, (+) the feed input, (-) the final mass in RT; (-) the permeate output; (-) adsorption and

(+) input from cell lysis. The difference between adsorption and lysis was normalised as expressed in equation 6.1:

$$\%(Ads-Lysis)=\frac{(V_0C_0+QtC-V_fC_f-\sum_{i=1}^{4}Q_{pi}t_iC_{pi})}{(V_0C_0+QtC-\sum_{i=1}^{4}Q_{pi}t_iC_{pi})}\times 100\% \qquad \text{(Equation 6.1)}$$

where V_0 and C_0 are the initial feed volume (L) and concentration (mg/L), respectively; Q is the feed flow (L/min) (make-up to balance the permeate flow output), t and C are the associated feed pumping time (min) and concentration (mg/L); V_f and C_f are the final volume (L) and concentration (mg/L); Q_{pi}, t_i and C_{pi} are the permeate flow (L/min), pumping time (min) and concentration (mg/L).

Results of the mass-balance application to the constant flow UF cycles with *M. aeruginosa* cells of 1 M and 3 M are depicted on Figure 6.6.

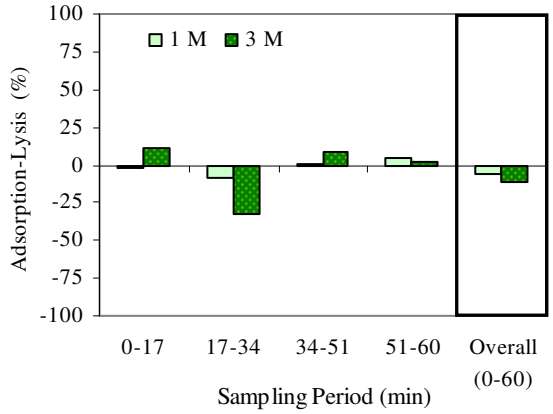

Figure 6.6 - Net contribution of adsorption and cell lysis computed from mass-balance equation developed for dissolved microcystins during constant flow UF cycles with *M. aeruginosa* cells (1 M and 3 M).

During the UF cycles with 1 M and 3 M cells, there were some periods when cell lysis exceeded the adsorption (negative bars) and others when adsorption was more important

(positive bars), but the overall net balance for both cultures indicated the cell lysis dominance over the adsorption, with greater expression for 3 M cells. Nevertheless, the cell damage did not compromise the permeate quality since the microcystins concentration in the permeate was always below its concentration in the feed (Figure 6.5).

The experiments were repeated one month latter with the same cultures, *i.e.*, by the time of the second set of trials the cultures were two months (2 M) and four months (4 M) old. Given the difficult interpretation of the dissolved microcystins results, it was decided to further assess cell lysis through the evolution of the intra-microcystin and chlorophyll-a contents in the feed during the filtration cycle. Results were analysed through concentration factors ($Cf = C/C_0$) for those two parameters, comparing the experimental Cf values with the expected ones. The latter were calculated by mass-balance equation, considering complete rejection, no adsorption and no cell lysis. Experimental Cf values lower than expected Cf values were therefore interpreted as cell lysis (Figure 6.7).

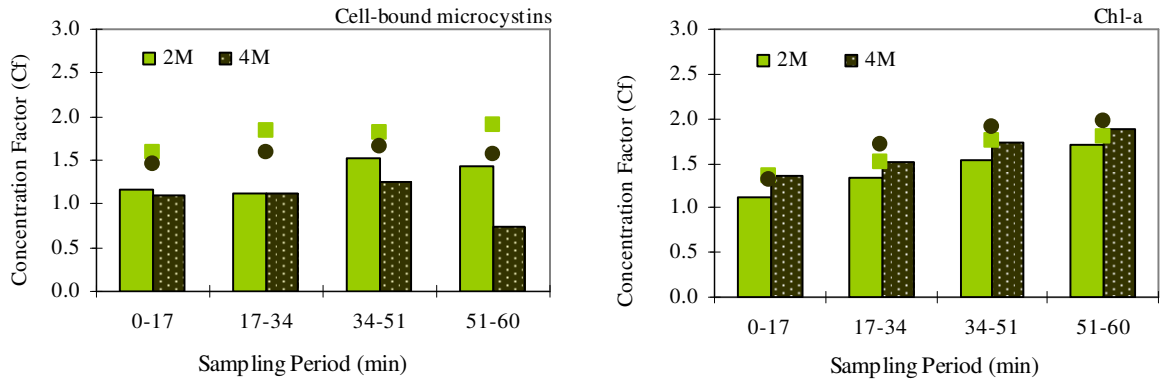

Figure 6.7 - Concentration factors for cell-bound microcystins (left) and chlorophyll-a (right) during constant flow runs with *M. aeruginosa* cells (2 M and 4 M): experimental (bars) and expected (symbols) values based on mass-balance equation.

Figure 6.7 (left) confirms the cell lysis occurrence for both cultures, but with greater significance for the older culture and at the end of the filtration cycle. Chlorophyll-a content (Figure 6.7, right) is less sensitive to cell lysis than the intra-microcystins content, which suggests the intra-microcystin/chl-a ratio as an indirect measure of cell lysis, but further experiments are necessary to confirm this finding.

Figure 6.8 presents the net contribution of adsorption and cell lysis for dissolved microcystins concentration during the experiments with 2 M and 4 M cells.

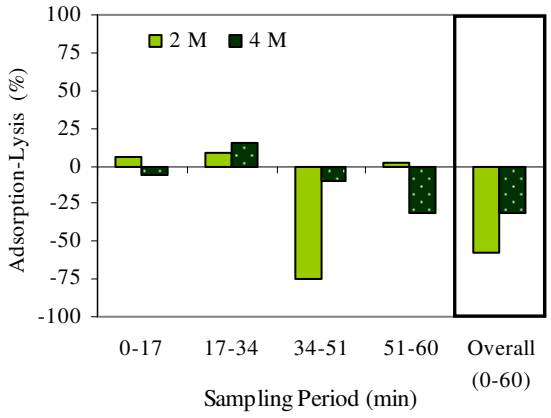

Figure 6.8 - Net contribution of adsorption and cell lysis computed from mass-balance equation developed for dissolved microcystins during constant flow UF cycles with *M. aeruginosa* cells (2 M and 4 M).

The overall net contribution continued to indicate a dominance of cell lysis over the adsorption for both 2 M and 4 M cells. The greater lysis of 4M cells, so clearly expressed in Figure 6.7 (left), was not evident in Figure 6.8, which indicates the importance of the adsorption phenomenon for this older culture. In parallel to cell damage, an enhancement of microcystins rejection by the UF cellulose acetate membrane must occur with ageing, probably associated with the greater content in segregated AOM (mucopolysaccharides) and/or protein lysed AOM of older cultures. For that matter, the permeate quality was not

degraded, not even for the older culture (Figure 6.9). Microcystins concentration on the permeate was similar or lower than its feed concentration, with cycle-averaged rejections of total (extra + intra) microcystins of 60% for 2 M and 80% for 4 M cells.

It can be therefore concluded that cell lysis happened during UF runs, especially for older cultures, but this phenomenon did not result in a net degradation of the permeate quality since in parallel to cell damage, an enhancement of microcystins rejection by the UF cellulose acetate membrane was observed with ageing, probably due to AOM-driven microcystin adsorption. Pietsch *et al.* (2002) also obtained higher values of *M. aeruginosa* cell removal and cell lysis by flocculation in the stationary growth phase compared with the exponential growth phase.

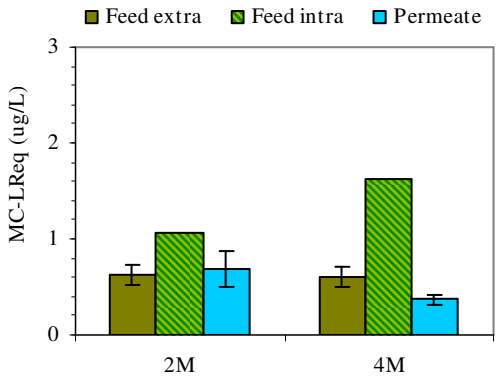

Figure 6.9 - Cycle-averaged concentration of microcystins (extra and intracellular) on the feed and permeate during constant flow runs with *M. aeruginosa* cells (2 M and 4 M). The error bars represents standard deviations.

6.3.5 Membrane Fouling by AOM

The distinct behaviour observed for microcystins rejection by the cellulose acetate UF membrane in the absence and in the presence of cyanobacterial culture (Figure 6.2 right *vs.* Figure 6.5; Figure 6.2 centre *vs.* Figure 6.11, bottom-right) is probably due to the AOM segregated during the metabolic activity (EOM) or resulting from cells decaying (IOM).

These organic substances include a wide range of compounds, such as oligo and polysaccharides, proteins, peptides, amino acids and also traces of other organic acids (Pivokonsky *et al.*, 2006). Many polysaccharides have inherent "stickness" and capacity for adsorption of other chemicals or particles and may act as thickening agents or bioflocculants (De Philippis *et al.*, 2001), which explains the enhancement of microcystins rejection in the presence of AOM. Aggregation or enmeshment of dissolved microcystins onto organic matter could result in its physical exclusion by the membrane or better adsorption onto the membrane surface. These polysaccharides and proteins were also found to be responsible for significant membrane fouling (Her *et al.*, 2004; Lee *et al.*, 2006).

The results of normalised flux *versus* WRR obtained in the UF concentration experiments with *M. aeruginosa* cells 1 M and 3 M old are presented on Figure 6.10. Figure 6.11 shows the feed and permeate quality (turbidity, chlorophyll-a, TOC and microcystins concentrations), as well as TOC and microcystins rejections.

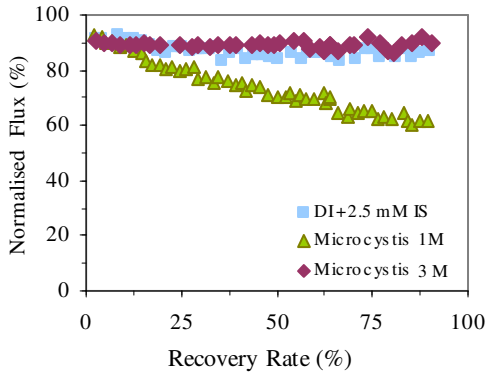

Figure 6.10 - Normalised flux during UF concentration runs with *M. aeruginosa* cells (1 M and 3 M).

With 1 M cell culture, flux decline with water recovery rate was very severe (normalised flux was 60% for 90% WRR), whereas for 3 M cell culture no significant flux decline was observed (Figure 6.10). However, the overall quality of the assayed 1 M and 3 M waters was

quite similar except for dissolved microcystins (parameters shown in Figure 6.11 and also pH and electric conductivity, which were not depicted but presented values of 7.3 and 301 μS/cm for 1 M, and 6.7 and 256 μS/cm for 3 M, respectively) and kept similar during the run in both the feed and the permeate. Therefore, the type rather than the overall concentration of salts and organics must explain the distinct membrane fouling observed. As previously referred, for 1 M water, a larger spiking volume was used (almost 6 times larger than the volume used for 3 M), which means that 1 M water had greater contribution of the dissolved microcystins and polysaccharide-type AOM, as well as of the growth media ions (yielding a conductivity of 190 μS/cm for 1 M *vs.* 28 μS/cm for 3 M). These mono- and multivalent ions may be responsible for NOM aggregation and precipitation, causing membrane fouling.

By other side, the assayed water containing 3 M cells, given the cell age and cell decaying vulnerability, probably have higher proportion of proteic organic matter compared to the assayed water with 1 M cells, whose AOM must predominantly consist of polysaccharide-type compounds resulting from cell secretion. Actually, Her *et al.* (2004) found that the characteristics of the organic matter are more influential than DOC concentration in the fouling potential.

For 1 M cells run, the TOC rejection gradually increases from 17% (0% WRR) to 36% at 90% WRR (Figure 6.11). The fouling potential of this water indicates high adsorption, cake formation or membrane pore blockage mechanisms, especially at higher recovery rates, which increase the membrane resistance and also the rejection. In the case of 3 M cells run, the TOC rejection at the beginning of the filtration exceeded the one found for the 1 M cells (26% *vs.* 17%) and has a rather insignificant increase with WRR (28% for 90% WRR).

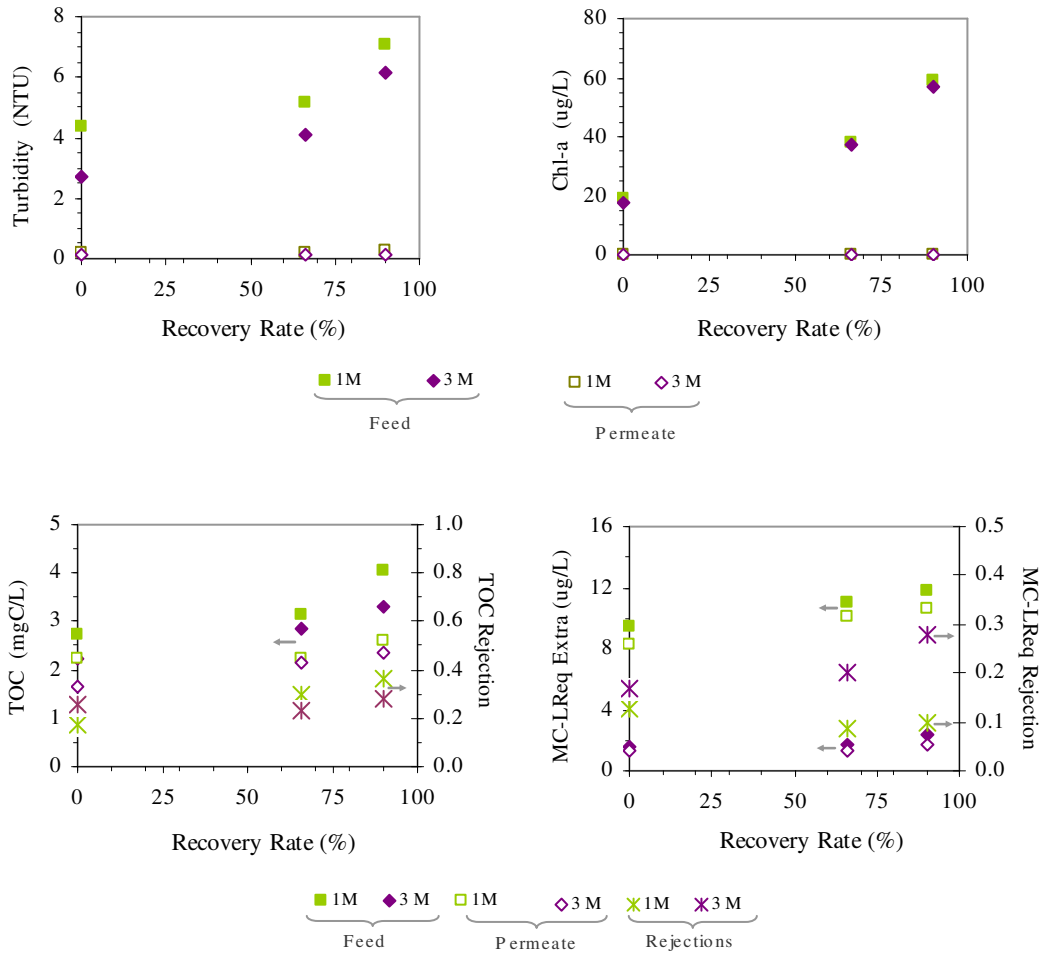

Figure 6.11 - Feed (closed symbols) and permeate quality (open symbols), and rejections (asterisks) during UF concentration runs with *M. aeruginosa* cells (1 M and 3 M).

The membrane fouling phenomenon and its relation with the organic and inorganic water matrices are further addressed in the following chapter.

6.4 CONCLUSIONS

This study demonstrated that ultrafiltration with a hollow-fibre cellulose acetate membrane of 100 kDa MWCO is a safe barrier against cyanobacteria, producing a permeate free from *M. aeruginosa* single cells (the smallest cells and therefore the most difficult to remove) and with a turbidity below 0.1 NTU, even with feed chlorophyll-a values as high as 60 µg/L.

Regarding the UF effect onto cell lysis, data indicated an increasing lysis with cell ageing, but the permeate quality was never deteriorated. In parallel to cell damage, an enhancement of microcystins rejection by the UF membrane was observed with ageing, probably associated with the greater content of the older cultures in segregated AOM (mucopolysaccharides) and/or protein lysed AOM. Actually, preliminary experiments in the absence of background organics showed no rejection of microcystins by this hydrophilic UF membrane. Due to the existence of AOM-driven microcystins adsorption, cell lysis evaluation based only on the time evolution of dissolved microcystins concentration in the feed and in the permeate underestimates the phenomenon, and mass-balance equations were derived to calculate the net contribution of adsorption and cell lysis. This methodology indicated the overall dominance of cell lysis over adsorption.

Results from UF concentration runs also indicate that the characteristics of water background organics and inorganics, rather than their overall concentration, must govern the membrane fouling. In this study, a strong foulant behaviour (60% normalised flux for 90% WRR) and an increase in the TOC rejection were observed for waters with 1 M cell culture, *i.e.*, polysaccharide-rich AOM in the presence of scaling multivalent ions, whereas no significant flux decline nor TOC rejection changes were observed for the analogous 3 M cells run (protein-rich AOM with much lower content of multivalent ions).

In conclusion, UF ensured an absolute cell removal with no degradation of permeate quality. However, cell damage results advise prudence when considering the use of UF for cyanobacterial cells removal. To minimise cell lysis and membrane fouling, in algal-rich waters UF should be preceded by a pre-treatment step of C/F/S or C/F/DAF (preferentially with enhanced coagulation for better removal of cyanobacteria and membrane foulant AOM)

and/or must be integrated with a process that efficiently removes the dissolved cyanotoxins and the AOM, *e.g.* powdered activated carbon.

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