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Effects of intracellular/dissolved ratios of microcystin-LR on its removal by ultrafiltration

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ABSTRACT

Microcystins (MCs) are dangerous toxins produced by cyanobacteria in eutrophic waters, that are increasingly used worldwide for human consumption after potabilization.In this study, we present the results of laboratory and pilot-plant experiments, aimed at deepening the knowledge of the mechanisms governing the equilibrium of dissolved and particulate-bound MCs, and exploring the possible use of ultrafiltration (UF) for their removal from eutrophic water within the potabilization treatment. Firstly, we analysed the presence of MCs in filtered water after three months of pure culture of *Microcystis aeruginosa* and different cycles of freezing and thawing, showing an increase of dissolved MCs due to cell breakage caused by the refrigeration cycles. Secondly, we performed filtration tests in a pilot UF plant, using samples of demineralised water and raw water from a eutrophic lake, both spiked with MCs. The tests demonstrated the possibility to remove MCs by adsorption rather than by mechanical seizing. The tests with demineralised water denoted a tendency to desorption after reaching the adsorption equilibrium, an adsorption isotherm less effective than the one observed in earlier lab-scale studies, and the need for very well controlled chemical washings to clean the membranes. Conversely, the raw lake water tests showed a higher removal efficiency, allowing to reach a final concentration of less than 1 μg/l, and a tendency to maintain the removal efficiency for longer cycles. These results allow us to discuss the role of the adsorption-release process on the efficiency of the UF process, and suggests the exploration of non-conventional operating rules aimed at maximizing the removal of MCs by UF.

Keywords: Microcystin-LR removal; Ultrafiltration membrane; Adsorption; Cyanobacteria; Drinking water treatment

1. Introduction

Eutrophication of freshwater bodies is increasing worldwide because of anthropization and climate changes [1], and often involves lakes and reservoirs exploited for human activities and drinking water withdrawal. The occurrence and intensity of cyanobacterial blooms are increasing as well [2], and approximately 50% of them are known to present some toxicity [3]. The most commonly occurring toxins produced by cyanobacteria are microcystins, of which microcystin-LR (MC-LR) is the most toxic, frequently detected, and studied variant.

MC-LR is a cyclic heptapeptide containing five amino acids (invariant in all MCs), and two specific amino acids, leucine and arginine, (L and R, respectively). It is an amphipathic molecule, with hydrophilic

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functional groups (carboxyl groups on glutamic and methylaspartic acids, and the amino group on arginine) and the hydrophobic ADDA (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4-6-dienole acid) residue. The net charge of MC-LR is negative (–1) at most pH values ($3 < pH < 12$) as a result of the dissociation of the carboxyl groups. MCs are approximately 3 nm in diameter, with a molecular weight of 900–1100 Da [4]. Since the ingestion of MCs can lead to liver damage and the possible promotion of liver tumours [5], these toxins must be removed from water supplies prior to use.

The production of MCs by cyanobacteria seems more seasonally dependent in some places than in others [6,7] as a response to ecological conditions [8]. The ratio between intracellular and dissolved microcystins is mainly considered to be related to the release of microcystins by cyanobacterial cells under stress conditions or during their senescent stages [9]. The stress caused by water level changes may induce the release of intracellular toxins into the water. The potential contribution of benthic communities to total MC production has been studied in shallow waters with high retention times [8].

Nevertheless, field statistical studies assume the possibility of further unknown mechanisms, since the release mechanisms alone do not seem to be clearly related to any ecological or climatic conditions. In most cases, it seems that while the highest cyanobacterial proliferation happens in highly eutrophic water bodies, the highest concentration of dissolved microcystins seems to happen mainly in oligotrophic or mesotrophic waters [6].

Conventional drinking water treatment processes are either ineffective at removing dissolved cyanotoxins, or have significant drawbacks:

- coagulation, flocculation, and sand filtration can remove cyanobacterial biomass, but not the produced dissolved toxins;
- oxidation processes and activated carbon adsorption are effective for MC removal, but present significant drawbacks such as requirement of accurate and expensive pre-treatment stages, risk of formation of dangerous by-products, and high operative costs due to high and continuously varying required dosages.

Pressure-driven membrane filtration has emerged as a promising treatment to effectively remove MCs from drinking water. Nanofiltration (NF) or reverse osmosis (RO), having molecular weight cut-off (MWCO) values lower than 900 kDa, can remove MCs via size exclusion [10–12]. However, the cost of membrane filtration is mainly related to pore size, which in turn determines the required transmembrane pressure and, as a consequence, both energy consumption and membrane working life. Hence, the use of low-density membranes, and specifically ultrafiltration, allows significant cost savings compared to NF and RO.

Previous studies reported that microfiltration (MF) and ultrafiltration (UF) with MWCO of less than 100 kDa reject cyanobacterial biomass but not cyanotoxins [11–14]. However, other researchers have found that UF membranes feature a significant removal of dissolved MCs, mainly by adsorption [13], at least at the early stages of filtration. The adsorption of MC-LR seems not to be influenced by natural organic matter (NOM) when both occur together in feed water, since MC molecules are apparently able to adsorb before significant amounts of NOM become associated with the membrane [4].

Gijsbertsen-Abrahamse et al. [11] investigated MC-LR rejection by UF membranes to determine the effect of membrane surface properties and operating conditions. The dominant rejection mechanism of MC-LR by UF membranes at early stages of filtration was found to be adsorption, presumably due to hydrophobic interactions or hydrogen bonding [4]. Membrane surface morphology, such as porosity, surface roughness, and thickness, may also play a role in controlling the extent of adsorption. For tight thin-film (TF) membranes, with similar MWCOs to the molecular weight of MC-LR, size exclusion was the dominant rejection mechanism once adsorption reached equilibrium. Lee and Walker [4] observed that higher permeate flux resulting from increased water recovery or operating pressure led to a greater adsorption of MC-LR on the membranes and to a decrease in size exclusion.

In this study, we present the results of several laboratory and pilot plant experiments, aimed at deepening the knowledge of the mechanisms governing the migration of MC-LR from particulate-bound to dissolved phase and vice versa, and the related effects on the UF removal of MCs.

The experimentation is divided in two parts.

Firstly, we analysed the presence of different levels of MC-LR in filtered water after three months of pure culture of two different strains of *M. aeruginosa* under different lighting regimes and different cycles of freezing and thawing. These tests were aimed to analyze the effect of cell growth, variable stress conditions caused by different lightings, and cell breakage caused by the refrigeration cycles on the presence of dissolved MC-LR in the analysed samples.

Secondly, we performed a number of filtration tests of different samples of water spiked with pure MC-LR, over a commercial spiral-wound UF polysulfone membrane element in a pilot scale plant. Specifically, we performed two comparable filtration tests on demineralised water and water taken from a eutrophic lake, both spiked with the same concentration of MC-LR. Then we performed a filtration test on increasing concentrations of MC-LR in demineralised water, with the aim of defining the adsorption isotherm of the adopted commercial membrane in the implemented operative conditions. The defined isotherm was compared with those defined

in earlier studies conducted with lab-scale filtration membranes. Finally, we tested several chemical washing techniques with the aim of assessing their effectiveness in removing the MC-LR adsorbed on the membrane.

2. Materials and methods

2.1. Culture of M. aeruginosa and extraction of MC-LR

M. aeruginosa strains (PCC 7806 and PCC 7820) were bought from the Institute Pasteur in Paris, France. The strains were cultivated according to the provider's instructions: flasks containing BG11 medium [15] with NaNO3 2 mm and with NaHCO3 10 mm (only for strain PCC 7820) were maintained at 24 ± 1°C in a regime of 12 h light/12 h dark for three months (June, July, and August 2008). In order to set up the best conditions to obtain microcystin production, the strains were exposed to two different light conditions: natural and artificial light (cool white lamp 230 V, 50 Hz, 17 W). To set up the best method of MC recovery, two different treatments were tested: in the first treatment, 100 ml of each of the four different culture solutions was filtered through a 45-μm cellulose nitrate filter to remove cells; in the second treatment, 100 ml of the same culture solution was subjected to three cycles of freezing and thawing to destroy the cells and release the toxins into the water, and then filtered through a 45-μm cellulose nitrate filter to remove the cell debris. The samples were then analysed for MC-LR presence according to the MC-LR detection method reported in Section 2.3. All samples were tested in triplicate.

2.2. Filtration tests over UF pilot plant

The filtration tests were performed using a UF pilot plant assembled according to the sketch diagram reported in Fig. 1. Both retentate and permeate were recirculated to the feed tank to maintain the feed solution constant while quantifying membrane adsorption and rejection by size exclusion. The volume of treated water in the feed tank was 20 l, while the amount of water contained in the plant (pipes, pump, valves, probes and UF vessel) was estimated at 1.66 l by previous hydrodynamic tracking tests. The main characteristics of the adopted commercial membrane element are reported in Table 1.

The filtration tests were conducted under constant pressure. The pressure was controlled and corrected continuously. Any pressure changes detected by the pressure gauges were compensated for by manual adjustment of valves V2 and V3.

The first filtration tests were performed using 20 l of water spiked with 0.5 mg of solid MC-LR. The toxin (MC-LR from *M. aeruginosa*, 95% HPLC pureness, bought from Sigma Aldrich) was dissolved in 2 ml of methanol and then transferred to the water contained in the feed tank (which was equipped with a magnetic stirrer) and homogenised for 10 min before each test start. Before each test the plant underwent chemical washing as described below. Each test started by switching the pump on, and continued for at least 24 h. Samples of 5 ml of feed, permeate and retentate fluxes were taken at fixed times and subjected to MC-LR detection analysis as described in Section 2.3.

Two tests were performed with the described methodology: the first one using demineralised water; and the second using a sample of raw water from the nearby eutrophic Lake Massaciuccoli (Tuscany, Italy), taken immediately before the test. The lake water was analysed for some main physical, chemical and biological parameters. The results of the characterization analysis are listed in Table 2 together with references of the adopted analysis methods.

Subsequently, another filtration test (concentration ramp test) was conducted on demineralised water with a similar method (flow rates, pressures, etc.), but adding

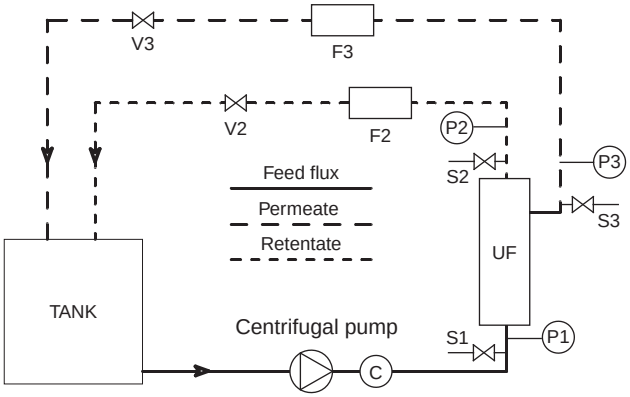

Fig. 1. Layout of the pilot UF plant used for the experiments. C = water meter; P1, P2, P3 = pressure gauges; F2, F3 = flow meters (rotameters); V2, V3 = valves; S1, S2, S3 = sampling points.

Table 1
Main properties of the adopted UF membrane filtration element

Type: Spiral-wound membrane	Active filtration area: 2.4 m^2
Manufacturer: GE Osmonics–Desal (USA)	Feed spacer: 28 mil = 0.711 mm
Model: EW3220T	Element length: 20″ = 508 mm
Membrane material: Polysulfone	Element diameter: 3.20″ = 81 mm
Mean pore size: 0.04 μm	Permeate pipe diameter: 1.187″ = 30 mm
MWCO: 20 kDa	Element weight: 2.3 lb = 1.0 kg

Table 2
Main characteristics of the Lake Massaciuccoli water used for the UF experiment

Parameter	Method	Unit	Value
Ammonia nitrogen	APAT CNR IRSA 4030 A2Man 29 2003	mg NH_4/l	<0.05
Nitric nitrogen	UNICHIM 1995 n. 169 m.u.940	mg N/l	6.5
Nitrous nitrogen	APAT CNR IRSA 4050 Man 29 2003	mg N/l	0.027
Total nitrogen	APAT CNR IRSA 4060 Man 29 2003	mg N/l	12
Total phosphorus	UNICHIM 1994 n. 169 m.u.947	mg P/l	<0.1
Sulphates	APAT CNR IRSA 4140 B Man 29 2003	mg SO_4/l	287
Chlorides	UNICHIM 1994 n. 169 m.u.931	mg Cl/l	600
Total hardness	UNICHIM 1994 m.u.935	°f	63
Total alkalinity	UNICHIM 1995 m.u.1071	meq/l	0.2
BOD_5	APAT CNR IRSA 5120-A-03	mg O_2/l	15
COD	APAT CNR IRSA 5130-0 Man 29 2003	mg O_2/l	79
Total fixed solids (180°C)	UNICHIM 1994 m.u.936	mg/l	1295
Total suspended solids (105°C)	APAT CNR IRSA 2090 B Man 29 2003	mg/l	5 (0.45μ) 13 (0.2μ)
Total silica	CAIM AQ2701	mg/l	5.6
Orthophosphates	APAT CNR IRSA 4110 A1Man 29 2003	mg/l	<0.02
Fixed & volatile solids (600°C)	APAT CNR IRSA 2090 D Man 29 2003	%	1.59 – 1.07
Turbidity	Secchi dish	m	0.65
pH	APAT CNR IRSA 2060 A1Man 29 2003	–	7.79
Temperature	APAT CNR IRSA 2100 A1Man 29 2003	°C	12.3
Electrical conductivity	APAT CNR IRSA 2030 A1Man 29 2003	μS/cm	242
Dissolved oxygen	EPA Method 360.1	mg O_2/l	7.1
Total bacterial count (22°C)	ISO 6222:1999	CFU/ml	9.05×10^3
Total bacterial count (37°C)	ISO 6222:1999	CFU/ml	4.4×10^3
Escherichia coli	ISO 9308-3	CFU/100 ml	61
Enterococcus	ISO/DIS 7899-2	CFU/100 ml	15
M. aeruginosa	Utermöhl's methods [16]	–	Presence

increasing concentrations of MC-LR with the aim of assessing the final adsorption equilibrium conditions, so as to define an adsorption isotherm of the membrane in the adopted experimental conditions.

In this test, a sample of 10 l of demineralised water was used, for a total of 11.66 l, including the water contained in the plant, estimated as described below. A brand new membrane element was used, of the same type as the one used for the previous tests.

The sample was initially spiked with 0.25 mg of pure MC-LR, with the same methodology described below. The filtration test was then started by switching on the pump, and continued by con tinuously recirculating both permeate and retentate to the feed tank. Samples were taken from retentate and permeate fluxes at time steps, and subjected to MC-LR detection analysis as described in 2.3 (since the previous tests showed no differences between feed and retentate fluxes, we did not take samples of the feed flux in this test).

After a time span (17 h) estimated as adequate to reach final adsorption equilibrium conditions, the pump was temporarily stopped and a further amount of 0.25 mg of MC-LR was added to the feed tank, so as to obtain a total amount of 1 mg. The pump was then started again for 24 h, with samples taken as described below.

The test continued with the same methodology as describe above, with two subsequent additions of 0.5 mg each. The total amount of added MC-LR at the end of the test was 1.5 mg.

The adsorption equilibrium conditions reached at the end of each step of the concentration ramp test allowed us to define the adsorption isotherm of MC-LR on a commercial spiral-wound polysulfone UF membrane in the described experimental conditions.

Finally, several chemical washing solutions were compared to select the most effective one for recovering the MC-LR adsorbed on the membrane. For the two most effective solutions a complete mass balance was performed to check the final recovery efficiency. The obtained results are as follows:

- Methanol-demineralised water 25% v/v. Recovered MC-LR after four stages of washing (total duration four days, total wash volume 26 l): 74%.
- Methanol-demineralised water 25% v/v with 2 mg/l of sodium carbonate (pH 10.3). Recovered MC-LR after four stages of washing (total duration four days, total wash volume 20l): 86%.

Hence, the chemical washing with methanol-demineralised water at pH 10.3 corrected by sodium carbonate

was evaluated as the most effective method to desorb MC-LR from the membrane, and was adopted as the standard washing method.

2.3. Analytical methods and instruments

MC-LR detection and quantification was performed on an Applied Biosystems Sciex API 4000 triple quadrupole mass spectrometer coupled with a Perkin Elmer Series 200 Micro HPLC system equipped with a "Synergy Hydro" reversed phase C18 column (4.6 mm × 250 mm, Phenomenex, USA).

Chromatographic method: a mix of 15% acetonitrile-85% water was used as eluent for 3 min, gradient to 95% acetonitrile-5% water in 1 min. The adopted flow rate was 250 µl/min. Water was added with 0.1% formic acid. The injection volume was 10 µl. The chromatographic column was thermostated to 35°C. Three replicates per sample were injected.

The acquisition was performed in multiple reaction monitoring scan type (positive polarity). MC was quantified by observing the following two ion transitions (expressed by their mass number/charge number ratios) : 498.20995.80; 861.80135.40.

The analyses of the lake water used for the UF pilot-plant experiment were performed according to the methods shown in Table 2, which also reports the results.

3. Results and discussion

3.1. Culture of M. aeruginosa and extraction of MC-LR

The detection of MC-LR in pure cultures of two strains of Microcystis aeruginosa, grown under natural and artificial light, and analysed after filtration with or without three prior cycles of freezing and thawing, showed that the concentration of MC-LR was always very low (from a minimum of 0.3 µg/l to a maximum of 2.6 µg/l).

The PCC 7820 strain, grown under artificial and natural light, produced more toxin than PCC 7806, but seemed to release less of it into the medium, as evidenced by the samples subjected to three cycles of freezing and thawing before filtration (Fig. 2).

Moreover, both the strains produced higher concentrations of MC-LR when grown under artificial light than when grown under natural light.

Therefore, artificial light seems to be a stress factor contributing to augmenting the production and release of MC-LR, while refrigeration cycles seem to enhance the recovery of MC-LR, thus increasing its presence in the analysed samples.

These results seem to confirm the mentioned field observations [69] that relate the dissolved MC production to stress conditions rather than to overall biomass development.

3.2. Filtration tests over UF pilot plant

The results of the first UF filtration test with demineralised water are presented in Table 3 and Fig. 3, while the results of the test with lake water are presented in Table 4 and Fig. 4.

These data show that, despite the membrane much higher MWCO compared to MC-LR molecular weight, the contained MC-LR was effectively removed from the permeate in both the tests. A better performance was observed for the lake water, where the permeate concentration reached a stable average value of 0.91 µg/l after three hours, compared to the value of 1.64 µg/l reached in the demineralised water test after the same amount of time.

Since the overall MC-LR concentration recirculated in the feed tank decreased significantly in the first three hours, reaching similar concentrations in feed, permeate, and retentate fluxes in both the tests, the main removal mechanism should be recognized in the adsorption onto the membrane, as already observed [3,11,14].

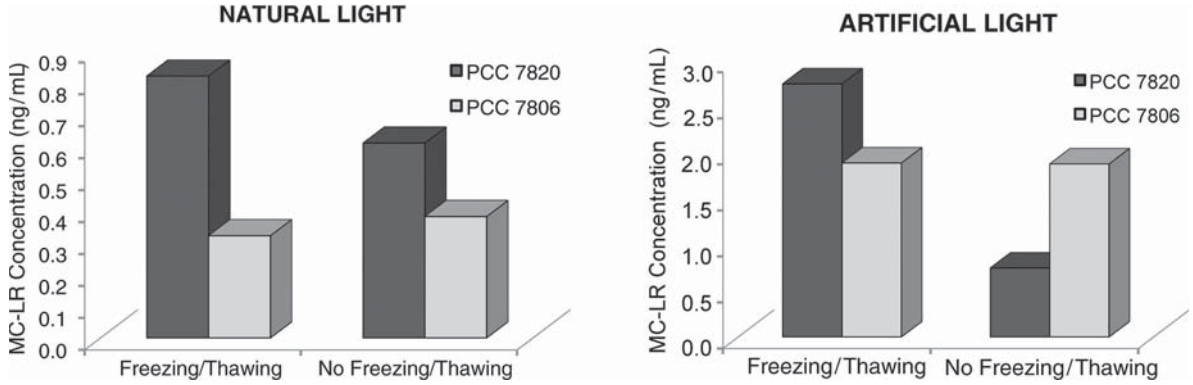

Fig. 2. Concentrations of MC-LR detected in pure cultures of *M. aeruginosa*.

Table 3
Data from the UF experiment with demineralised water

Time (h:m)	Feed			Permeate			Retentate	
	Flux (l/h)	Press. (bar)	MC-LR conc. (µg/l)	Flux (l/h)	Press. (bar)	MC-LR conc. (µg/l)	Flux (l/h)	MC-LR conc. (µg/l)
0:00	114	1.0	13.3	52	0.1	0.1	60	13.3
0:05	114	1.0	9.0	52	0.1	0.1	60	8.6
0:30	114	1.0	4.6	53	0.1	0.3	60	4.7
1:30	114	0.9	1.6	55	0.1	1.0	50	1.7
3:30	111	0.9	1.6	62	0.0	1.5	38	1.7
5:30	114	0.9	1.7	64	0.1	1.5	38	1.8
20:30	102	1.1	1.6	68	0.2	1.6	38	1.8
24:00	102	1.1	1.7	67	0.2	1.7	38	1.9
27:30	102	1.0	1.9	66	0.1	1.9	38	2.1

Table 4
Data from the UF experiment with lake water

Time (h:m)	Feed			Permeate			Retentate	
	Flux (l/h)	Press. (bar)	MC-LR conc. (µg/l)	Flux (l/h)	Press. (bar)	MC-LR conc. (µg/l)	Flux (l/h)	MC-LR conc. (µg/l)
0:00	158	1.0	14.0	79	0.3	0.0	81	13.8
0:15	147	1.0	10.9	78	0.3	0.1	75	14.1
0:30	145	0.9	3.0	75	0.2	0.1	75	2.4
0:45	127	1.0	1.9	81	0.3	0.2	50	1.6
1:00	133	1.0	1.0	84	0.3	0.2	49	0.9
3:00	133	0.9	0.7	84	0.3	0.7	49	0.8
5:00	136	0.9	0.8	85	0.3	0.9	48	1.0
7:00	133	0.9	1.0	88	0.3	0.9	38	0.9
22:00	170	1.1	0.8	95	0.5	0.7	79	0.8
24:15	168	1.1	0.7	95	0.5	1.3	77	0.8

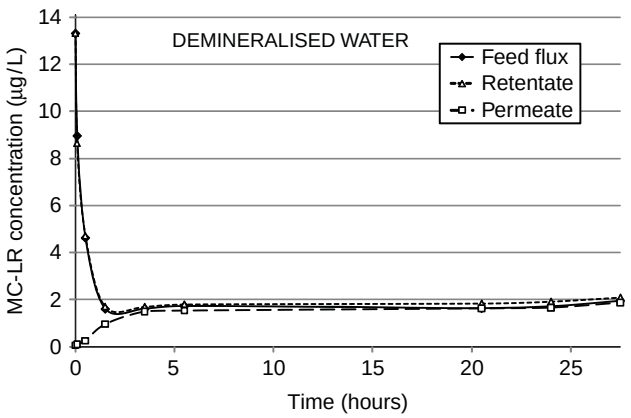

Fig. 3. MC-LR concentrations in samples of feed flux, retentate and permeate during the UF experiment with demineralised water spiked with pure MC-LR.

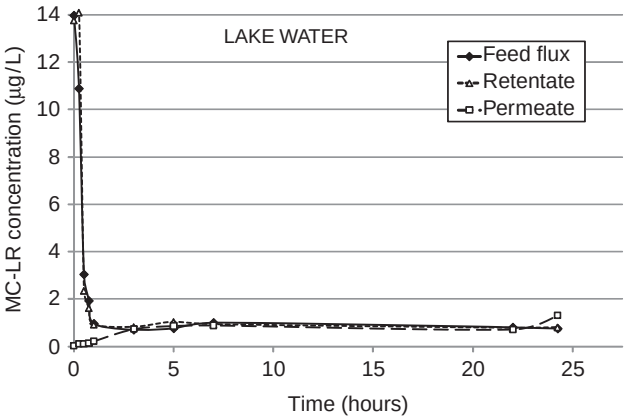

Fig. 4. MC-LR concentrations in samples of feed flux, retentate and permeate during the UF experiment with lake water spiked with pure MC-LR.

The significant observed removal (that allowed us to achieve compliance with the 1998 WHO drinking water standard [17] of 1 μg/l in the lake water test) is presumably due to hydrophobic interactions with the highly hydrophobic polysulfone membrane [4].

The better performance shown in the lake water test is worth further analysis. The adsorption paths shown in the two tests are compared in Fig. 5, where the adsorbed amounts of MC are put in relation to the MC concentrations observed in the feed flow at the same times. The linearity of the paths is related to the balance among the concentrations in feed, retentate and permeate fluxes. The path of demineralised water shows a good linearity until the reaching of the final equilibrium, when e clear tendency to the desorption takes over. The path of lake water shows an opposite trend: a divergence from the linearity is observed mainly in the earlier part, and the final tendency to the desorption is visibly less evident than in deionised water. Finally, the final equilibrium points belong to two different adsorption isotherms, with the one relative to lake water being located at a lower feed flux concentration, and more stable in time.

Taking into account the characterization of the adopted lake water shown in Table 2, the following hypotheses can be stated:

- the ionic strength due to high sulphate and chloride concentrations can improve the adsorption features of the membrane;
- a certain amount of MC-LR can be adsorbed to the colloidal and suspended solids present in the lake water and then kept on membrane fouling;
- a specific role can be assumed by the cyanobacteria detected in the lake water, due to possible adsorption by cell-bound extracellular polymeric substances (EPS).

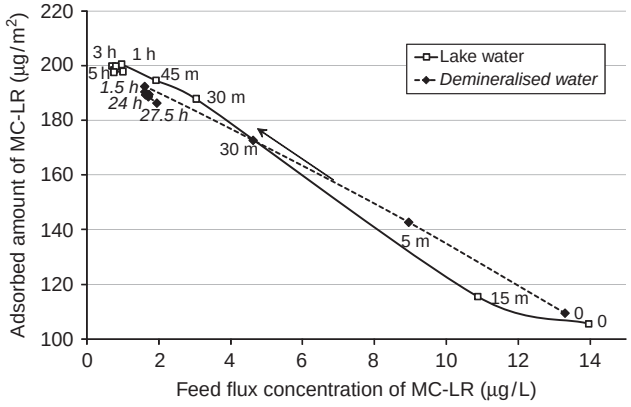

Fig. 5. Adsorption paths detected during the UF experiments with demineralised water and lake water spiked with pure MC-LR (the arrow shows the direction of the paths).

Since the results of the presented tests don't allow us to investigate the contribution of these three possible effects, specific tests will be conducted to continue this research.

Nevertheless, the better performance reached with lake water is a significant achievement for two reasons:

- the final equilibrium is reached at a lower water concentration, which has the same magnitude as the WHO drinking water standard for microcistine-LR;
- the final equilibrium is more stable, and, being related to the treated water rather than to the membrane, can maintain its stability for larger volumes of treated water between subsequent washings.

The results of the UF test with demineralised water spiked with increasing concentrations of pure MC-LR (concentration ramp test) are presented in Table 5 and Figs. 6 and 7.

These data allowed us to set up the adsorption isotherm of the adsorption of MC-LR onto the commercial spiral-wound polysulfone UF membrane in the described pilot test conditions. In the isotherm, which is presented in Fig. 8, the adsorbed amount of MC-LR has been referred to the specific membrane filtration surface, as already presented in earlier studies for MC-LR [4], an estrogenic hormone [18] and a protein [19]. As for the water concentration reported on the X axis, it is a mean of concentrations detected in feed, permeate and concentrate fluxes.

The final equilibrium points of the first two tests with demineralised water and lake water spiked with 0.5 mg of MC-LR are plotted on the same graph.

The linear regression of the experimental points is plotted as well, together with the related equation coefficients and R^2 parameter (0.9714), which denotes a good approximation of the linear regression assumption. The good match of the linear regression assumption was also reported in the three above-mentioned earlier studies.

As a comparison, the linear regression of the adsorption isotherm reported by Lee and Walker [4] for their laboratory study on the adsorption of MC-LR onto a small (140 cm^2) flat polyamide 4 kDa MWCO membrane is also plotted in Fig. 8.

The comparison of the two regression lines shows that the isotherm calculated in this study denotes a slightly lower adsorption efficiency than the one reported in [4], despite the higher affinity of MC-LR with polysulfone than with polyamide. We attribute this difference to the fact that the pilot-scale conditions (spiral-wound membrane element, large filtration surface, higher flow rates with generally lower concentrations) don't allow us to reach an overall adsorption efficiency comparable to the one obtained in laboratory-scale conditions (flat membrane element, small filtration surface, small flow rate with higher concentrations).

Table 5
Data from the UF test with demineralised water spiked with increasing concentrations of pure MC-LR

Time (h:m)	MC-LR conc.(µg/l)		Time (h:m)	MC-LR conc.(µg/l)	
	Retentate	(h:m)		Retentate	(h:m)
Initial MC-LR spiking: 0.25 mg			Second MC-LR addition: 0.50 mg		
0:00	21.4*		0:00	44.6*	
0:05	5.7	0.0	0:05	29.8	1.8
0:15	4.5	0.2	0:35	4.2	2.6
1:30	0.4	0.2	1:35	4.4	4.3
16:45	1.4	1.4	7:05	4.5	4.7
First MC-LR addition: 0.25 mg			119:35	4.8	7.4
0:00	22.8*		Last MC-LR addition: 0.50 mg		
0:05	13.5	0.6	0:00	49.0*	
0:20	10.3	0.7	0:05	21.3	5.0
1:35	3.7	1.2	24:00	9.3	9.1
24:00	1.7	1.7			

*The initial retentate concentrations after each addition are theoretically calculated by adding the dosage to the final concentration of the previous test.

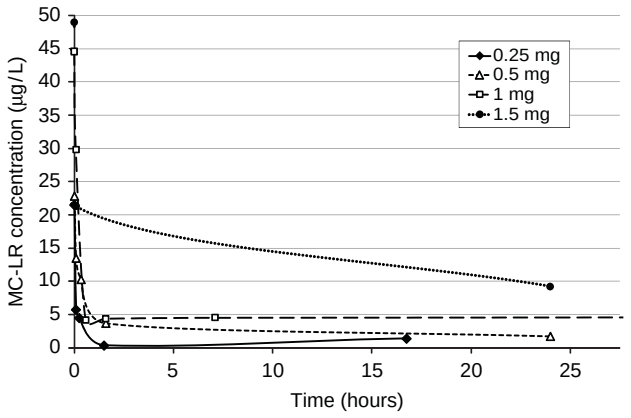

Fig. 6. MC-LR concentrations in samples of retentate during the ultrafiltration test with demineralised water spiked with increasing concentrations of pure MC-LR.

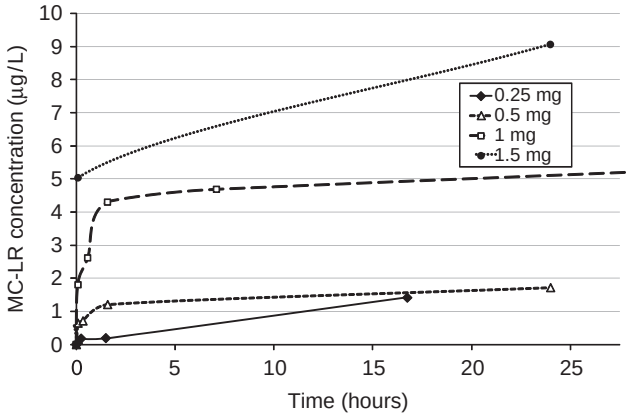

Fig. 7. MC-LR concentrations in samples of permeate during the ultrafiltration test with demineralised water spiked with increasing concentrations of pure MC-LR.

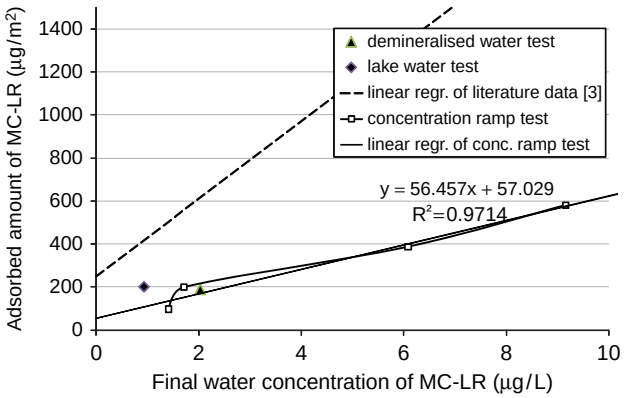

Fig. 8. Adsorption isotherm of MC-LR onto a commercial spiral-wound polysulfone UF membrane calculated with the results of the UF test with demineralised water spiked with increasing concentrations of pure MC-LR. The graph also reports as a comparison: final equilibrium points reached at the end of filtration tests with demineralised water and lake water; linear regression of Lee and Walker's adsorption isotherm [4] for Polyamide 4 kDa MWCO membrane; linear regression of concentration-ramp-test points with related equation and R^2 parameter.

4. Conclusions and future perspectives

The first results of this ongoing research to evaluate safe and cost-effective policies to control cyanobacterial threats in drinking water treatment of eutrophic water by low-density membrane filtration, are as follows:

- the tests on pure cultures of *M. aeruginosa* show opposite trends in the production of MC and its release in dissolved form: stress conditions seem to reduce the overall MC production but increase the release of the more dangerous dissolved form;

- the polysulfone membrane features a significant MC-LR removal potential in early filtration stages, presumably due to adsorption driven by the affinity between the hydrophobic polysulfone membrane and the amphipathic MC-LR molecule;
- the adsorption isotherm calculated from the presented pilot-plant test results shows a slightly poorer performance compared to the isotherm calculated after the lab-scale tests conducted in an earlier study [4], causing us to conclude that the operative conditions of full-scale plants play a very important role in the reachable removal performance;
- in contrast with observations in earlier studies for other membranes [13], simple water flushing did not desorb MC-LR from the commercial spiral-wound polysulfone membrane tested, but washing with a 25% methanol solution at pH 10 obtained good desorption performance;
- the removal performance was found to be significantly higher in the treatment of raw lake water as compared to demineralised water. Some possible reasons for this effect have been hypothesized as being related to changes in ionic strength, presence of inorganic and organic particulate fractions, or a role of EPS produced by cyanobacteria themselves. These possible contributions will be investigated in the continuation of this research;
- the better performance observed for lake water partially compensates for the poorer performance of real scale plants, allowing them to treat larger amounts of water with general removal performances that can be realistically expected to be maintained between subsequent chemical washes thanks to the adsorption capacity related to the treated water itself. This possibility can be exploited in emergency situations simply reducing the washing intervals of existing treatment plants equipped with polysulfone ultrafiltration stages.

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