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# Nanofiltration membranes applied to the removal of saxitoxin and congeners

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**ABSTRACT**

Cyanobacterial toxins pose a potential threat to human health and wildlife. Effective treatment processes are essential for the removal of these constituents in drinking water. The efficiency of two nanofiltration membranes (NF-270 and NF-90) in the removal of cyanotoxins of the saxitoxin group from water was investigated. In this work, these toxins were extracted from a laboratory culture of *C. raciborskii* and added to surface water. A working pressure of 8 bar was applied. A filtration time of 180 min was used for each experiment. Total removal (100%) of the identified toxins was obtained with the NF-90 membrane throughout the filtration period. A lower degree of toxin removal was observed with the NF-270 membrane, decreasing with filtration time. Different rejection mechanisms can be considered in the process: size exclusion, electrostatic interactions and hydrophilic interactions, as well as the mechanism of concentration polarization. A larger influence of fouling phenomena was considered for the NF-270 membrane, with a larger drop in the permeate flux. A more stable permeate flux was observed for the NF-90 membrane. These results indicate that the use of nanofiltration technology is effective in the removal of dissolved cyanotoxins in water intended for human consumption.

*Keywords:* Water supply source; Nanofiltration; Cyanobacteria; Saxitoxin and congeners

## 1. Introduction

Saxitoxin (STX) and congeners represent a group of neurotoxic alkaloids. They are also known as paralytic toxins (PTs), or paralysing shellfish poison (PSP), due to their occurrence in association with seafood [1]. These toxins are mainly produced by some marine dinoflagellate species such as *Alexandrium spp.*, *Gymnodinium catenatum* and *Pyrodinium bahamense*. They are also produced by cyanobacterial species such as the *Anabaena*, *Aphanizomenon* and *Cylindrospermopsis* genera. As highlighted by Donovan et al. [2], these PTs accumulate in filter-feeding bivalve molluscs such as mussels, clams, oysters and scallops, which feed on dinoflagellates and thus concentrate these toxins in their digestive organs. The phenomenon of food poisoning by the consumption of shellfish contaminated with PSP toxins is one of the most severe forms of intoxication mediated by molluscs and some crustaceans and fish [3].

The main mode of action of PTs in mammals occurs through the connection of the sodium channels of neural membranes, even in small concentrations. This process results in the inhibition of impulses along the nerves and, therefore, in paralysis, respiratory depression and

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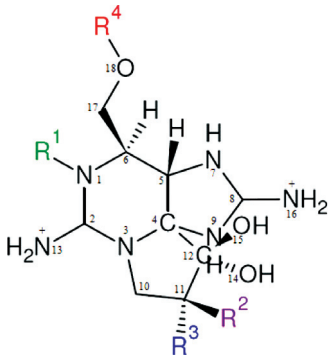

| R1 | R2 | R3 | R4 | | |
|---|---|---|---|---|---|
| | | | $CO–NH_2$ | $–CO–NH–SO_3^-$ | –H |
| H | H | H | STX | GTX5 | dcSTX |
| H | H | $OSO_3^-$ | GTX2 | C1 | dcGTX2 |
| H | $OSO_3^-$ | H | GTX3 | C2 | dcGTX3 |
| OH | H | H | neoSTX | GTX6 | dcNEO |
| OH | H | $OSO_3^-$ | GTX1 | C3 | dcGTX1 |
| OH | $OSO_3^-$ | H | GTX4 | C4 | dcGTX4 |

Fig. 1. Chemical structures of the paralytic toxin STX and its congeners (adapted from Donovan et al. [2]).

circulatory failure [2,4], which can cause death within a few minutes after exposure.

PTs possess a tetrahydropurine skeleton with two guanidinium groups (Fig. 1) [2,3]. Saxitoxin and its analogues are generally grouped into three categories: the carbamate compounds (which include saxitoxin, neosaxitoxin and goniautoxins 1–4); the *N*-sulphocarbamoyl compounds (including the C and B toxins); and the decarbamoyl compounds (which may be related to the presence or absence of 1-*N*-hydroxyl, 11-hydroxysulphate and 21-*N*-sulphocarbamoyl, as well as to epimerisations at the C-11 position) [5]. According to Donovan et al. [2], compounds derived from saxitoxin are formed when the STX molecule is modified by the addition or removal of 1-*N*-hydroxyl, 11-hydroxysulphate or 21-*N*-sulphocarbamoyl groups. To date, more than 20 paralytic toxin derivatives have been identified [2,6,7], of which saxitoxin is considered the parent and most potent compound.

Saxitoxin is the best-studied toxin among its analogues. In mice, its peritoneal lethal dose ($LD_{50}$) is 3–10 mg · $kg^{-1}$ of body weight, and the oral lethal dose is 263 mg · $kg^{-1}$ of body weight. The oral lethal dose for humans is 1–4 mg, depending on the gender and physiological condition of the patient [5].

As previously mentioned, some species of cyanobacteria are also responsible for the production of PTs. The occurrence of cyanobacterial blooms in aquatic environments, including water supplies, has become a constant concern. The release of toxins into drinking water can affect human and animal health and is consequently a matter of public health. The occurrence of cyanobacterial blooms producing PTs, especially blooms of *Cylindrospermopsis raciborskii*, has been reported with high frequency in various Brazilian water sources [8–10].

Different processes have been used for the treatment of drinking water to remove cyanobacteria and the toxins produced by these organisms. Conventional treatment technologies (involving stages of coagulation, flocculation, sedimentation or flotation and filtration) can efficiently remove cyanobacteria but not dissolved toxins [11–13]. Hrudey et al. [14] listed a number of studies showing the low efficiency of different coagulants for the removal of dissolved toxins; these coagulants can, in high doses, cause cell lysis and the additional release of toxins into the water.

Due to their high solubility, these toxins tend to permeate easily through the treatment system, reducing the efficiency of the process. The use of oxidants, such as chlorine or ozone, in treatment, in correct dosages, allows for the removal of both organisms and toxins. At high dosage, they can, however, cause cell lysis and the consequent release of these compounds. As highlighted by Henderson et al. [15], algal cells and associated algogenic materials are the precursors of trihalomethanes (THMs), and therefore, the use of chlorine and other oxidants should be avoided. The activated carbon adsorption process has been well studied with satisfactory results in the removal of toxins from water. However, the presence of organic matter in water can reduce the adsorption capacity of activated carbon, thus reducing its efficiency [16].

Membrane technology has been proven to be effective in removing cyanotoxins, particularly nanofiltration (NF). Nanofiltration membranes have properties that are intermediate between those of ultrafiltration and reverse osmosis membranes. They have been proven to be efficient for toxin removal, once they present a low porosity and a capacity for the retention of dissolved organic solutes with molecular weights between 200 and 1000 Da (Daltons) [17]. This range allows for the removal of both saxitoxin and its analogues, as the molecular weights of these compounds vary between 258 Da (dcSTX) and 492 Da (C3 and C4) [18]. The high efficiency of nanofiltration for the removal of cyanotoxins, mainly concerning variants of microcystin, has been reported by several studies in the literature [11,19,20]. Pontié et al. [21] highlighted the advantages of nanofiltration: relatively low operating pressure, high permeate flux and high retention of the anions of multivalent salts.

Regarding the high efficiency of nanofiltration in removing microcystin, this study was intended to assess the behaviour of two nanofiltration membranes for the removal of PTs. The samples used for the nanofiltration tests were collected after previous treatment by dissolved air flotation (DAF). This procedure was adopted to reduce the load of suspended material in the water, with the aim of decreasing the level of membrane blockage during the experiment.

## 2. Methodology

### 2.1. Water characteristics

The water used in this study was collected from Lagoa do Peri (Peri Lagoon), a freshwater coastal lagoon located in Florianopolis, Santa Catarina, Brazil. Monitoring indicated the presence of high densities of microalgae and potentially toxic cyanobacteria in this environment, with a predominance of the species *C. raciborskii*. The presence of these organisms has resulted in operational difficulties in the currently employed treatment system (rapid filtration with a descending flux).

Although the cell density of *C. raciborskii* in the studied water is high, the concentration of dissolved toxins is relatively low, as the majority of the toxins are intracellular. Considering this aspect, we opted to cultivate the *C. raciborskii* cyanobacteria to promote the extraction of toxins and to increase their concentration in the water for the removal trials.

### 2.2. Extraction of cyanotoxins

In the extraction of cyanotoxins from the *C. raciborskii* culture, the organisms were subjected to multiple cycles of freezing and thawing. This procedure caused complete disruption of the cells, releasing intracellular toxins and other substances. In this step was not realized any kind of separation process for residual removal of cells from the lysis. The aim was to consider a real occurrence of toxins release, due the senescence of microorganisms or by external agent's action.

The water used in the studies of DAF and NF was obtained from the mixing of surface water and *C. raciborskii* culture after the cell lysis process, using equal proportion (1:1). For DAF experiments, the prepared water was used directly in the tests. The treated water by DAF was applied on NF tests after. The characteristics of the surface and prepared water are given in Table 1. The culture addition showed higher average values for the analytical parameters evaluated. The increase in colour and turbidity values is associated with the characteristics of strong colour in the culture and the presence of the cellular residue after lysis. In relation to the DOC (dissolved organic carbon), as observed by Miao and Tao [22] in studies of oxidation of cyanobacteria by ozonation, the damage at cell wall in this case, due to the process of freezing and thawing resulted in the release of cell cytoplasm, with increment of the concentration of DOC at solution. The initial toxin concentrations are also listed in Table 1. The analytical conditions for the identification and quantification of the toxins are described in Section 2.5.

### 2.3. Nanofiltration membranes

Two flat-sheet nanofiltration membranes, NF-270 and NF-90 were used in this investigation. These membranes were supplied by Dow Chemical Company. The characteristics and behaviour of these membranes with respect to their efficiency in the removal of different

Table 1
Mean values of colour, turbidity, absorbance, DOC, cyanobacterial density and toxin concentration in the different study steps

| Analytical parameters | | NF-270 test membrane | | | | NF-90 test membrane | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Surface water | Prepared water | DAF | NF | Removal (%)[1] | Prepared water | DAF | NF | Removal (%)[1] |
| Colour (uH) | 65.00 | 193.00 | 75.00 | 1.00 | 98.7 | 199.00 | 79.00 | 1.00 | 98.7 |
| Turbidity (NTU) | 5.72 | 12.23 | 5.56 | 0.20 | 96.4 | 12.98 | 5.44 | 0.27 | 95.0 |
| $Absorbance_{254\,nm}$ ($cm^{-1}$) | 0.1347 | 0.2421 | 0.1200 | 0.0072 | 94.0 | 0.2452 | 0.1191 | 0.0058 | 95.1 |
| DOC ($mg \cdot l^{-1}$) | 4.70 | 6.02 | 4.74 | 1.30 | 72.7 | 5.44 | 4.46 | 1.30 | 70.8 |
| Cyanobacterial density ($ind \cdot ml^{-1}$) | 45,900 | 31,920 | 17,958 | – | 100.0 | 27,925 | 13,667 | – | 100.0 |
| Toxin concentration ($\mu g \cdot l^{-1}$) | –[2] | 14.26 | 13.41 | 11.89[3] | 11.3 | 16.65 | 16.46 | 0.00[3] | 100.0 |

[1]Removal values using the values after DAF as a reference.
[2]Value below the instrumental detection limit.
[3]Quantified values at the end of the filtration period (180 min).

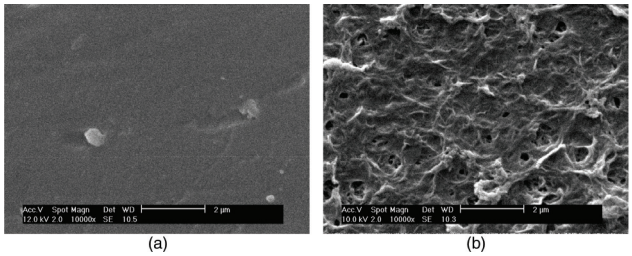


Fig. 2. SEM images of the surfaces of NF-270 (a) and NF-90 (b) membranes in a clean state.

contaminants, fouling characteristics and their dependence on different factors such as pH, temperature and membrane surface charge, have been described elsewhere [23–28].

The membranes are composed of a thin-film polyamide composite with a microporous polysulfone supporting layer. The active layer of the NF-270 membrane is a very thin semiaromatic piperazine-based polyamide layer, whereas the NF-90 membrane has a fully aromatic polyamide active layer [29]. According to Pontié et al. [21], the NF-270 membrane has a contact angle of 38° and is thus more hydrophilic than the NF-90 membrane, with a contact angle of 64°. These membranes can also be differentiated by their differing porosities. Nghiem et al. [30] and Nghiem and Hawkes [27] reported average pore sizes of 0.68 nm for the NF-90 membrane and 0.84 nm for the NF-270 membrane. Regarding the roughness of the membranes, examination by scanning electron microscopy (SEM) indicated, as in other studies [30,31], that the NF-270 membrane has a smoother layer than the NF-90 membrane, which presents a rough surface (Fig. 2). López-Muñoz et al. [29] indicated molecular weight cut-off values of 300 and 200 Da for the NF-270 and NF-90 membranes, respectively. According to Nghiem et al. [28], the pure water permeability for NF-270 and NF-90 membranes are 13.5 $l \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ and 6.4 $l \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, respectively.

For each filtration test, a piece of the membrane was cut from a larger piece to a diameter corresponding to the internal diameter of the filtration unit (5.7 cm). The effective filtration area of the membranes was thus $5.81 \times 10^{-4}$ $m^2$.

### 2.4. Nanofiltration experiments

In a joint study, the toxin-removal efficiency of dissolved air flotation (DAF) combined with nanofiltration was evaluated. The prepared water was initially treated by DAF to reduce the amount of organisms and suspended material in the surface water and in the lysed cyanobacterial culture. As observed by Teixeira and Rosa [32], DAF not is a very efficient process for toxin removal, only for cell removal. Thus, this process was expected only to reduce the impact on the membrane due to suspended material.

The nanofiltration experiments were carried out in a dead-end filtration system. The nanofiltration cell was made of stainless steel with a volume of 450 ml. The pressure was supplied by a cylinder of compressed liquid $N_2$. The permeate was collected and the volume was monitored continuously during the experiments. The permeate flux was calculated based on the volume of water collected as a function of time and of the effective area of membrane, as shown in Eq. (1).

$$J_0 = \frac{V}{A \bullet t} \quad (1)$$

where $J_0$ is the permeate flux ($l \cdot m^{-2} \cdot h^{-1}$), $V$ is the volume collected (l), $A$ is the effective area of the membrane ($m^2$), and $t$ is the collection time (h).

The effluent resulting from DAF was transferred to the filtration equipment and subjected to a constant pressure of 8 bar for both membranes. This pressure value was adopted as a result of previous studies in our laboratory to evaluate the retention of toxins by the NF-270 and NF-90 membranes at different working pressures. The total filtration time was 180 min, with sampling to quantify the presence of toxins at 0 (initial), 30, 60 and 180 min of filtration. After collection, the samples were stored under refrigeration and in the absence of light. For chromatographic analysis, the samples were brought to room temperature.

To evaluate the behaviour of the permeate flux through the clean membrane during and after the filtration of samples, the permeate volume was measured every 10 min. Initially, the hydraulic permeabilities for the clean membranes were monitored for 60 min. The permeate-flux behaviour was then evaluated during the filtration of the sample (180 min). With these measurements, it was possible to characterise the flux behaviour with regards to the sample quality and the filtration time. After passing the sample, the filtration of ultrapure water was again performed for 60 min. The occurrence of membrane fouling was evaluated according to the difference between the permeate flux obtained in the initial hydraulic permeability test and the pure water flux after sample filtration. All tests were performed in triplicate. The analytical parameters colour, turbidity, DOC, absorbance and cyanobacterial density were evaluated. The density ($ind \cdot ml^{-1}$) of cyanobacteria was obtained by counting the cells using a Sedgewich Rafter chamber and an optical microscope.

*2.5. Analytical conditions for the identification and quantification of cyanotoxins*

The identification and quantification of toxins were carried out using high-performance liquid chromatography (HPLC) with post-column derivatisation and fluorimetric detection, based on the method of Oshima [33] with a few modifications [34]. The chromatographic system consisted of a Shimadzu HPLC pump (LC-10AD), an auto injector (SIL-10AF) with a 500 ml loop and a reverse-phase column (Phenomenex C8; 5 mm Luna, 4.6 × 250 mm), warmed to 30°C in an oven (CTO-10A) [34]. The chromatographic tests were operated under isocratic conditions using a mobile phase consisting of 2 mM 1-heptanesulphonic acid in 30 mM (pH 7.1) ammonium phosphate and acetonitrile (100:5). The chromatographic flow was 0.6 $ml \cdot min^{-1}$.

Before injection, the samples were filtered through a cellulose acetate membrane (0.45 μm pore size) to remove particulate material.

## 3. Results and discussion

*3.1. Characteristics of water after dissolved air flotation and nanofiltration*

Table 1 shows the values obtained for the evaluated analytical parameters (colour, turbidity, absorbance, DOC and cyanobacterial density) for the water after DAF and nanofiltration for the two membranes. As mentioned in the methodology section, the DAF was used to reduce the load of suspended material in the sample. This effect can be observed mainly in the reduction in the values of colour (60.72%), turbidity (47.78%) and cyanobacterial density (47.40%). After filtration, the values were even lower, with total removal of the organisms. In terms of the percentage of removal/reduction, the two membranes showed very similar results in relation to the analytical parameters. Similar values of DOC concentration were obtained for the two membranes. This similar retention of organic matter has also been reported by Nghiem et al. [30].

*3.2. Permeate fluxes for the NF-270 and NF-90 membranes*

Regarding the filtration of the samples, the NF-270 membrane presented a decrease in the permeate flux with increasing filtration time (Fig. 3), with an average flux decrease of 100 $l \cdot m^{-2} \cdot h^{-1}$. This flux reduction was likely associated with the deposition of suspended or dissolved material on the membrane surface or inside the pores. This behaviour is characterised by the occurrence of phenomena such as concentration polarisation and fouling. Agenson and Urase [35] affirmed that the permeate flux reduction for their studied membranes was mainly due to the adsorption of organic particles onto the membrane, resulting in clogged pores. For the NF-90 membrane, in contrast, the permeate flux remained stable (Fig. 3).

It has been reported that chemical fouling depends on hydrophobic interactions and electrostatic interactions between organic matter (humic substances) in the feed water and the membrane surface [36]. As reported by Nghiem et al. [37] and Choi et al. [38], the isoelectric point of the NF-270 membrane is approximately 3.5. This means that the membrane is negatively charged at pH values above 3.5 and positively charged at lower pH values. As the pH of the sample was close to 6, both the membrane and the organic matter (negatively charged at pH of natural waters) were negatively charged. In this sense, electrostatic repulsion as well as size exclusion would be responsible for the retention of organic matter by the membrane. This removal can be observed according to the quantification of DOC obtained after filtration (Table 1). In a study with the NF-270 membrane, Choi et al. [38] found that the adsorption of humic acids onto the membrane surface resulted in an increase of the hydrophilicity of membrane surface, with a slight increase in permeate flux at 1 h of filtration. After this period, the flux decline became more pronounced as a result of the concentration polarisation effect. According to Hong and Elimelech [39], a low ionic strength (strong electrostatic repulsion between the membrane surface and natural organic matter NOM) hinders NOM deposition on the membrane surface. Moreover, the fouling layer becomes more flexible. However, with increasing filtration time, the thickness of the adsorbed humic acid layer increases, and hence its hydraulic resistance to permeate flux tends to increase. As a result, the permeate flux tends to decrease.

The greater decline of permeate flux for the NF-270 membrane as compared to the NF-90 membrane may be directly related to the phenomenon of size exclusion. As described by Eagles and Wakeman [40], particles smaller

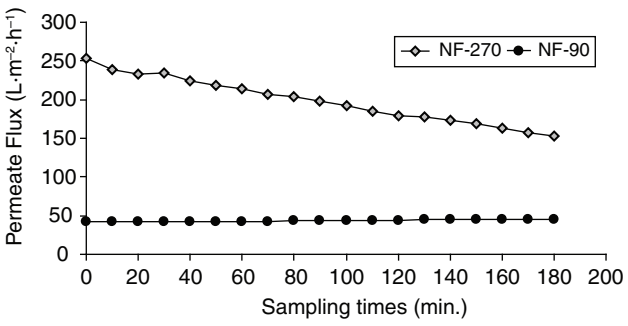


Fig. 3. Flux behaviour for NF-270 and NF-90 membranes for the sample filtration (time evaluated: 180 min; working pressure: 8 bar).

than the membrane pores tend to deposit on the walls of the pore. This can cause an effective reduction in pore diameter and reduce the flux. The flux decline observed for the NF-270 membrane can be attributed to the accumulation of organic matter on the membrane surface as well as clogging of the membrane pores by particles that are similar to or smaller than the average pore diameter. For the NF-90 membrane, with a smaller average pore diameter, surface deposition was likely, with a correspondingly lower influence on the permeate flux.

The roughness of the membrane is also considered an important factor in the occurrence of fouling and in the reduction of permeate flux. Vrijenhoek et al. [41] reported a relation between a decrease in permeate flux and membrane roughness in the filtration of colloidal solutions. The presence of valleys on the surface of rough membranes induces preferential deposition of particles at these sites. This results in a blockage of valleys and in a severe reduction in flux. On smooth membranes (where there are no valleys on the surface), even if the same number of particles were deposited, they would more likely be evenly spaced, reducing the effect of permeate-flux decline. Although the NF-90 membrane exhibited a rough surface (Fig. 2b), this feature was not considered, as the permeate flux was relatively constant.

The fouling effect on the membranes can be evaluated by assessing the reduction of permeate flux with pure water after sample filtration. Fig. 4 compares the behaviour of the permeate flux when measuring the pure-water hydraulic permeability of the membrane and the flux after sample filtration. A significant reduction in permeate flux after a sample filtration of ~120 $l \cdot m^{-2} \cdot h^{-1}$ was observed for the NF-270 membrane. This reduction may be associated with obstruction of the membrane by the material deposited in the membrane pores and on the surface, reducing its efficiency. The reduction of the permeate flux for the NF-90 membrane after sample filtration was not significant, indicating a low deposition of material suspended or dissolved in the membrane.

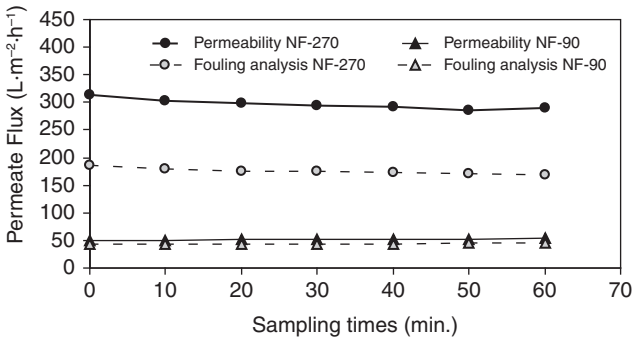


Fig. 4. Pure-water hydraulic permeability in clean membranes and after sample filtration at a pressure of 8 bar.

The presence of a fouling layer can significantly influence the removal of various trace contaminants, e.g., it can result in an increase or decrease in the retention of the membrane as compared to a clean membrane or in the absence of fouling [42].

### 3.3. Rejection of cyanotoxins by the nanofiltration membranes

The toxins neosaxitoxin (neoSTX) decarbamoyl saxitoxin (dcSTX) and saxitoxin (STX) were identified and quantified in the chromatographic analysis. For the NF-270 and NF-90 membranes tests, respectively, the initial concentrations of the toxins were 6.81 and 9.90 $\mu g \cdot l^{-1}$ for neoSTX, 3.15 and 3.21 $\mu g \cdot l^{-1}$ for dcSTX and 3.45 and 3.35 $\mu g \cdot l^{-1}$ for STX. A characteristic chromatogram of the identified toxins is shown in Fig. 5.

The percentages of toxin removed by the NF-270 and NF-90 membranes at the filtration times evaluated are listed in Table 2. For the NF-270 membrane, the passage of toxins through the membrane was observed, with increasing values with increasing filtration time. This increase in the toxin concentration in the permeate can be associated with a saturation of the membrane with a corresponding reduction in toxin retention. Braeken et al. [43] observed a decrease in the retention of compounds with high hydrophobicity by hydrophobic membranes. This behaviour was due to adsorption of material onto the surface and to the membrane structure. Likewise, it can be associate the decrease at toxin retention due to the increasing concentration of feed solution with the passage of filtration time and, the formation of a fouling layer by the deposition of toxins and other substances on the membrane. The presence of the

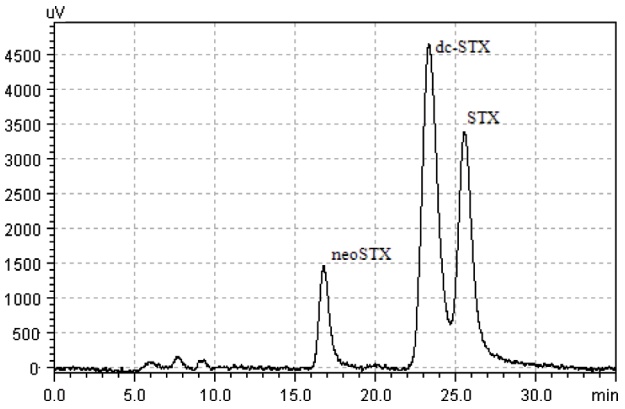


Fig. 5. Characteristic chromatogram of the neoSTX, dcSTX and STX cyanotoxins. Chromatographic conditions: HPLC with post-column reaction and fluorimetric detection; Phenomenex C8 reverse-phase column (Luna 5 µm, 250 × 4.6 mm), mobile phase consisting of 1-heptanesulphonic acid, ammonium phosphate and acetonitrile, chromatography flow: 0.6 $ml \cdot min^{-1}$.

Table 2
Concentrations and removal percentages of neoSTX, dcSTX and STX toxins by NF-270 and NF-90 membranes

| Membrane | Sample | neoSTX | | dcSTX | | STX | |
|---|---|---|---|---|---|---|---|
| | | Conc. ($\mu g \cdot l^{-1}$) | Removal (%) | Conc. ($\mu g \cdot l^{-1}$) | Removal (%) | Conc. ($\mu g \cdot l^{-1}$) | Removal (%) |
| NF-270 | Initial[1] | 6.81 | – | 3.15 | – | 3.45 | – |
| | $t$ = 0 | 3.12 | 54.2 | 2.17 | 31.1 | 2.02 | 41.2 |
| | $t$ = 30 | 3.77 | 44.6 | 2.40 | 23.8 | 2.24 | 35.1 |
| | $t$ = 60 | 4.21 | 38.2 | 2.38 | 24.6 | 2.20 | 36.3 |
| | $t$ = 180 | 5.87 | 13.7 | 2.95 | 6.2 | 3.07 | 11.0 |
| NF-90 | Initial | 9.90 | – | 3.21 | – | 3.35 | – |
| | $t$ = 0 | 0.00 | 100.0 | 0.00 | 100.0 | 0.00 | 100.0 |
| | $t$ = 30 | 0.00 | 100.0 | 0.00 | 100.0 | 0.00 | 100.0 |
| | $t$ = 60 | 0.00 | 100.0 | 0.00 | 100.0 | 0.00 | 100.0 |
| | $t$ = 180 | 0.00 | 100.0 | 0.00 | 100.0 | 0.00 | 100.0 |

[1]Initial concentration after DAF.

fouling layer may have contributed for the toxins release adhered at membrane and their passage for permeate. Nghiem et al. [28] also observed a lower retention of hydrophilic compounds by the NF-270 membrane due to organic fouling caused particularly by deposition of alginates and humic substances. This fouling layer would also be responsible for the reduction in permeate flux of the membrane, as noted previously and also considered by Nghiem et al. [28]. Higher removal values for the neoSTX and STX toxins were verified by comparing them to the dcSTX toxin, but with equivalent reductions in removal efficiency. For the NF-90 membrane, conversely, total removal of the toxins was observed over the whole filtration period (180 min).

Apart from the adsorption of toxins onto the membrane surface and inside the pores, various solute-membrane-interaction effects may have occurred, especially with the NF-270 membrane. The effects of size exclusion, electrostatic interactions and hydrophilic interactions were considered in an attempt to understand this process. For the NF-90 membrane, the size-exclusion phenomenon can be regarded as the predominant mechanism of removal, as also observed by Nghiem et al. [28]. This is likely because the molecular weights of the identified toxins (neoSTX, 317 Da; dcSTX, 258 Da; and STX, 301 Da) [18] are greater than the molecular weight cut-off of the membrane (200 Da). Therefore, the toxins would be more easily retained by membrane. The higher hydrophobicity of the NF-90 membrane may also have contributed to the retention of toxins by this membrane.

Regarding the NF-270 membrane, considering that both, membrane and the toxins have similar characteristics (hydrophilicity and negative surface charge) and a similar molecular weight, it is believed that all three removal mechanisms described above may contribute to the rejection of toxins by the membrane. The higher values for the removal of the neoSTX and STX toxins indicate that the mechanism of size exclusion could have exerted more influence on the removal process. The decline of toxins retention by the membrane with increasing filtration time suggests that other effects act on the retention process, as the effects of hydrophilic or electrostatic interaction, or the interference of a concentration polarization layer. Hoek and Elimelech [44] argue that the formation of a concentration polarization layer results in an increasing trans-membrane osmotic pressure and thus in a measurable decline at salt rejection, after a few hours filtration. The rejection decline is attributed to the high concentration of solutes on the membrane surface. As seen by Hoek and Elimelech [44] the presence of a concentration polarization layer had contributed to the reduction of the permeate flux of NF-270 membrane.

Due to its molecular weight (258 Da), a lower initial retention of the dcSTX toxin compared with the other toxins studied was expected. Braeken et al. [43] related the rejection behaviour of molecules with molecular weights lower than the molecular weight cut-off of the membrane based on the structure of the compound. According to the authors, molecules with low (negative) octanol-water partition coefficients (*log* P), which are therefore hydrophilic, usually have more –OH or =O groups, which allows for the formation of hydrogen bonds with water molecules. Thus, hydrophilic compounds tend to have a higher affinity for the water phase and are less permeable through the membrane structure (which is hydrophobic). The association of organic molecules with water molecules results, therefore, in an increase in the effective diameter of the molecule due to its hydration shell [43,45]. This increase in the effective size of the dcSTX molecules could have induced the retention of toxins by the membrane. Meantime due to

the high hydrophilicity of the toxin studied [3,46], their affinity with aqueous phase may have contributed for a higher permeation of toxins through the membrane, also hydrophilic [23], which could explain the decrease retention, indicated initially. This behaviour ratio may be considered for other toxins.

Figs. 6 and 7 show chromatograms indicative of rejection behaviour for the evaluated toxins by the two membranes. The plots were obtained from the overlap of the peaks generated by HPLC analysis. The arrows in the figure indicate the peaks corresponding to each filtration time evaluated. As mentioned before, higher peaks for the identification of toxins as a function of filtration time can be observed for the NF-270 membrane. This indicates the presence of higher toxin concentrations in the permeate. For the NF-90 membrane, the absence of peaks in the chromatogram indicates the absence of toxins in the permeate.

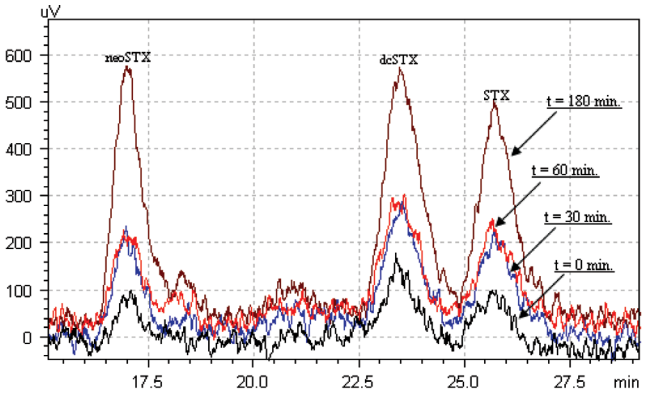


Fig. 6. Chromatographic evolution of neoSTX, dcSTX and STX toxin concentrations during the filtration period ($t$ = 0 to $t$ = 180 mins) for the NF-270 membrane.

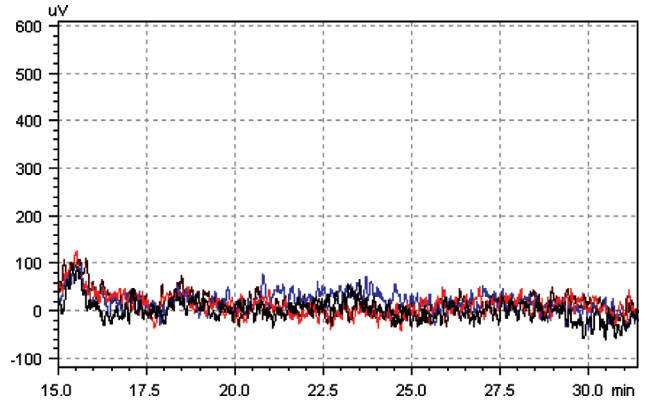


Fig. 7. Chromatographic evolution of neoSTX, dcSTX and STX toxin concentrations during the filtration period ($t$ = 0 to $t$ = 180 mins) for the NF-90 membrane.

## 4. Conclusions

The NF-270 membrane presented a higher permeate flux of toxins than the NF-90 membrane, which was mainly due to its higher porosity and larger average pore diameter. The hydrophilicity of the NF-270 membrane can also be considered a relevant factor in this behaviour. The larger decline in permeate flux for the NF-270 membrane indicates that this membrane may have suffered more severe fouling effect due to the deposition of both organic substances and toxins on the membrane surface and inside the pores. The larger pore diameter seems to have contributed, to a large degree, to the membrane obstruction by organic matter. The hydrophilic character of the NF-270 membrane and its relation to the toxins (also hydrophilic) could also be responsible for the flux decrease. Because the NF-90 membrane has a smaller average pore diameter and a higher hydrophobicity, this membrane suffered no significant influence of this interaction.

The results reported here on the efficiency of toxin removal indicate that the NF-90 membrane is more efficient than the NF-270 membrane. A total removal of toxins was obtained by application of the NF-90 membrane. For the NF-270 membrane, a lower removal was observed, with a trend of decreasing rejection as the filtration time increased. This behaviour may be related to the formation of a concentration polarization layer which results in an increased trans-membrane osmotic pressure, with a rejection decline. The mechanisms of size exclusion, electrostatic interactions and hydrophilic interactions are considered to be important in the degrees of rejection observed. Based on these data, the NF-90 membrane is not only more effective in the removal of cyanotoxins but also shows a tendency toward a longer useful life. The results obtained indicate that it is feasible to use nanofiltration as an effective technology in removing toxins from drinking water.

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