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Application of Clay Minerals and Polymeric Resins to Remove Dissolved Microcystin-LR from Water

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ABSTRACT: This study aimed to develop techniques for purification of cyanotoxins dissolved in water - particularly those produced by *Microcystis aeruginosa* species - using natural clay, chemically modified natural clay, commercial clay and ion exchange and adsorbent resins. Except for natural clay, chemical pre-treatment was required to increase the adsorption capacity of the treatment materials. All the clays were exposed to microcystin solutions of $0.5x10^{-3}$, $1.0x10^{-3}$ and $10.0x10^{-3}$?g/mL in batch purification processes. The same solutions were used in purification processes with polymeric resins packed in columns. The microcystin-LR samples were quantified using ELISA immunoassay and removal capabilities and saturation of the treatment materials were evaluated. The modified clays showed a microcystin-LR removal efficiency rate between 82.90% and 99.86%, while the resins a rate between 87.6% and 99.74%. This study shows that the materials have technologically and economically promising properties for the removal of microcystins from water.

Key words: Toxin removal, Microcystin, Natural clay, Commercial clay, Commercial resins, Purification

INTRODUCTION

Eutrophic lakes, ponds, and reservoirs worldwide are recurrently dominated by toxic cyanobacteria blooms, mainly at summer time in temperate regions and at different points in time throughout the year in tropical and subtropical areas of Australia, South America and China (Dörr *et al*., 2010; Paldavièiené *et al*., 2009; Van Apeldoorn *et al*., 2007). These events are occurring increasingly due to difficulties in controlling nutrients, especially in developing countries, and water temperature increases associated with global warming (Aboal and Puig, 2005; Bartram *et al*., 1999; Delpla *et al*., 2009; Kardinaal *et al*., 2007; Paerl and Huisman, 2008; Skulberg *et al*., 1984). The presence of cyanotoxins in the water, in particular microcystin, can impair water uses for multiple purposes (Amado and Monserrat, 2010; Barrington and Ghadouani, 2008; McElhiney *et al*., 2001). One of the most common cyanobacteria, *Microcystis aeruginosa*, can produce several variants of microcystins, which are responsible for acute hepatotoxicity, liver damage and the growth of liver tumors (Azevedo *et al*., 2002; Carmichael *et al*., 2001; Gupta *et al*., 2003; Haider *et al*., 2003; Jochimsen *et al*., 1998; Pflugmacher 2002). More recently, the identification of microcystin in the serum of chronically exposed fishermen found by Chen (Chen *et al*., 2009) increased the relevance of risk assessment of some types of exposure to this type of toxin. The spreading of the occurrence and severity of harmful cyanobacteria blooms in aquatic ecosystems has become a worldwide public health concern, as many countries use surface water as their main source of drinking water. In this scenario, companies responsible for the production and distribution of potable water are faced with the challenge of improving purification processes to provide safe drinking water for consumers (Ho *et al*., 2007; Rosa *et al*., 2005). Conventional water treatment methods such as coagulation, flocculation and sand filtration are known to remove cyanobacteria but are ineffective at removing or destroying dissolved cyanotoxins, particularly in water supplies with high levels of organic material (Haider *et al*., 2003; Lawton and Robertson, 1999; Morris *et al*., 2000; Rosa *et al*., 2005; WHO, 1998). Most of the strategies used for removal of cyanobacterial toxins from water such as ultrafiltration combined with adsorption on powdered activated carbon and oxidation techniques (Gijsbertsen-Abrahamse *et al*., 2006; Lee and Walker, 2008; Onstad *et al*., 2007) present disadvantages, since most of them are expensive, are dependent on the specific features of the water to be treated and require commitment to maintenance (Antoniou *et al*., 2010; Campinas and Rosa, 2006; Gijsbertsen-Abrahamse *et al*., 2006; Lambert *et al*., 1996; Rodríguez *et al*., 2007; Shawwa and Smith, 2001). Therefore, more affordable and low-

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maintenance methods of drinking water treatment should be developed (Gurbuz and Codd, 2008; Sathishkumar *et al*., 2010). Natural clays show an elevated capability of organic compounds adsorption through different types of interactions (Guarino *et al*., 1997), and may be used in isolation or in combination with the soil as barriers against organic pollutants. These barriers are reactive to positive charges presented by organic pollutants (cation exchange) and by polar pollutants (dipole-dipole interactions) (Rodriguez-Cruz *et al*., 2007). The adsorption of microcystin-LR by sediments with a high percentage of clay and naturally-occurring clay particles has already been reported (Chen *et al*., 2006; Eynard *et al*., 2000; Miller *et al*., 2001; Morris *et al*., 2000; Tsuji *et al*., 2001; Zakaria *et al*., 2007). On the other hand, ion-exchange resins are a promising tool for selective separation of micropollutants from water. The potential of chelating ion-change resins for removal and recovery of pollutants from industrial waste waters has been very well established since the 1970s (Crook *et al*., 1975; Nabi *et al*., 2007).

The objective of this study was to investigate the potential use of Brazilian naturally-occurring clay (smectite), commercial clay (smectita-montmorillonit K-10), commercial ion-exchange and adsorbent resins (Amberlite) for the removal of dissolved microcystin-LR from water. The clays, because of their availability and low cost (U$0.15. Kg^{-1}) in Brazil, should be considered for use in water treatment stations, and resins for use in hemodyalysis facilities.

MATERIALS & METHODS

Natural clay (smectite) and Commercial clay (smectite - montmorillonit K-10) were supplied by Bentonisa – Bentonita do Nordeste (Paraiba State, Brazil) and Sigma-Aldrich® (São Paulo State, Brazil), respectively. Ion Exchange Resin (Amberlite 200cNa) and an Adsorbent Resin (Amberlite XAD-16) were supplied by Rohm and Haas® (São Paulo State, Brazil). A stock solution of microcystin-LR (7.55μg/mL) was obtained from a unialgal mass culture of the *Microcystis aeruginosa* from the Laboratório de Ecofisiologia e Toxicologia de Cianobactérias of the Universidade Federal do Rio de Janeiro. Solutions were prepared from the stock solution in order to reach concentrations of $10.0x10^{-3}$μg/mL, $1.0x10^{-3}$ μg/mL and $0.5x10^{-3}$μg/mL.

The Amberlite 200cNa (R1) cationic resin was treated with an aqueous solution of HCl (20% v/v) under shaken for 30 minutes at room temperature. The resin was intensively washed with distilled and deionized water, at 15-minute intervals, until a constant conductivity, measured with a conductivity meter (Handylab LF1). After the washing, the modified resin was dried at 50°C for 48hours. The Amberlite XAD-16 (R2) adsorbent resin was also treated with HCl (20% v/v) solution and washed with distilled/deionized water until constant conductivity. Next, it was treated with an aqueous NaOH (2.0% w/v) solution for 30 minutes under shaken and washed with distilled/deionized water until a constant water conductivity. The resin was finally kept for 1hour in commercial ethanol, washed with distilled/deionized water and dried at 50°C for 48h.

A dispersion of natural clay 1.0% (w/v) was prepared with 500mL of HCl (0.1 mol/L) and mixed for 24hours at room temperature. The solution was centrifuged at 4000 RPM for 30 minutes and the supernatant discarded. The clay was re-suspended in distilled/deionized water, shaken for one hour and centrifuged again. This process was repeated until a constant conductivity. Afterwards, the sample was dried at 50°C, triturated and kept in desiccators until put into use. This treated material was called modified natural clay (MNC). To evaluate the removal of dissolved MCYST-LR by resins, 50mL of each diluted solution of microcystin-LR ($10.0x10^{-3}$, $1.0x10^{-3}$ and $0.5x10^{-3}$μg/mL) was carefully eluted through the column previously packed, in duplicate for each resin at 1.2mL/min flow rates. To evaluate the removal of dissolved MCYST-LR from water by clays, 50mL of the same diluted solutions of MCYST-LR were prepared with each type of clay - commercial clay (CC), natural clay (NC) and modified natural clay (MNC) in order to give dispersions at a ratio of 1% (w/v). The batch experiments were conducted in duplicate. A contact time of 30 minutes was chosen for the adsorption experiment. The samples were then centrifuged at 4,000 RPM for 15 min, the precipitate was separated and the liquid was frozen for further microcystin quantification. To assess the effect of contact time upon the adsorption of dissolved MCYST-LR by clays, a batch experiment was carried out with the MNC. A MCYST-LR concentration of $1.0x10^{-3}$ μg/mL was used and the batch experiments were run with different contact times (5, 10, 15, 20, 25, 30, and 120 minutes). All these batch-time evaluation experiments were later replicated.

To investigate the saturation capacity of MCYST-LR absorption by the two treatment materials used in the experiments, additional sets of trials were conducted using the procedures for column and batch experiments. The Amberlite 200cNa (R1) ion-change resin and the MNC were selected because they yielded better MCYST-LR removal results. To assess the effect of the R1 adsorption saturation, ten consecutive elutions with 50mL of $10.0x10^{-3}$ μg/mL MCYST-LR solution were conducted in the same column. Samples for MCYST-LR concentration quantification were taken

at the end of each elution. To assess the effect of the MNC adsorption saturation, ten consecutive batch experiments were performed, using 50mL of suspended dispersions of MNC 1% (w/v) prepared with $10.0x10^{-3}$ µg/mL MCYST-LR solutions. A 30-minute contact time was decided for the saturation experiment. The solution was centrifuged at the end of each batch trial, and a sample was taken for MCYST-LR concentration quantification. The MNC was once more suspended using a 50mL of $10.0x10^{-3}$ µg/mL MCYST-LR solution and the procedure repeated again. Both experiments were performed in duplicates.

All samples were analyzed by the immunoassay method ELISA (ENVIROLOGIX.INC®). One-way ANOVA was performed to determine whether there was a statistically significant difference between initial and final MCYST-LR concentrations in the eluted experiments. The relationship between different MCYST-LR concentrations removal for all treatment materials, contact times and adsorption capacities were assessed using the ANOVA (Tukey and Scheffé) correlation test (Maxwell and Delaney, 2004; Milliken and Johnson, 2009).

RESULTS & DISCUSSION

Microcystins are cyclic heptapeptides that share a general structure containing ionic features. Anion-exchange has been used successfully to semipurify mycrocistin on a small scale due to its ionic interactions with these groups (Cremer and Henning, 1991; Cremer *et al*., 1991). This work focused on the functionalization of the starting materials, clay minerals and polymeric resins, by the change of the functional groups present in these materials to hydrogen counter-ions. The functionalized materials provide specific sites for the adsorption of the microcystins in the interlamelar region of the clay or by ion exchange of these biomolecules in the matrix of the polymeric resins.

The results of column elution experiments of MCYST-LR removal in the initial and in the final eluates, with the three different concentrations, are shown in Table 1. Efficiency rate of removal using R1 was between 88.8 and 95.7% with $0.5x10^{-3}$ µg/mL MCYST-LR solutions, between 89.6 and 94.8% with $1.0x10^{-3}$ µg/mL MCYST-LR solution, and 99.2 and 99.7% with $10.0x10^{-3}$ µg/mL MCYST-LR solution. Using R2, efficiency rate was between 87.6 and 89.5% with $0.5x10^{-3}$ µg/mL MCYST-LR solutions, between 91.5 and 98.1% with $1.0x10^{-3}$ µg/mL MCYST-LR solution and 89.6, and 99.4% with $10.0x10^{-3}$ µg/mL MCYST-LR solution. The Tuckey test showed that there were no significant differences in the removal efficiency between the initial and the final eluate.

Table 1. Results of microcystin-LR range of adsorption (%) at the start(i) and end (f) points of the elution experiments within columns using the two resins (R1= Amberlite 200cNa; R2= Amberlite XAD-16) and with different microcystin-LR solution concentration

RESIN	Concentrations of microcystin-LR (µg/mL)	% of microcystin-LR absorption
1	$0.5x10^{-3}$	(i) 88.8 – 91.0
		(f) 89.3 – 95.7
	$1.0x10^{-3}$	(i) 90.4 – 91.6
		(f) 89.6 – 94.8
	$10.0x10^{-3}$	(i) 99.4 – 99.7
		(f) 99.2 – 99.2
2	$0.5x10^{-3}$	(i) 87.6
		(f) 89.5
	$1.0x10^{-3}$	(i) 93.7 – 98.1
		(f) 91.5 – 98.1
	$10.0x10^{-3}$	(i) 99.3 – 99.3
		(f) 89.6 – 93.4

Initial and final eluates were considered in combination (Fig.1) in order to allow a graphic presentation of data regarding the results of MCYST-LR adsorption by the resins in the column experiments. Both resins were efficient in the retention of the cyanotoxin. R1 presented a better adsorption rate with the $10.0x10^{-3}$µg/mL MCYST-LR solution, whereas R2 had a better adsorption with the $1.0x10^{-3}$µg/mL MCYST-LR solution. These results are indicative of excellent removal rates, ranging from 87.6 to 99.7%, by the commercial polymers that were used, which were modified chemically to the hydrogen form by the acid activation.

The results of the batch experiments concerning adsorption of MCYST-LR by clays also showed variations in the efficiency rate and such variations relate to the MCYST-LR solution concentration used (Fig.2). All the three types of clay showed lower adsorption with the $0.5x10^{-3}$ µg/mL MCYST-LR solution but a higher adsorption rate with the $10.0x10^{-3}$ µg/mL MCYST-LR solution. Acidified clay, i.e., modified natural clay (MNC) and commercial clay (CC), absorbed MCYST-LR with higher efficiency than natural clay. It should be noted that commercial clay was already previously acidified. The acidification treatments increased the presence of H^+ in the clays and facilitated ionic exchanges between these hydronium ions and MCYST-LR.

The pioneer study of (Morris *et al*., 2000) with the potential of naturally occurring clay minerals to scavenge MCYST-LR from solution succeeded with more than 81% of MCYST-LR removal. Differently, (Yan *et al*., 2006) using the same conditions of the absorbing

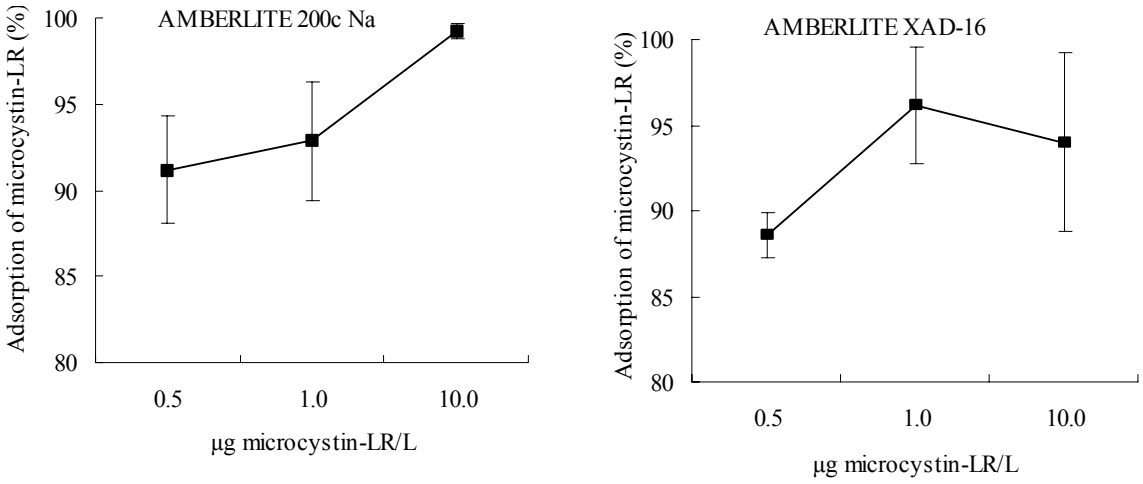

Fig. 1. Microcystin-LR adsorption by resins (a) R1(Amberlite 200cNa) and (b) R2 (Amberlite XAD-16) at different microcystin-LR solution concentrations (0.50x10^{-3}µg/mL, 1.0x10^{-3}µg/mL and 10.0x10^{-3}µg/mL)

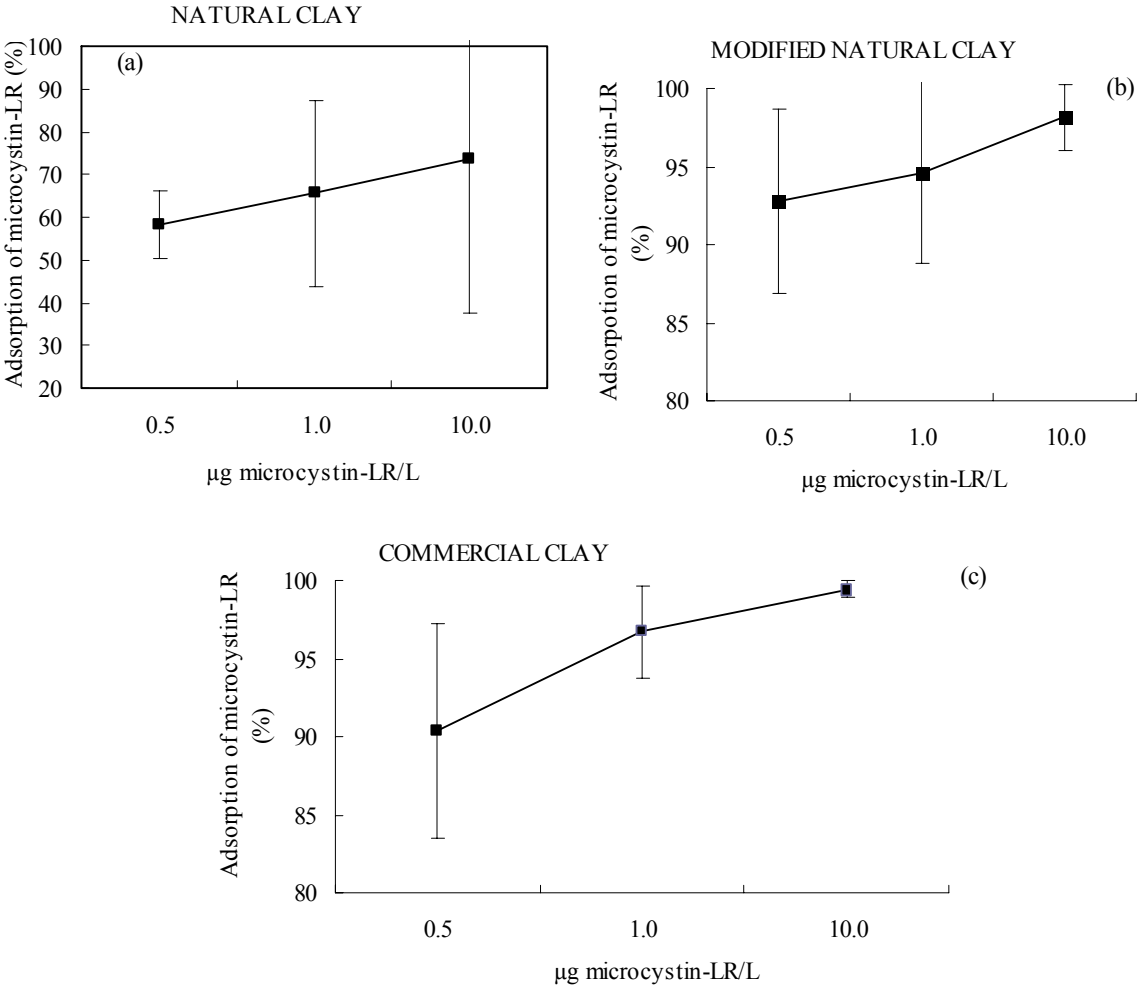

Fig. 2. Microcystin-LR adsorption by (a) natural clay, (b) modified natural clay and (c) commercial clay at different microcystin-LR solution concentrations (0.50x10^{-3}µg/mL, 1.0x10^{-3}µg/mL and 10.0x10^{-3}µg/mL)

materials, such as activated carbon, carbon nanotubes (CNTS), concluded that the lowest rate of absorption by clay was a result of the lack of a previous treatment. In the present study, the lowest MCYST-LR absorption capacity of natural clay can also be related with the absence of a previous treatment. Despite the lower MCYST-LR adsorption capacity revealed here, natural clays are acknowledged as having an important role in the retention and transportation of pollutants, including microcystins in aquatic ecosystems. According to (Morris *et al*., 2000) the structure of part of the microcystins may make them susceptible to scavenging by one-grained particles, particularly certain clays, and this feature may have important implications for the geochemical fate of these compounds once they are released into natural waters, including their rate of degradation and pathways available for re-entry into the food chain.

Clays can also have a protective function for otherwise quite labile adsorbed organic compounds, being the nature of the association with clay to significantly slow normal biological and chemical degradation processes (Morris *et al*., 2000). This is particularly the case for smectite, the group of clay minerals, because their structures can be expanded along the "c" axis by the adsorption of organic compounds as well as of water molecules in interlayer positions (Vaccari 1998). Because of their small size, clay minerals remain in suspension for long periods in aquatic environments and hence can act as a major vector for adsorbed compounds, transporting them over considerable distances (Morris *et al*., 2000). Moreover, as the pH can interfere on the adsorption capacity of natural clay, (Miller *et al*., 2001) in sediments with low oxygen content, high organic matter decomposition and low pH an accumulation of microcystin is expected, such as in anoxic bottoms of some lakes. The comparison between the adsorption of MCYST-LR by resins and by clays in column elution and batch experiment is shown in Fig. 3. Modified clays (MNC and CC) and resins attained better adsorption efficiencies than natural clay (NC), with the three MCYST-LR concentrations, which was countersigned by the Scheffé test.

Comparing all adsorption efficiencies attained with all the treatment materials used in connection with the three concentrations of MCYST-LR, an evident improvement can be seen regarding the adsorption capacity with increased concentration. Likewise, (Lambert *et al*., 1996) found low MCYST-LR removal rate with activated carbon at low concentration of the cyanotoxins. Zakaria (Zakaria *et al*., 2007) also concluded that the low microcystin concentration used could explain low adsorption by activated carbon and clays in their experiments. Similarly, (Lee and Walker, 2008) using a hydrophobic membrane that adsorbed 91% of MCYST-LR, observed an increase in the adsorption capacity with higher concentration.

Linear increase of adsorption with concentration suggests an initial surface adsorption followed by a deposition of more MCYST-LR. As suggested by (Lee and Walker, 2008) it seems that a unique layer of MCYST-LR is adsorbed and after a deposition of more MCYST-LR, a multi-layer bond is formed. The same authors showed an influence of both MCYST-LR concentration and membrane-solution interface gradient upon the deposition of the toxin in the membrane. A higher concentration of MCYST-LR in the solution may increase the interactions among

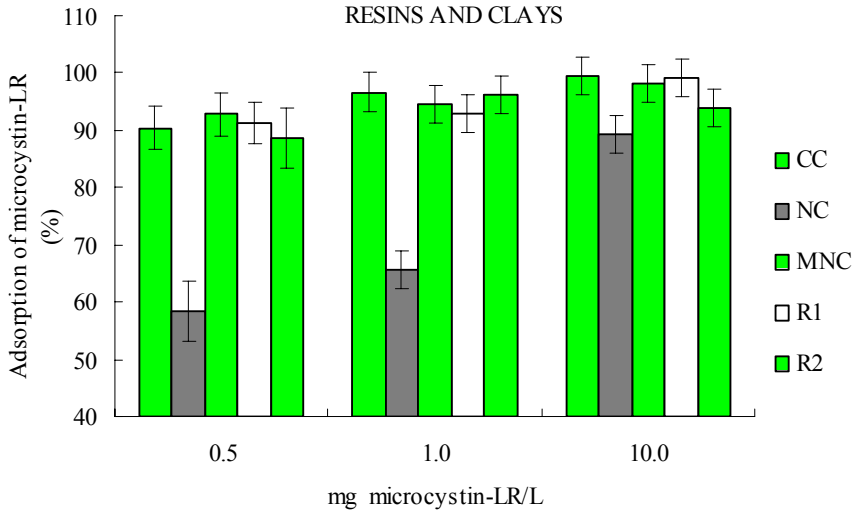

Fig. 3. Estimated standard error in the adsorption efficiency of microcystin-LR by Amberlite 200cNa (R1) resin, Amberlite XAD-16 (R2) resin, natural clay (NA), modified natural clay (MNC) and commercial clay (CC) in the experiments with the three different microcystin-LR Concentrations

molecules and as a consequence, enhance the adsorption capacity of the treatment materials. This could explain improved MCYST-LR removal rates found in our study using resins and clays with the $10.0x10^{-3}$ µg/mL MCYST-LR solution. In an attempt to optimize the time of contact with the treatment material in the solution used for microcystin purification, batch tests were carried out with times lower than 30 minutes in samples with chemically modified natural clay (NMC) and a $1.0x10^{-3}$µg/mL MCYST-LR solution. Fig. 4 shows the results of the adsorption efficiency rate for the different contact times and estimated standard error.

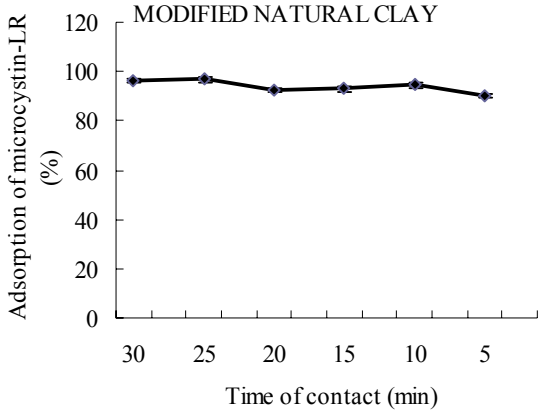

Fig. 4. Estimated standard error in the adsorption efficiency of microcystin-LR by modified natural clay at different contact times with the $1.0x10^{-3}$µg/mL of microcystin-LR solution

Two recent publications reported results about the adsorption kinetics of MCYST-LR onto activated carbon materials. Pyu and Moon, (Pyu and Moon, 2005) using activated carbon synthetic fibers to remove MCYST-LR, obtained up to 97% removal of the toxin from a solution with an initial concentration of $1.0x10^{-3}$µg/mL of the toxin, after a contact time of 5 minutes. Yan (Yan *et al*., 2006), showed that a solution with a concentration of $9.6x10^{-3}$µg/mL of the same toxin, when treated with nano tubes of activated carbon, decreased the concentration after 24 hours of contact time, with a removal of MCYST-LR of 61.46%. In this study, contact times of 30 and 25 minutes attained higher removal efficiency, but times up to 10 minutes can be used to remove more than 90% of the cyanotoxins dissolved in water. These results could be translated in practical terms into savings in time and money, taking into account a possible application of NMC in large scale treatments such as water treatment plants.

The evaluation of the saturation capacity of resins and clays was performed with R1 and MNC, in column elution and batch experiments, respectively. The MCYST-LR solution used had a $10.0x10^{-3}$µg/mL concentration, aiming to reach the saturation of the treatment materials with a low number of repetitions. The solution containing MCYST-LR was placed in contact with the same treatment material for 10 repeated trials and the results are shown in Fig. 5.

Recently, isotherms pertaining to the sorption of MCYST-LR onto peat were determined at different initial MCYST-LR concentrations ranging from 100 to 1000 ?g/L (Sathishkumar *et al*., 2010). The maximum adsorption capacity was 255.7?g/g and 93.7% of MCLR could be desorbed with 2N NaOH as eluting media. Adsorption capacity of peat used in the same study completely out-performed almost all the wood and coal based powdered carbon reported by (Donati *et al*., 1994). Adsorption capacity performed in the present study show that after ten repeated trials in column elution, the resin showed no significant variation concerning MCYST-LR adsorption. Thus, it can be concluded that it would take more than ten trials to achieve an exchange capacity decreased by R1. However, the obtained results allow us to state that this treatment material can be reused up to ten times with losses as low as 2% of its adsorption capacity with a MCYST-LR concentration of $10.0x10^{-3}$µg/mL. MNC, nevertheless, showed a sharper drop (~ 10%) in adsorption capacity after the consecutive batch experiments. However, up to the fourth repeated trial this variation was not as significant, which shows that this material could be reused up to four times and still remove ~ 94% of dissolved microcystins.

CONCLUSION

In conclusion, this study has demonstrated the feasible use of clay, an abundant and low-cost naturally occurring material for the removal of microcystin-LR from water. These findings should encourage academic and technological research into the use of this treatment material as an interesting alternative method to the traditional purification process in treatment plants. Ion exchange and adsorbent resins also showed good results by elution in columns, and they are a promising treatment material to be used in further techniques to eliminate toxins from aqueous media, mainly in hemodialysis clinics. Moreover, it would be profitable to carry out experiments with these treatment materials for the removal of other types of cyanotoxins.

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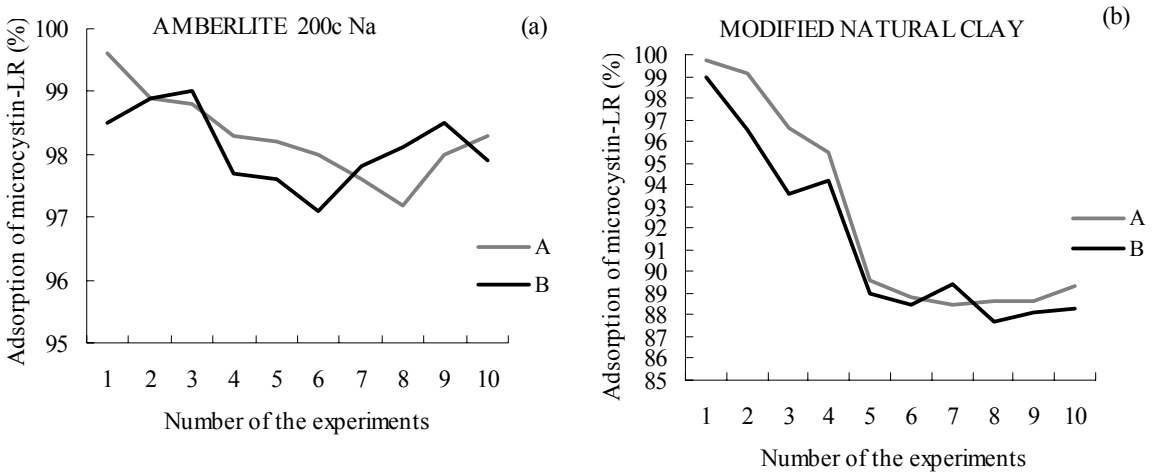

Fig. 5. Adsorption of microcystin-LR along the 10 consecutive experiments in (a) elutions with Amberlite 200c Na resin and in (b) batches where modified natural clay A and B are the replicates

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