# Adsorption of Microcystin LR by Activated Carbon Fibers

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Blooms of toxic microorganisms are commonly found in freshwater in several areas of the world. Freshwater poisonings are mainly caused by species of cyanobacteria, also known as blue-green algae.[1,2] They develop in eutrophic water wherever proper conditions for their growth are found, including a mild wind, a water temperature between 15 and 30 °C, a neutral or alkaline pH and a rather high level of mineral nutrients. As a consequence of the extended use of nitrates and phosphates, blooms occur more and more frequently.[3] There is a risk that these blooms contaminate water supplies that are used as recreational areas or as drinking water reservoirs. This is a serious water quality problem because many of the cyanobaterial species are able to produce potent toxins.

The most frequently reported toxins are hepatotoxins. They are classified as microcystins and nodularins, the former representing the largest group. All microcystins have a common cyclic heptapeptide structure consisting of (-D-Ala-L-X-D-MeAsp-L-Z-Adda-D-Glu-Mdha), where MeAsp stands for erythro-$\beta$-methylaspartic acid, Mdha for N-methyldehydroalanine, Adda for 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, and X and Z for the variable amino acids that give its name to the molecule.[4] For example, microcystin LR has X = Leusine and Z = Arginine, microcystin RR has X = Arginine and Z = Arginine. Microcystins can be synthesized by various genera of cyanobacteria such as *Microcystis*, *Oscillatoria*, *Anabaena* or *Nostoc*. They have been responsible for the poisoning of fish, birds, wild and domestic animals in many countries.[5,6] Adverse effects on human health have also been recognized. Recent researches have actually shown that microcystins act as type 1, 2A and 3 protein phosphatase inhibitors, as well as tumor promoters when present in nanomolar concentrations.[7,8,9] Nowadays, WHO guidelines have been established for the monitoring of microcystins in water. Maximal values of 0.1 $\mu$g/L in drinking water for a long term exposure or 1 $\mu$g/L for a short term exposure have been proposed on the basis of laboratory experiments of toxicity on mice and pigs.[10]

Blooms of blue-green algae, which have been increasing in recent years, have the potential to cause problems for the water supply industry. Recent occurrences of toxic algal blooms have highlighted the potential risk of toxins entering drinking water supplies, and posing a threat to public health. This necessitates a need for research to determine the effectiveness of different water treatment processes for removing algal toxins.

At present, slow sand filtration has shown some potential to remove toxins by biodegradation. However, not all types of toxin were successfully removed. The limited amount of work on ozonation has shown that it is an effective process for removing toxins, even with relatively low ozone doses. Ozonation does produce by-products, but these have been shown to be non-toxic. Activated carbon adsorption has been shown to be capable of effectively removing different toxins. With powdered activated carbon (PAC),[11] high PAC doses were generally required. However, most of the tests were conducted with higher toxin concentrations than would probably be found in raw waters. Granular activated carbon (GAC)[12] can effectively remove toxins, but the studies have not fully assessed the most suitable GAC. In addition, the PAC doses required may be impractical to dose at a treatment works. For GAC adsorption to be effective, contact times of more than 15 minutes should be considered, assuming that microcystin does not biodegrade on the GAC.

Activated Carbon Fiber (ACF) is a recently developed adsorbent which has more superior adsorbing capacity to active carbons. Recently, Chungbuk National University (Chungju, South Korea) has developed a manufacturing process of ACF by using various precursors of fabrics, cottons, viscous rayons and mixed fabrics etc.[13] ACF made by fabrics and cottons has never been used to remove microcystins in water sample. There is no published data available that has investigated water treatment processes using ACF made by fabrics and cottons, In this study we aimed to obtain basic data on the adsorption of the toxic peptide, microcystin LR to the ACF that was prepared in Chungbuk National University. Our future goal would be to identify and develop ACF treatment processes suitable for removal of algal toxins and to provide a basis for estimating potential water treatment usage.

## Experimental Section

The three ACFs, KF1500, HPC10400, NPV55400 were chosen to test the characteristics of microcystin LR adsorption. All these three ACFs were supplied from Chungbuk National University. KF1500 was manufactured by Kuraray–Futamura Chemical in Japan and HPC10400 and NPV55400 were manufactured by Chungbuk National University. Some

**Table 1**. ACFs characteristics

| ACFs | Producing Country | Adsorbed Iodine (mg/g) | BET surface area ($m^2$/g) |
|---|---|---|---|
| KF 1500 | Japan | 1000 | 750 |
| HPC 10400 | Korea | 1580 | 1538 |
| NPV 55400 | Korea | 1890 | 1557 |

physical data for these ACFs were obtained and listed in Table 1. Iodine adsorption data were determined according to AWWA(American Water Works Association) standard procedures. The procedures include that After adding iodine solution on the ACFs, the supernant is titrated by $Na_2S_2O_3$ standard solution. Surface areas were determined with BET analyzer (Micrometrics, ASAP 2400, USA) by measuring adsorption isotherms of liquid nitrogen at 77 K.

Granular activated carbon (GAC) was purchased from Leifheit aqualette Co. (Leifheit, Nassau, Germany) and Octadodecyl silicate (ODS) was purchased from Waters Co. (Milford, MA, USA)

Cultures of *Microcystis aeruginosa* NIES 293 were grown in MA medium under continuous illumination by cool white fluorescent lights. Microcystin LR was purified by the method of Pyo and Lee[14] and stored in methanol at −20 °C until required.

Microcystin RR is a potent hepatotoxin containing arginine and arginine moiety. Microcystin RR was separated from the mixture of Microcystin LR and Microcystin RR.

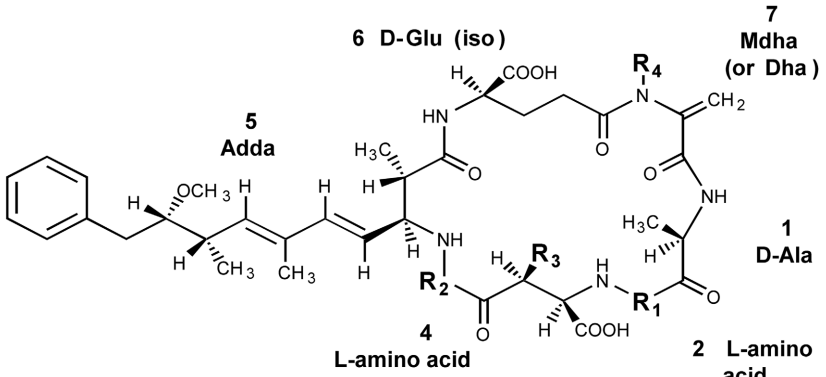
Microcystin analyses were carried out by reverse phase, high performance liquid chromatography (HPLC) with photo-diode array detection at a wavelength of 237 nm. The mobile phase was Methanol + Acetonitrile/0.025 M phosphate buffer (52 : 48) at a flow rate of 1 mL/min. The system used was a Beckmann liquid chromatograph equipped with a Beckmann 116 pump (System Gold programmable solvent module 126), a 10 mm × 15 cm ODS column and Hewlett Packard HPLC 1100 series diode array detector coupled in series. Microcystin content of water samples before and after filtration was determined by trace enrichment on $C_{18}$ cartridge.[15]

To measure an adsorption rate of microcystin LR, the ACF was made with a disk form and put into a 3 mL-vial. Then, an aliquot of 10 $\mu$L from the standard solution of microcystin LR (1 $\mu$g/mL) was injected to HPLC and its response was measured. 1 mL of the standard solution of microcystin LR (1 $\mu$g/mL) was pipetted into the 3 mL-vials containing 31 mg of ACF sdsorbent, then were stirred at constant rate (100 rpm) using magnetic stirrer. At certain time intervals, 30 $\mu$L of the equilibrated solution were sampled, where 10 $\mu$L of the sample solution was injected to HPLC.

For the flow–through experiment, 0.6 mg of ACF was packed in the microcolumn (i.d.: 0.085 mm). The microcolumn was washed by methanol and deionized water. Microcystin LR solution (1 $\mu$g/mL) was passed through with a flow rate of 0.05 mL/min. The solution after passing through the microcolumn was collected and analyzed at the regular time intervals.

## Results and Discussion

To evaluate the removal of microcystins by three kinds of activated carbon fibers, a testing protocol using purified microcystin LR and RR was adopted. All activated carbon fibers tested in this study were found to significantly reduce




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | MW |
|---|---|---|---|---|---|
| Microcystin - LR | Leu | Arg | $CH_3$ | $CH_3$ | 994 |
| Microcystin - YR | Tyr | Arg | $CH_3$ | $CH_3$ | 1044 |
| Microcystin - RR | Arg | Arg | $CH_3$ | $CH_3$ | 1037 |

**Figure 1**. Structure of microcystins. A characterisitic of microcystins and related cyanobacterial toxins is the hydrophobic amino acid Adda which contains in position 5 two conjugated double bonds. Numbers represent the positions of the corresponding amino acid.

**Table 2**. Microcystin LR, RR removal by three kinds of Activated carbon fibers

| ACFs | Removal of microcystin LR (%)[a] | Removal of microcystin RR (%) |
|---|---|---|
| KR 1500 | 39.1 (2.4) | 42.3 (4.2) |
| HPC 10400 | 98.1 (8.2) | 97.2 (6.5) |
| NPV 55400 | 99.5 (7.8) | 99.4 (5.9) |

[a]Relative standard deviation (n = 3) in parenthesis

levels of microcystin LR and RR in water (Table 2). The vials used in this study had a volume of 3 mL and were packed as a disk with three kinds of ACFs (HPC 10400, NPV 55400, KF 1500). Among these, HPC 10400 and NPV 55400 ACFs removed more than 97% of purified microcystin LR, RR which had been added to the water sample. Especially, NPV 55400 showed an excellent results of removing microcystin LR, RR in water sample. It removed almost 99.5% of microcystins (Table 2). In contrast, KF 1500 removed about 39-48% of microcystins in water sample. This result could be explained by the differences of BET surface areas of ACFs. KF 1500 had a small BET surface area compared to the other two ACFs. The BET surface area is an important factor affecting the adsorption capacity of activated carbon fibers. The BET surface areas of ordinary GACs is about 400-700 $m^2/g$ .

The time dependent behaviors of microcystin LR adsorption were measured by varying the contact time between adsorbate and adsorbent in the range of 0–300 min. The amount adsorption of microcystin LR plotted in Figure 2 as a function of contact time. Figure 2 shows that ACF showed the fastest adsorption rate among three adsorbents (ACF, GAC and ODS) tested. The sorption equilibrium of microcystin LR and ACF were attained within 5.0 min, whereas GAC took about 50 min and ODS took more than 200 min. These results indicate that ACF can be a more effective adsorbent than the others. In Y axis, Co means the HPLC peak area of the standard solution of microcystin LR (1 $\mu$g/mL), C means the HPLC peak area of microcystin LR at a

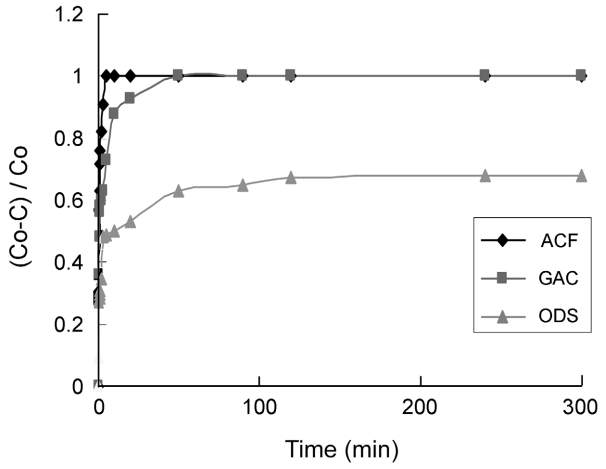



**Figure 2**. The time dependent adsorption behaviours of micricystin LR onto three different adsorbents (ACF, GAC and ODS).

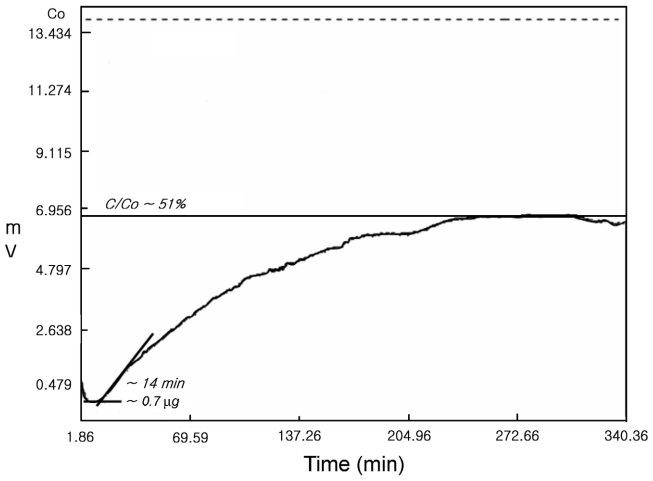



**Figure 3**. Breakthrough Curve of Microcystion LR over ACF ACF was NPV 10400 (0.6 mg), adsorbate was microcystin LR (1 $\mu$g/mL) and flow rate was 0.05 mL/min.

certain time interval and ODS means octadecylsilicate which is the same material as used in the HPLC analytical column.

Figure 3 shows the breakthrough curve of microcystin LR over ACF under conditions of a flow rate of 0.05 mL/min of 1 $\mu$g/mL microcystin LR aqueous solution and 0.6 mg adsorbent. Based on the observation (Fig. 3), it is proposed that the sorption process consisit of two different steps. The first step is the adsorption of microcystin LR to the surface of ACF, while the second step is the adsorption of microcystin LR into the fine micropore of ACF. The results shown in Figure 3 indicated that the amount of microcystin LR to 0.6 mg of ACF by the initial adsorption was 0.7 $\mu$g (14 min $\times$ 0.05 mL/min = 0.7 mL; 0.7 mL $\times$ 1 $\mu$g/mL = 0.7 ug). After that, the adsorption of microcystin LR continued until about 300 min. The amount of sobate mass removed between 14 min and 300 min has to be calculated as follows;

$$sorbed\ mass = \int_{4\ \mathrm{min}}^{300\ \mathrm{min}} (Co - C)dt$$

Where V is the elution volume (time $\times$ flow rate), Co is the influent concentration (1 ug/mL) and C is the effluent concentration. The amount of adsorption was 9.45 $\mu$g. Since the amount of adsorbent is 0.6 ug, the total amount of adsorption by ACF was about 17 $\mu$g microcystin LR/1 mg ACF. Compared to the PAC data of 50 $\mu$g microcystin LR/12 mg PAC,[16] these results show that ACF is a very effective adsorbent for the removal of microcystin LR from the aqeous solution.

## Conclusion

The adsorption of microcystin LR by three different activated carbon fibers (ACFs) was investigated. NPV 55400 and HPC 10400 were clearly very effective microcystin LR adsorbents. KF 1500 was the relatively poor microcystin LR adsorbent. This study has shown that ACF

has a great adsorption ability and ACF can be utilized as an exellent adsorbent to reduce the amount of microcystin LR in water sample. If ACF is to be employed as remedial water treatment during episodes of toxic cyanobacterial blooms, it would show a great removal efficiency.

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