

Adsorption of Microcystin LR and LW on Suspended Particulate Matter (SPM) at Different pH

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Abstract The occurrence of microcystins (MC) during aquatic blooms of cyanobacteria in eutrophicated freshwater body has increasingly become an environmental and health concern worldwide. An experimental study was conducted to investigate the adsorption of two common variants of microcystins, MCLR and MCLW, onto SPM at different pH values. The results showed that most microcystins spiked into an SPM solution with the concentration of 0.25g/l was adsorbed onto solids at pH3 (>95%) and 7 (>85%).

At pH13, the proportion of adsorbed microcystin decreased to 8–29% for MCLR and 38–47% for MCLW. The adsorption of MCLR and MCLW onto SPM fitted well with S type of Freundlich isotherm except for pH3 at which L type of Freundlich isotherm was suitable for MCLW. At pH3 and 7, linear isotherm was also able to describe the adsorption of MCLR and MCLW. The adsorption of MCLR and MCLW decreased significantly with the elevated pH, which is consistent with the pH-dependent hydrophobicity.

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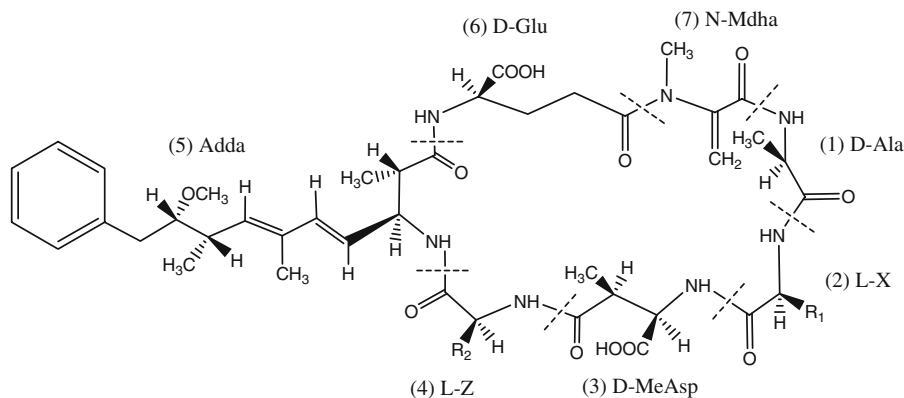
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1 Introduction

Microcystins (MC), a group of monocyclic heptapeptide hepatotoxins, are produced by various freshwater cyanobacteria such as *Microcystis*, *Anabaena*, *Nostoc* and *Oscillatoria* species (Carmichael 1992). There are three D-amino acids, namely alanine (Ala), methylaspartic acid (MeAsp) and glutamic acid (Glu), two unusual amino acids, N-methyldehydroalanine (Mdha) and 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda), and two variable L-amino acids in a MC molecule. The general structure of microcystins is cyclo[–D-Ala–L-X–D-MeAsp–L-Z-Adda–D-Glu–Mdha–] as illustrated in Fig. 1, where X and Z are variable amino acids and

Fig. 1 Typical structure of microcystins (after Carmichael 1992 and Gert-Jan de Maagd et al. 1999). *X* and *Z* represent two variable L-amino acids



give different name to the toxin molecule. To date more than 60 closely related microcystin variants have been reported. Of these variants, microcystin-LR (MCLR) and microcystin-LW (MCLW) are the commonly occurred members of this family of compounds, which could make up between 45.5 and 99.8% of total microcystin concentration in natural blooms (Vasconcelos et al. 1996; Craig et al. 1993; Sano et al. 1998; Sivonen and Jones 1999). The two variable amino acids are leucine (L) and arginine (R) for MCLR while leucine (L) and tryptophan (W) for MCLW, respectively (Carmichael 1992, 1994).

The occurrence of these hepatotoxins during aquatic blooms of cyanobacteria has become an increasingly severe global health hazard. Microcystins are toxic to mammalian liver (Carmichael 1994; Mackintosh et al. 1990) and are also extremely potent tumor promoters (Nishiwaki-Matsushima et al. 1992; Fujiki and Suganuma 1993). Microcystin toxins are reported to be responsible for the deaths of birds, wild animals, agricultural livestock (Carmichael 1992; Rinehart et al. 1994; Mez et al. 1997) and fish (Andersen et al. 1993; Rodger et al. 1994). The effect of microcystins on human health, e.g. severe liver damage, has been recognized when water supplies contain cyanobacteria (Falconer et al. 1983; Yu 1989; Jochimsen et al. 1998; Pouria et al. 1998).

Microcystins are synthesized and retained in cyanobacteria cells, but are released into the surrounding water during bloom senescence and cell lysis. Once released in the water, microcystins, like other water contaminants, will go through a variety of transport and transformation processes in aqueous environment. Previous studies dealing with microcystins have primarily focused on the isolation and

identification, analytical methods, removal technology from drinking water supply, and toxicological effect of microcystins (Carmichael 1992, 1994; Fujiki and Suganuma 1993; Song et al. 2005). Few studies have been carried out to study environmental processes microcystins undergo in aquatic environment (Morris et al. 2000; Hyenstrand et al. 2003). As a result, limited information is available with respect to the fate of microcystins after being released into water environment so far. The microcystin concentration in water may decrease as a result of many environmental processes, such as dilution by uncontaminated water masses, adsorption on particulate material, chemical decomposition, photolysis and biological degradation (Harada and Tsuji 1998). However, microcystins are generally considered to be chemically stable in natural water (Tsuji et al. 1994).

Suspended particulate matter (SPM) in aquatic ecosystem is an important environmental medium because it regulates the transport of all types of water pollutants in dissolved and particulate phases (Håkanson 2006). Adsorption of chemicals on SPM is one of dominant processes determining the fate of pollutants, including microcystins, in water environment. Microcystins adsorbed onto SPM could have limited bioaccessibility to fish and agricultural livestock and thus pose environmental risk to a less extent. During water treatment process, microcystins adsorbed onto SPM could more easily be removed by passing through conventional filtration system, in comparison to the dissolved microcystins the removal of which may require advanced techniques. However, adsorption onto SPM could make microcystins more resistive to chemical and microbial degradation and lead to a longer residence time in water environment. Despite

the importance of adsorption onto SPM, the partitioning of microcystins between water and SPM has not been studied in detail in previous studies. We investigated the adsorption behavior of the abundant hepatotoxins, MCLR and MCLW, in SPM solutions at different pH. The results, including adsorption constants, obtained here will be useful to further investigate and predict the fate of microcystins in aquatic environment.

2 Materials and Methods

2.1 Materials

Microcystin LR standard (MCLR > 98%) was provided by Linda A. Lawton of the School of Applied Sciences, Robert Gordon University, Aberdeen, U.K.. Microcystin LW standard (MCLW > 95%) was purchased from Alexis (Switzerland). Both standard chemicals were used only for calibration curves. Microcystin LR and LW, used for adsorption experiments, were isolated from *Microcystis aeruginosa* FACHB7820 cultured in our laboratory. The strain was purchased from Institute of Hydrophytic Biology, Chinese Academy of Science, Wuhan, China. Methanol was obtained from Fisher (Tianjin, China). The HPLC-quality grade water used in mobile phase was prepared on a Milli-Q filtration system (Millipore, Bedford, MA, USA).

2.2 Extraction of Microcystins

Cultures of the *Microcystis aeruginosa* strain were maintained in a 100-l glass aquarium containing 20l of BG11 growth media at 25°C under white light (2,500lx, 12h light/12h dark). Cultures of microcystin strain were harvested and lyophilized after an incubation period of 6 weeks.

The lyophilized algal cells (33.7g) were extracted with 500ml of methanol–water (75:25) by continuous stirring using an orbital shaker (model THZ82, Jintan, China) at 400rpm for 1h at room temperature. The sample was then centrifuged on a TDL5A centrifuge (Shanghai, China) at 5,000×g for 15min. The extraction procedure was repeated five times. The supernatants combined from all five extractions were pooled and reduced to approximately one-tenth of its initial volume by rotary evaporation (RE52C rotary

evaporator, Shanghai, China) at 50°C and 0.075MPa. The liquid concentrate was centrifuged at 5,000×g for 40min. Then the microcystin-rich concentrate was applied to a preconditioned homemade C18 cartridge (15 × 2.0cm) at 1ml/min. The preconditioning steps included washing with 50ml of neat methanol and followed by 50ml of 20% aqueous methanol. The loaded cartridge was washed with 50ml of 20% aqueous methanol, after which the microcystins were eluted using 30ml of 75% aqueous methanol at 1ml/min. The sample was analysed for microcystin contents (38.3mg/l for MCLR and 64.4mg/l for MCLW) and stored at –18°C for adsorption experimental use. Further purification of this crude extract was not attempted because the application of crude extract, rather than pure microcystins, would mimic the realistic environmental conditions more closely.

2.3 Analysis of Microcystins

The analysis of MCLR and MCLW was conducted using high performance liquid chromatography (HPLC). The Waters HPLC system consisting of a binary pump (Model 1525) and dual wavelength UV detector (Model 2487) was controlled by a personal computer using Waters Millennium³² software. The samples were injected in a 20µl loop connected with a Suntek Kromasil-C18 analytical column (250 × 4.6mm) which was isocratically eluted with methanol–water (70:30) at 1ml/min. The wavelength of UV detector was set to 238nm and the range of sensitivity was adjusted to 2.0.

2.4 Preparation of SPM Solution

A sediment collected from a lake located in Nankai University campus was used for preparation of SPM solution. After the sediment was air-dried, a size-fraction (<0.064mm) was separated by dispersing and sifting the sediment through a sieve of 200 mesh. An appropriate amount of sifted particulate matter was weighed and thoroughly dispersed in distilled water to prepare a suspension of 0.25g of SPM in 1 liter distilled water 24h before the adsorption experiment. In order to inhibit the growth of microbe 0.1g of sodium azide (analytical grade) was added into the suspension. The organic carbon (OC) content of the sediment used was 3.2%, determined by potassium dichromate–sulfuric acid oxidation method.

2.5 Adsorption Experiment

The prepared SPM solution was divided into three aliquots which were calibrated to the selected pH (3, 7 or 13) with NaOH or HCl. The adsorption experiments were carried out using 5-ml polycarbonate ester centrifuge tubes. For each pH, five aliquots of 2ml of suspension were added to five tubes. Then, 0, 0.1, 0.125, 0.15, 0.2, 0.25ml of microcystin-rich extracts were transferred into each tube, respectively. To avoid head-space distilled water was added to give a final volume of 5ml in each tube. The pH of the suspension system was checked again and adjusted if necessary. The final concentration of SPM was 100mg/l for each tube. The initial concentrations of MCLR and MCLW in each tube were calculated from their concentrations in raw extract which were determined separately. Previous studies and our preliminary results showed that the adsorption equilibrium of microcystins onto particulate matter could be achieved in 24h (Huang et al. 2007; Qian 2003). Therefore, after being shaken vigorously for 24h at 25°C, all sealed tubes were centrifuged at 11,000×g for 5min. The supernatants were filtered through cellulose acetate membranes (pore size 0.45µm) and analysed for solution MCLR and MCLW concentrations. The amount of microcystins adsorbed onto SPM was calculated from difference between the initial and equilibrium solution concentration. For each pH, a tube containing 0.2ml of microcystin-rich extracts was served as the control sample without adding SPM. The recoveries for the spiked microcystins in control samples ranged from 84–107% for MCLR and 81–93% for MCLW, indicating significant degradation of microcystins was not occurred during adsorption experiments. All adsorption experiments were carried out in duplicate. The relative percent difference (RPD) between two replicates was always better than 15% so that the average was reported throughout unless otherwise stated.

3 Results and Discussion

3.1 Adsorption Capacity

After adsorption equilibrium (24h), the concentrations and percentages of MCLR and MCLW adsorbed onto SPM at different suspensions pH were illustrated in

Figs. 2 and 3. It can be seen from Figs. 2 and 3 that both MCLR and MCLW showed different adsorption affinities at different pH values. Strong adsorption was observed at acidic (pH = 3) and neutral pH (pH = 7) while weak adsorption at basic condition (pH = 13). Over 95% (96–98%) of microcystins added into the suspension system was adsorbed onto SPM at pH3. The proportion of the adsorbed microcystin decreased slightly at pH7, but still, more than 85% (85–90%) of the spiked microcystin was adsorbed onto SPM (Figs. 2b and 3b). At pH 13, the adsorption of microcystins decreased dramatically. Only 8–29% of the spiked MCLR was adsorbed by SPM while the proportion for MCLW ranged from 38 to 47% at this pH. The differences in proportions of microcystins adsorbed by SPM between different pH values were highly significant ($p < 0.01$, t -test). It appeared that pH played a vital role in the adsorption process of microcystins onto SPM. This was true for MCLR as well as for MCLW in our experiment. It

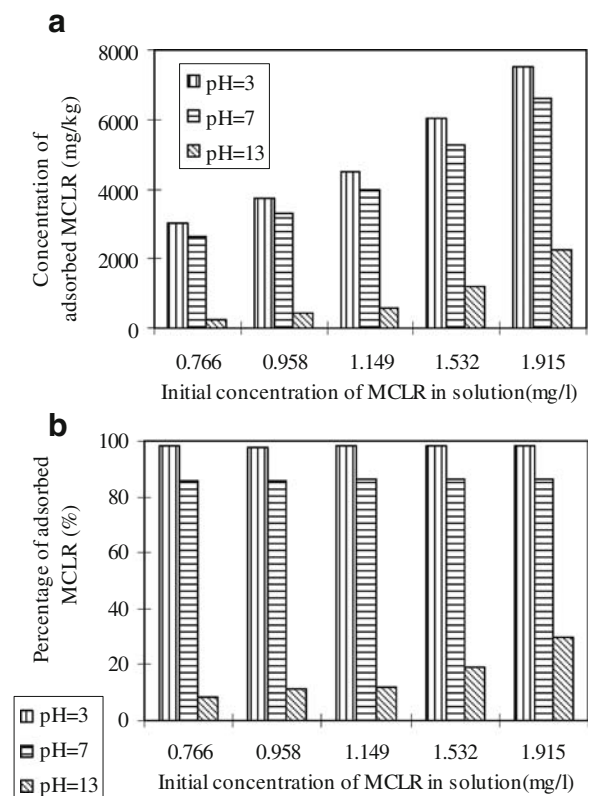


Fig. 2 MCLR adsorption onto suspended particulate matter (SPM) at different pH. The concentration of SPM was 100 mg/l. **a** concentration of MCLR adsorbed; **b** percentage of MCLR adsorbed

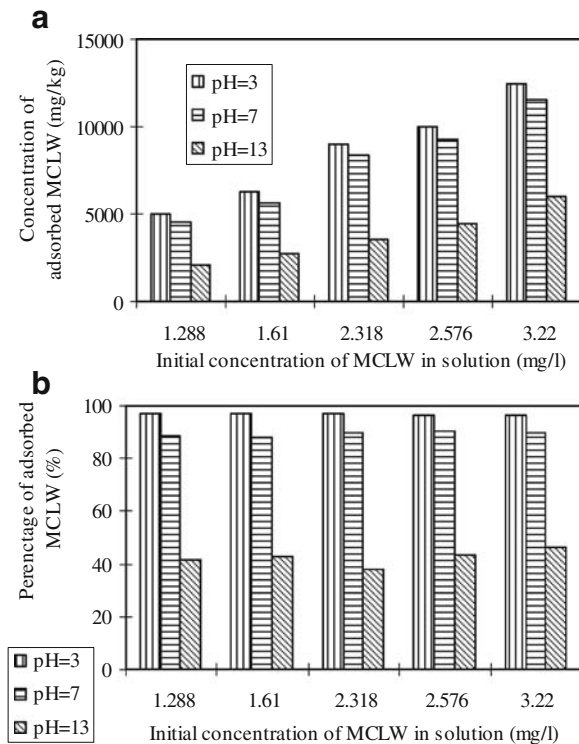


Fig. 3 MCLW adsorption onto suspended particulate matter (SPM) at different pH. The concentration of SPM was 100 mg/l. **a** concentration of MCLW adsorbed; **b** percentage of MCLW adsorbed

seemed that SPM in suspensions with higher pH had lower capacities to adsorb microcystins. This might be due to the alterations in physical or chemical properties of suspension system caused by different pH or, more likely due to the shift in speciation and properties of microcystin itself.

Suspended particulate matter in aquatic environment can significantly affect the fate of toxicants such as organic and inorganic contaminants through the adsorption process. Our results revealed that a considerable proportion of microcystins present in water would be associated with SPM, rather than in the dissolved form. Similar with our findings, Morris et al. (2000) found that more than 81% of MCLR could be scavenged from water by fine-grained particles (<2 and <0.3 μ m) of marine sediment with a high content of clay minerals, such as montmorillonite and kaolinite. However, in another laboratory experiment studying the fate of dissolved MCLR using 14 C-radiolabelled chemicals, Hyenstrand et al. (2003) observed that most of the spiked MCLR remained in the dissolved fraction after the 24-h

incubation period. Although they did observe significant increases in radioactivity in the particle fraction throughout the incubation period, the proportion of the particle fraction MCLR was able to be accountable only for 2–24% of the radioactivity initially introduced. The lower proportion of MCLR in the particulate fraction observed in that study, in comparison to our results, could be ascribed to the differences in suspended particles. We introduced lake sediment, which is believed to be rich in clay mineral and organic matter and to have high capacity to adsorb organic compounds, into the suspension system to produce SPM. The particle fraction in Hyenstrand et al. (2003), however, was referred to photoplankton and zooplankton communities, which could assimilate, rather than adsorb directly, MCLR.

3.2 Adsorption Isotherms

The adsorption isotherms which were widely used to characterize retention of chemicals in soil, sediment, or particulate matter could be constructed based on adding known amounts of adsorbate to adsorbent and measuring the amount of adsorbate left in solution after equilibrium. Giles et al. (1974) classified adsorption isotherms based on their initial slopes and curvatures. They distinguished between high affinity H, Langmuir L, constant partition C, and sigmoidal-shaped S isotherm classes. To account for plateaus, points of inflection, and maxima, they further distinguished between subgroups in each class. Simple equations, such as Linear (C class), the Freundlich (L or S class) or Langmuir (L class) isotherms are commonly used to describe adsorption data, although these equations do not accurately describe the data in some cases.

The linear, Freundlich, and Langmuir adsorption isotherms were expressed as the following equations (Eqs. 1, 2, and 3), respectively.

$$q = K_P \times C = K_{OC} \times OC \times C \quad (1)$$

$$q = K_F \times C^N \quad (2)$$

$$q = q_{\max} \times \frac{K_L \times C}{1 + K_L \times C} \quad (3)$$

In these isotherms, q (mg/kg) is the amount adsorbed per unit mass of adsorbent and C (mg/l) is the solution phase concentration at equilibrium. K_p , K_F , and K_L are linear, Freundlich, and Langmuir adsorption isotherm coefficients related to adsorption affinity, respectively. The linear partition coefficient, K_p , can be normalized to the soil organic carbon content (OC), as K_{OC} . In Freundlich isotherm, N is a constant related to adsorption intensity which ranges from 0.7 to 1.2 in most cases. In Eq. 3 q_{max} (mg/kg) is the Langmuir adsorption maximum.

Both for MCLR and MCLW, the adsorption data could not be fitted with Langmuir equation (very poor correlation, data not shown). At all pH values tested, the adsorption of MCLR and MCLW fitted well with Freundlich or linear isotherms (correlation coefficients $r^2 > 0.90$), except for pH = 13 at which linear isotherm is unable to describe the adsorption of MCLR ($r^2 = 0.4690$) as well as that of MCLW ($r^2 = 0.8481$). Regression data and adsorption parameters obtained for Freundlich or linear isotherms were illustrated in Table 1. It should be noted that the Freundlich isotherm of MCLW at pH = 3 was an L type of isotherm (indicated by $N = 0.88 < 1$), which differed from all other S type Freundlich isotherms (indicated by $N > 1$).

The adsorption isotherms coefficients, K_p or K_F , can be used to characterize adsorption capacities of SPM toward microcystins. Again, it is suggested that adsorption capacities of SPM decreased remarkably with the increasing pH of suspension systems (Fig. 4).

The adsorption behaviour of microcystins onto sediments or suspended solids in aquatic environment has not been extensively investigated so that limited information is available in the literature with respect

Table 1 Isotherm parameters of MCLR and MCLW adsorption onto suspended particulate matter at different pH

pH	MC	Linear isotherm		Freundlich isotherm		
		K_p	R^2	K_F	N	R^2
3	LR	215,079	0.9409	269,631	1.06	0.9414
	LW	117,673	0.9266	87,976	0.88	0.9662
7	LR	24,737	0.9980	26,252	1.04	0.9976
	LW	34,692	0.9366	51,768	1.29	0.9673
13	LR	1,003.3	0.4690 ^a	687.9	3.16	0.9498
	LW	3,038.8	0.8481 ^a	2,889.9	1.10	0.9014

^a Correlations are not statistically significant whereas all others are significant at $P < 0.01$.

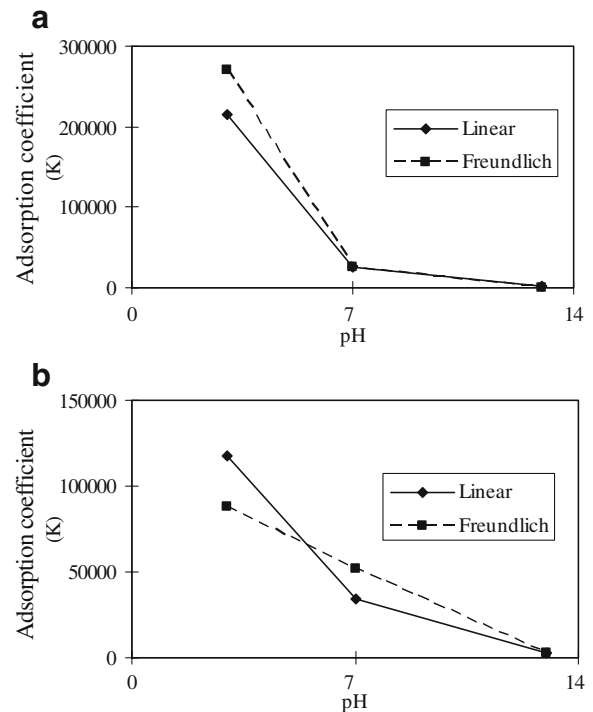


Fig. 4 Linear (K_p) and Freundlich adsorption coefficients (K_F) of **a** MCLR and **b** MCLW onto suspended particulate matter (SPM) at different pH values

to the adsorption isotherms of microcystins so far. Several studies dealing with microcystin adsorption used activated carbon as adsorbent with the purpose of evaluating the removal effect of water treatment processes on microcystins from aqueous solution (Pendleton et al. 2001; Warhurst et al. 1997; Huang et al. 2007). These studies suggested that the adsorption of MCLR onto various activated carbon fitted well with Langmuir isotherm, which is inconsistent with our results. We speculated that the difference in adsorbents, activated carbon versus SPM from natural lake sediment, could be one of possible reasons leading to the different adsorption behaviour of MCLR. The adsorption of MCLR onto activated carbon appeared to be through electrostatic forces, in addition to physical adsorption owing to the high surface area of activated carbon. Support for this came from the fact that activated carbons with higher pH_{zpc} values (the pH at the zero point of charge), which exhibit positive charges under typical pH conditions, had higher capabilities to bind MCLR with negative charges (Huang et al. 2007). The adsorption through electrostatic forces, which is similar to that through ligand exchanges, generally follows Langmuir iso-

therm (Stumm 1992). The adsorption of microcystins onto SPM from lake sediment, which is negatively charged in general, could be via chemical adsorption, such as hydrophobic interactions between microcystin molecules and organic matter in the sediment. These types of adsorption can usually be described by linear or Freundlich isotherm (Stumm 1992).

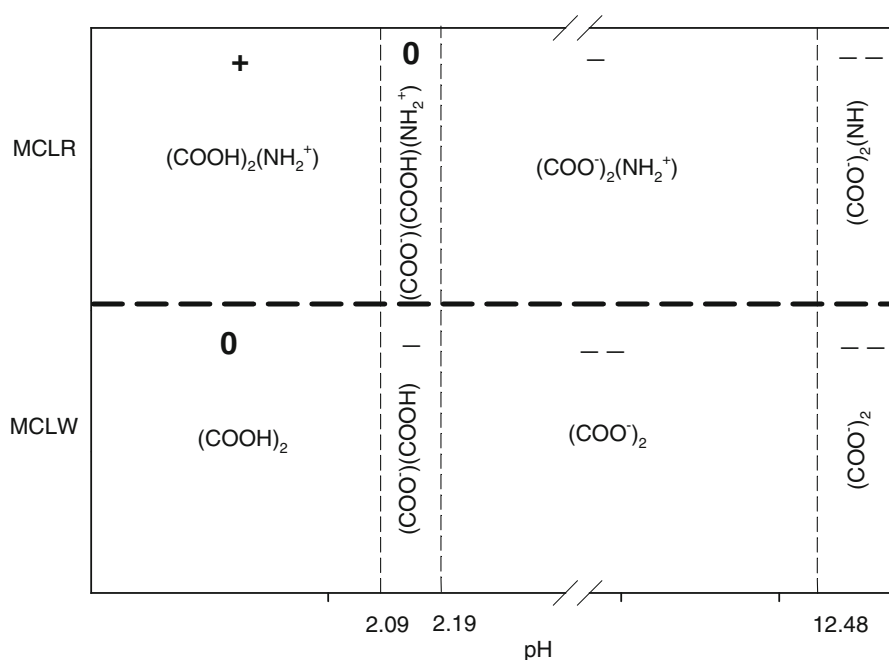
3.3 Adsorption and Hydrophobicity

Since the adsorption of microcystins onto SPM was presumably, at least in part, related to the organic matter, the hydrophobicity would be expected to play an important role in this process. It has been known that for many organic compounds the hydrophobicity, generally expressed as octanol–water partition coefficient (K_{ow}), positively correlates with the tendency to adsorb in the organic matter of sediments, soils, and SPM (Karickhoff et al. 1979; Mackay 1982). A significantly negative correlation between pH and the hydrophobicity of MCLR was clearly observed by Gert-Jan de Maagd et al. (1999), which could be partially accountable for the pH-dependent adsorption we observed in this study. Because multiple MCLR species are present and each contributes to the partitioning between octanol and water (Ward and Codd 1999), Gert-Jan de Maagd et al. (1999) employed octanol–water distribution ratio (D_{ow}),

instead of K_{ow} , to characterize the hydrophobicity of MCLR. They observed that the $\log D_{ow}$ decreased from 2.18 at pH = 1 to -1.76 at pH = 10. With the decrease of hydrophobicity accompanying the increase in pH, the reduced adsorption of MCLR onto SPM was expected. Because of the similarity in structure between MCLR and MCLW, it was expected that MCLW should have similar properties, including the decreased hydrophobicity with the elevated pH.

The pH-dependent hydrophobicity of microcystins seemed to be closely related to their chemical structures. Both MCLR and MCLW contain two ionizable carboxyl groups, one of which is in the D-MeAsp segment and the other is in the D-Glu (Fig. 1). In addition to these two carboxyl groups, MCLR also contains one ionizable amino group in the L-Arg segment (Fig. 1). These carboxyl and amino groups are not part of peptide bonds making up the cyclic peptide structure so that they can be protonated or deprotonated to make MCLR positively or negatively charged depending on the pH (Rudolph-BoÈhner et al. 1994). Based on the measurement in corresponding free amino acids, the pK_a -values of carboxyl in D-MeAsp and D-Glu and amino group in L-Arg are 2.09, 2.19 and 12.48, respectively (Gert-Jan de Maagd et al. 1999). Therefore, both for MCLR and MCLW, different species are dominant at different pH values (Fig. 5). The neutral species with no net charge would

Fig. 5 Dominant species and net charge of MCLR and MCLW at different pH values. *Plus sign* Singly positively charged; *0* zero charged; *negative sign* singly negatively charged; *double negative sign* doubly negatively charged



be present at $\text{pH} < 2.09$ for MCLW while at a narrow pH range between 2.09 and 2.19 for MCLR. This neutral species may be expected to have the highest hydrophobicity and subsequent tendency to partition into organic matter. At higher pH (> 2.19), both carboxyl groups could be deprotonated, which produces more negative charges, and therefore the hydrophobicity of microcystins would be decreased with the elevated pH. However, it should be borne in mind that (1) the $\text{p}K_{\text{a}}$ -values for these functional groups may vary when amino acids containing these groups are incorporated in a peptide, including microcystin; (2) other species, in addition to the dominant species, are present at the different pH ranges in water. The relationship between the hydrophobicity of microcystins and pH will become more complicated when all species are taken into account.

3.4 Environmental Considerations

After being released into the aquatic environment, microcystin could cause contamination to the surrounding water and pose environmental and human health hazard. The physical, chemical, and biological transport and transformation processes occurred in water affect significantly the residence time, stability, and fate of microcystin, which may subsequently influence its bioavailability and toxicity. Unfortunately, the fate of microcystin and its relationship with various biochemical/geochemical processes occurred in particular aquatic ecosystems are not well understood yet. Little is known in respect of the partition of microcystin between water and SPM or sediment nor the chemical/biological availability of microcystin partitioned into solids.

The research results in this paper, in addition to those reported in few publications (Morris et al. 2000), clearly showed that microcystins were adsorbed strongly onto sediment and SPM in the natural pH range (6–9) at which cyanobacteria may flourish (Carmichael 1994). This has important implications for the environmental fate of these compounds once they are released into natural waters. The adsorption onto SPM can facilitate the movement of microcystin because SPM is fine in particle size and can remain in suspension for a long period. This facilitate transport becomes more important taking into account the fact that the

adsorption onto SPM also can protect microcystin from chemically or microbially mediated degradation which occurs primarily in the water column (Christoffersen et al. 2002). The adsorption process could reduce the bioavailability of microcystins to aquatic organisms, such as phytoplankton and zooplankton, and thus prevent them from entering the food web. This might mitigate the environmental and health risk of microcystins. However, the reduced bioavailability and subsequent toxicity by adsorption onto suspended solids appeared to be questionable in some cases. In addition to direct uptake from water, consumption of particles could be another pathway for microcystin to enter the food web. Recent studies have shown that microcystins, even bound onto particles, can be assimilated and accumulated by various aquatic invertebrates such as zooplankton (Ferrão-Filho et al. 2002; Thostrup and Christoffersen 1999; Hyenstrand et al. 2003).

4 Conclusion

Both for MCLR and MCLW, a considerable proportion of microcystin (over 95% at pH 3 and 85 at pH 7) was adsorbed onto solids in an SPM solution with the concentration of 0.25 g/l. At pH 13, the proportion of adsorbed microcystin decreased to 8–29% for MCLR and 38–47% for MCLW, respectively. The adsorption isotherm of MCLR and MCLW onto SPM fitted well with linear and/or S type of Freundlich equation, except for pH=3 at which L type of Freundlich isotherm was suitable for MCLW. The adsorption of MCLR and MCLW decreased significantly with the increasing of pH, indicated by the reduction in amounts of adsorbed microcystin as well as in adsorption isotherm coefficients (K_{P} or K_{F}). The pH-dependent adsorption behaviour of microcystins was consistent with the pH-dependent hydrophobicity.

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