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pH effect on adsorption of saxititoxin on powdered activated carbon

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PH EFFECT ON ADSORPTION OF SAXTITOXIN ON

POWDERED ACTIVATED CARBON

by

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ABSTRACT

The presence of algal toxins, or cyanotoxins, in surface water has been increasingly reported. These highly hazardous toxins are potential threat to both human health and animals by contaminating drinking water sources over the world. In this study, the removal efficiency of saxitoxin from water using powered activated carbon was tested at different pH values for deionized water and natural surface water. The results of this study showed that pH has a strong impact on removal efficiency of saxitoxin using powdered activated carbon. Specifically, the removal efficiency of saxitoxin is significantly increased at higher pH from 5.7 to pH 10.7. This is explained by chemical speciation of the saxitoxin with changing pH. Natural organic matter in natural water was shown to exhibit significant adsorption competition with saxitoxin at the neutral pH.

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1. REVIEW OF LITERATURE

1.1 CYANOBACTERIA AND CYANOTOXINS

In the past two decades, algal blooms were reported frequently over the world and a variety of algal toxins have been identified. These algal toxins are produced by cyanobacteria which are also referred as blue-green algae due to their capability of photosynthesis. Cyanobacteria are found worldwide in water bodies either used as drinking water resource or for recreational purposes and cause great concern for water quality through contamination of water supplies. They are not only responsible for taste and odor issues of drinking water, but they also release toxic compounds, which are known as cyanotoxins or algal toxins, to surface water. Cyanobacteria grow and produce toxins in surface waters throughout the world, particularly under eutrophic conditions, but also in the metalimnion of deep mesotrophic reservoirs. Many of these surface waters are used for drinking water production. Thus, presence of these cyanobacteria and their toxins in the drinking water source poses a great threat to long-term population health.

The most frequently found cyanobacteria are microcystis, anabaena and planktothrix strains (Sivonen, 1990). Cyanobacteria produce hepatotoxic cyanotoxins, such as microcystins and cylindrospermopsins, and neurotoxic cyanotoxins that consist mainly of anatoxins and saxitoxins. These cyanotoxins are widely considered as a potential hazard to human health and other marine animals. Among the four major groups of algal toxins, microcystin-LR (MC-LR) is the most frequently occurring structural variant of microcystin. World Health Organization (WHO) derived a provisional

guideline value of 1 µg/L for daily exposure to this hepatotoxic cyanotoxin (WHO, 1998). The National Health and Medical Research Council of Australia set a guideline value of 1.3 µg/L in drinking water (NHMRZ/ARMCANZ, 2001), based on all MC-congeners as MC-LR equivalents. Saxitoxin (STX) is the most toxic agal toxin with an acute oral LD_{50} of 531 µg/kg body weight of mice (Watts, et al., 1966; ICPS, 1984). Although the study on saxitoxin is not sufficient to help setting a statutory guideline yet, an informal guideline of 3 µg/L saxitoxin in drinking water has been adopted in Australia (NHMRC, 2001; Orr et al., 2004). In New Zealand, a maximum saxitoxin concentration of 1 µg/L in drinking water is currently required by New Zealand Ministry of Health (Orr et al., 2004).

1.2 SAXITOXIN

Among all the major algal toxins, saxitoxin poses one of the most serious threats to human health due to extreme toxicity. Saxitoxin is commonly associated with marine dinoflagellates and cyanobacteria. Three genera of marine dinoflagellates that produce saxitoxin are *Alexandrium*, *Gymnodinium*, and *Pyrodinium* (Pomati et al., 2001; Shimizu, 1977; Harada et al., 1982; Oshima et al., 1987) and the species of cyanobacteria that can produce saxitoxin include *Aphanizomenon flos-aquae*, *Anabaena circinalis*, *Lyngbya wollei*, and *Cylindrospermopsis raciborskii* (Pomati et al., 2001; Alam et al., 1973; Humpage et al., 1994; Carmichael et al., 1997; Lagos et al., 1999).

Saxitoxin is a kind of paralytic shellfish poisoning (PSP) toxin, which may cause neurological illness. Record of PSP as a public health problem dates back to 1793 (Indrasena et al., 1999; Prakash et al., 1971). It acts by blocking voltage-gated sodium

channels of mammalian nerves, skeletal muscle fibers and most cardiac fibers (Okumura, 2005; Kao, 1993), and thus preventing the signal conducting between neurons, which has a final result of muscular paralysis or death due to respiratory failure (Pereira et al., 2004; Baden and Trainer, 1993; Pomati et al., 2001; Catterall, 1980; Strichartz, 1981). Lately it has also been proven that saxitoxin can block Ca^{2+} (Su et al., 2004) and K^+ (Wang et al., 2003) channels in heart cells. Symptoms of saxitoxin toxication usually include numbness, nausea, vomiting, ataxia, dizziness, and complete paralysis which results in death due to a respiratory failure (Lefebvre et al., 2004; Kao et al., 1967; Hughes and Merson, 1976; Zwahlen et al., 1977). There are still no known antidotes to saxitoxin (Indrasena et al., 1999). Saxitoxin caused death of wild and domestic animals has been reported in several countries (Pereira et al., 2004; Sawyer et al., 1968; Bowling, 1992; Negri et al., 1995; Pomati et al., 2000).

Saxitoxin has been reported in fresh and brackish water in many countries including Danish (Kaas and Henriksen, 2000), Thai (Kungsuwan, 1997), Brazil (Molica et al., 2005), Venezuela (Sevcik et al., 2002), Paraguay (Sevcik et al., 2002; Sevcik et al., 1993), Bangladesh (Zaman et al., 1997), Australia (Humpage et al., 1994; Negri et al., 1997), and USA (Carmichael et al., 1997). The concentrations of saxitoxin in the aforesaid countries have a range of 5 to 3,400 μg saxitoxin equivalents/g dry weight of cells and saxitoxin concentration in water is over 15 μg/L.

Saxitoxin is very water soluble and stable in surface water. Rositano et al. (2001) showed that saxitoxin was not susceptible to ozonation. Ozonation or treating with hydrogen peroxide is not efficient to remove saxitoxin from drinking water (Orr et al.,

2004). In the same study, Orr et al. found that saxitoxin can be effectively removed from water with granular activated carbon (GAC).

Saxitoxin molecular has several amine groups that may either lose or get protons depending on different pH. As a result, up to 10 different species of saxitoxin may present based on the pH of the solution (Hilal et al., 1995). And of all these species, only neutral species of saxitoxin can be adsorbed easily by activated carbon. This suggests that the removal efficiency of the saxitoxin by activated carbon would be affected by changing water pH. Therefore, systematic investigation of pH impact on the removal of saxitoxin using activated carbon is important and should greatly benefit the drinking water treatment industry.

1.3 ADSORPTION OF SAXITOXIN ON ACTIVATED CARBON

Adsorption processes are usually used in drinking water treatment to remove the compounds that cause taste and odor issues, synthetic organic chemicals such as herbicides and pesticides, color-forming organics, disinfection byproducts and some other dissolved organics in water. Inorganic constituents including perchlorate, arsenic, and some heavy metals, can also be removed from water by adsorption. Activated carbon is widely used as primary adsorbent material in the adsorption process for drinking water treatment.

Adsorption mechanisms include physical adsorption and chemisorption. Physical adsorption is the most common type of adsorption mechanism, and it is usually used to remove organics from water in water treatment. Water is polar solvent and activated carbon is non-polar material, which has an affinity for non-polar organics in water, so

neutral organics can be easily adsorbed on the surface of activated carbon. However, when an organic compound either gets or loses a proton and thus is either positively or negatively charged, it becomes more polar and poorly be adsorbed by non-polar activated carbon. Speciation of saxitoxin in the water can be changed by changing water pH and the molar fraction of neutral species of saxitoxin is changed due to the change of water pH. So the removal efficiency of saxitoxin using activated carbon in water may be affected by different water pH.

In this study, adsorption isotherm experiment was done to compare the adsorption capacity of powdered activated carbon for saxitoxin at different water pH. Freundlich adsorption isotherm was used to demonstrate the adsorption results at equilibrium. Freundlich adsorption isotherm was originally proposed by Heinrich Freundlich in 1906 as an empirical equation, which is used to describe the data for heterogeneous adsorption. It's a curve that relates the concentration of a solute on the surface of an adsorbent at adsorption equilibrium, to the equilibrium concentration of the solute in the liquid with which it is in contact.

The Freundlich adsorption isotherm is mathematically expressed as

$$q_e = K C_e^{1/n}$$

where K = Freundlich adsorption capacity parameter, $(mg/g)(L/mg)^{1/n}$

$1/n$ = Freundlich adsorption intensity parameter, unitless

q_e = μg STX/g PAC at equilibrium

C_e = equilibrium concentration of STX, $\mu g/L$

The linear form of this equation is

$$\log (q_e) = \log (K) + (1/n) \log (C_e)$$

The parameter K was used as an indicator of adsorption capacity of powered activated carbon for saxitoxin in this study.

2. GOAL AND OBJECTIVES

The overall goal of this study is to determine the removal efficiency of saxitoxin by powdered activated carbon at different water pH in deionized water and surface water, respectively. Specific objectives of this study are as following:

1. Evaluate the adsorption efficiency of saxitoxin on powdered activated carbon at different pH in deionized water and surface water, respectively.

2. Develop the adsorption isotherm model for saxitoxin at different pH in deionized water and surface water, respectively.

3. Evaluate the adsorption competition of natural organic matters in surface water at different water pH.

4. Compare the adsorption efficiency of three different powered activated carbons for saxitoxin at pH 8.2 in deionized water.

PAPER

PH EFFECTS ON THE ADSORPTION OF SAXITOXIN BY POWDERED ACTIVATED CARBON

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ABSTRACT

The presence of cyanobacterial toxins, or cyanotoxins, in surface water has been increasingly reported. These highly hazardous toxins are the potential threaten to both human health and animals by contaminating drinking water sources over the world. In this study, the removal efficiency of saxitoxin from water using powered activated carbon was tested at different pH values for deionized water and natural surface water. The results of this study showed that pH has a strong impact on removal efficiency of saxitoxin using powdered activated carbon. Specifically, the removal efficiency of saxitoxin is significantly increased at higher pH from 5.7 to pH 10.7. This is explained by chemical speciation of the saxitoxin with changing pH. Natural organic matter in natural water was shown to exhibit significant adsorption competition with saxitoxin at the neutral pH.

Keywords

1. INTRODUCTION

In the last two decades, a variety of cyanobacterial toxins (also known algal toxins) have been identified. These cyanobacterial toxins are produced by cyanobacteria which are also referred as blue-green algae due to their capability of photosynthesis. Cyanobacterial toxins are widely considered as a potential hazard to human health and other marine animals. Cyanobacteria grow and produce toxins in surface waters throughout the world, particularly under eutrophic conditions, but also in the metalimnion of deep mesotrophic reservoirs. Many of these surface waters are used for drinking water production. Thus, presence of these cyanobacteria and their toxins in the drinking water source greatly threaten human health.

The most frequently found cyanobacteria are microcystis, anabaena and planktothrix strains (Sivonen, 1990). The major cyanobacterial toxins include microcystins, saxitoxins, anatoxins, and cylindrospermopsin. Among these four groups of cyanobacterial toxins, microcystin-LR (MC-LR) is the most frequently occurring structural variant of microcystin. The World Health Organization (WHO) derived a provisional guideline value of 1 μg/L for daily exposure to this hepatotoxic cyanobacterial toxin (WHO, 1998). The National Health and Medical Research Council of Australia set a guideline value of 1.3 μg/L in drinking water (NHMRZ/ARMCANZ, 2001), based on all MC-congeners as MC-LR equivalents. Saxitoxin (STX) may be the most toxic of the cyanobacterial toxins with an acute oral LD_{50} of 531 μg/kg body weight

(Watts et al., 1966; ICPS, 1984). Although the study on STX is not sufficient to help set a statutory guideline, an informal guideline of 3 µg/L STX in drinking water has been adopted in Australia (NHMRC, 2001; Orr et al., 2004). In New Zealand, a maximum STX concentration of 1 µg/L in drinking water is currently required by New Zealand Ministry of Health (Orr et al., 2004).

STXs have been reported in fresh and brackish water in many countries including Danish (Kaas and Henriksen, 2000), Thai (Kungsuwan, 1997), Brazil (Molica et al., 2005), Venezuela (Sevcik et al., 2002), Paraguay (Sevcik et al., 2002; Sevcik et al., 1993), Bangladesh (Zaman et al., 1997), Australia (Humpage et al., 1994; Negri et al., 1997), and USA (Carmichael et al., 1997). The concentrations of STX in the aforesaid countries have a range of 5–3,400 µg STX equivalents/g dry weight of cells, and STX concentrations in water of over 15 µg/L.

STXs are both stable and soluble in water. Rositano et al. (2001) showed that STXs were not susceptible to ozonation. Ozonation alone and the ozone/peroxide advanced oxidation process was not effective at removing STX from drinking water (Orr et al., 2004). In the same study, Orr et al. found that STX can be effectively removed from water with granular activated carbon (GAC).

Adsorption mechanisms for STX include both physical adsorption and chemisorption. Physical adsorption is the most common type of adsorption mechanism, and most often controls the remove neutral organics via carbon adsorption from water in water treatment. However, when an organic compound has cationic or anion character, it becomes more polar, and may be more poorly adsorbed by activated carbon.

The molecule structure of the STX has several amine groups that may potentially gain protons and become cationic depending as a function of pH (Figure 1) (Hilal et al., 1995). As a result, up to 10 different species of STX exist depending on the pH of the solution (Hilal et al., 1995). Species 1 and 2 are the two neutral species of STX , which would most likely have the highest adsorption onto most activated carbons based on the physical adsorption mechanism. The two neutral species predominate at pH levels above 8.8. From pH 7.0–8.8, the monocationic species 3 and 4 predominate, whereas di-, tri- and tetra-cationic species predominate below pH 7.0.

Due to the speciation behavior of STX, it was hypothesized that pH would have a strong effect of the treatability of STX in drinking water treatment. The purpose of this research was to test this hypothesis and to determine quantitively the effect(s) of pH on sorption capacity, and to provide guidance to the water industry with respect to STX treatability.

In this study, the sorption of STX by powdered activated carbon (PAC) was investigated at different pH levels in both deionized water and surface water at a variety PAC doses. The effect of pH on equilibrium, and on the kinetics of adsorption, was investigated, as was the impact of natural organic matter (NOM) for the STX adsorption. The adsorption efficiency of three different kinds of powdered activated carbon was also compared.

2. MATERIALS AND METHODS

2.1 Chemicals and standard preparation

HPLC-grade acetic acid, HPLC-grade acetonitrile, A.C.S.-certified grade ammonium phosphate (dibasic), phosphoric acid, and sodium phosphate (dibasic) were purchased from Fisher Scientific (Pittsburgh, PA, USA). A.C.S.-reagent grade periodic acid, sodium 1-heptanesulfonate were purchased from Sigma-Aldrich (St. Louis, MO, USA). The STX standard solution in 0.003M HCl was purchased from the Institute for Marine Biosciences (National Research Council of Canada, Ottawa, Ontario, Canada). Deionized (DI) water was produced with a Millipore Simplicity 185 water system (Billerica, MA). The surface water was collected from Bray Pond (Rolla, MO, USA), and was filtered through 0.45-μm filter and stored at 4°C for later use.

STX standard solutions for calibration were prepared by the dilution of stock standard solution with 0.05 M acetic acid. They were all stored in amber vials in the dark at 4°C. Three activated carbons were studied, specifically: WPH (Calgon Carbon Corporation), HydroDarco B (NORIT Americas Inc.), and Aqua Nuchar (Meadwestvaco Corporation).

2.2. HPLC detection of STX

A post-column derivatization liquid chromatography with fluorescence detection (FLD) method was used for analysis of the cyanobacterial toxins detection following a published method by Yasukatsu (1995) with some modifications. High pressure liquid chromatography (HPLC)/FLD was conducted using a Waters[TM] (Milford, MA, USA) HPLC system including 717 plus Autosampler, 600 Controller, 2475 Multi λ

Fluorescence Detector, and a computer with Empower software. The HPLC column was a reverse phase Keystone Betabasic-C8 column, with dimension of 250×4.6 mm and particle size 5μm (Thermo Electron Corporation). The post-column derivatization setup including a Shimadzu Model LC-10AD pump with modified flow path for delivering both oxidation reagent and acidifying reagent simultaneously. The post column reaction tubing was a 10-m Teflon tubing with 0.5 mm i.d. The reaction tubing was kept in a water bath held at a temperature of 80°C. Due to the instability of the periodic acid in the post-column derivatization solution, a cooler with ice was used to keep this reagent at low temperature.

HPLC mobile phase contained 30 mM ammonium phosphate with 2 mM sodium 1-heptanesulphonate as ion pairing reagent, at pH 7.1, plus 4.8% acetonitrile. It was filtrated through 0.45-μm membrane filter after preparation.

For post-column derivatization, the oxidizing reagent was 7 mM periodic acid in 50 mM sodium phosphate buffer at pH 9.0. The acidifying reagent was 0.5 M acetic acid aqueous solution. Both of oxidizing reagent and acidifying reagent were filtrated through 0.45-μm membrane filter.

The operational conditions were as follows: sample injection volume was 50 μL; separation mobile phase flow rate was 0.8 mL/min; both oxidation reagent and acidifying reagent flow rates were 0.4 mL/min; and post-column reaction temperature was 80°C in water bath. The FLD detector excitation wavelength was 330 nm, and the emission wavelength was 390 nm.

2.3. Adsorption Experiments

The PAC was dried in an oven at 105°C for overnight prior to use. 800 mg/L PAC stock suspension solution was prepared by stirring the PAC in D.I. water for at least 20 minutes. The water samples were buffered with 10 mM phosphate to pH 5.7, pH 7.5, pH 8.2, and pH 10.7. The adsorption experiments were initiated by adding 200 μL of 1.25 mg/L STX stock solution and 8.8 mL buffered water solution to 12-mL glass vials. Next, a total volume of 1 mL of PAC suspension solution and D.I. water were added into each treatment vial to make 10 mL total final volume with 25 μg/L STX and different dosage of PAC. The vials were quickly placed in LABQUAKE tumbler clips, and tumbled continuously at 8 rpm in a temperature controlled chamber at 22°C. To protect STX from the photo degradation, the tumbler with the adsorption samples was covered with a box. Aliquots of 1.5 mL of samples were taken from each vial at specifed times and transferred into centrifuge tubes. After centrifugation at 1000 rpm for 5 minutes to remove the PAC, the clear supernatant was then transferred into HPLC autosampler vials, and stored at 4°C until HPLC analysis (conducted within 72 hours of sample collection).

Short-term adsorption experiments were conducted to study the adsorption rate and efficiency of STX with different dosages of PAC, at different pH levels, within a period of 24 hours. PAC dosages of 0, 1, 2, 5, 10, 20, 40, 80 mg/L, and sampling times of 0, 0.5, 1, 2, 4, 7, 24 hours, were used in the short-term adsorption experiments. Adsorption of STX by PAC was tested in buffered water at pH 5.7, 7.05, 8.2 and 10.7.

The equilibrium adsorption experiments were conducted with a 5-day duration to study the equilibrium capacity of PAC for STX at different pH levels. The appropriate PAC dosages for each pH was determined based results from short-term adsorption

experiments. For pH 5.7, the PAC doses were 0, 20, 30, 40, 50, 65, and 80 mg/L; for pH 7.05 and pH 8.2, the PAC doses were 0, 0.5, 1, 2, 4, 7, and 10 mg/L; for pH 10.7, the PAC doses were 0, 0.5, 0.75, 1, 1.5, 2.5, and 4 mg/L. Samples were taken at pre-selected times of 2 hours and 5 days.

The experiments to compare different PAC types on the adsorption efficiency were conducted using WPH, HydroDarco B, and Aqua Nuchar. For these experiments, PAC dosages of 0, 1, 2, 5, 10, 20, 40, and 80 mg/L, and a sampling time of 2 hours, was used.

2.4. Total organic carbon detection

TOC concentrations of samples were measured using a SHIMADZU TOC-5000A Total Organic Carbon Analyzer (Columbia, MD, USA). At set time of each adsorption isotherm experiment, 1.5 mL of sample was taken from each treatment vial and was centrifuged at 1000 rpm for 5 minutes to precipitate PAC. Then 1 mL of supernatant was transferred to TOC tube and diluted to 40 mL with D.I. water for TOC analysis using TOC analyzer.

3. RESULTS AND DISCUSSION

3.1. Deionized water systems

The removal efficiency of STX with PAC was first tested using deionized water to exclude other factors in water that may affect the experimental result. This test was done at four different pHs. Water pH was controlled by buffered with 10 mM phosphate. For

each pH, PAC dosages ranging from 1 mg/L to 80 mg/L were used to determine STX removal efficiency for different PAC dosages.

For laboratory deionized (D.I.) water, the removal efficiency of STX by PAC was poor at low pH, and increased significantly at increasing water pH (Figure 2), even with long contact time of 24 hours. By contrast, typical PAC contact times in drinking water treatment are commonly 2–4 hours. At pH 5.7, STX removal was less that 20% even for PAC dosages up to 40 mg/L (Figure 2a). At pH levels of 7.05, 8.2, and 10.7, increasing STX removal was observed. For a 4-hour contact time, and typical source water pH levels of 7.05 and 8.2, typical taste-and-odor PAC dosages of 1–2 mg/L, only provided <20% STX removal, while at higher dosages of 10 mg/L >50% STX removal was achieved (Figures 2b and 2c). For a 4-hour contact time and a pH of 10.7 (as may occur in lime-softening operations), even greater removals were observed. Specifically, with a low PAC dosage of 2 mg/L (and 4-hour contact), 30% removal was observed, while a higher dosage of 10 mg/L resulted in approximately 90% removal (Figure 2d). These results are explained by the shift of the predominant net charge on STX from cationic forms (e.g., species 3 and 4) at neutral pH, the neutral forms at higher pH (e.g., species 1 and 2) (Figure 1). Specifically, at pH 5.7, the neutral species of STX present in water is very low, and the dominant species is the di-cationic species 5 (Figure 1). At pH 7.05, the neutral forms of STX account for less than 5% of the species, rising to about 30% at pH 8.2 (Figure 1). At pH 10.7, essentially all of the STX is in one of the neutral forms (Figure 1). These results confirm the hypothesis that pH plays a significant role in removal of STX using PAC.

3.2. Natural water systems

The effects of competition with NOM were investigated using natural water collected from Bray pond (in Rolla, MO, USA) in May 2006. The water pH was controlled by buffering with 10 mM phosphate. The dissolved organic carbon (DOC) concentration of the water was 28 ±2 mg/L as C. This represents a relatively high DOC level for a drinking water source. After five days of incubation at 22°C (with no PAC present), the DOC decreased due to biodegradation by 27, 53, 20, and 0% at pH levels, of 5.7, 7.05, 8.2, and 10.7, respectively.

For pH 5.7, 8.2, and 10.7 (Fig. 3a, 3c, 3d), the results were similar with the results observed using of D.I. water (described above) suggesting that compounds in the surface water did not affect the adsorption of STX significantly. These trends may be interpreted through the speciation of STX and NOM. At pH 5.7, STX is predominantly di-cationic. NOM, on the other hand, will have a range for ionization states. At pH 5.7, some acidic groups (e.g., carboxylic, 4<pKa<5) will tend to be ionic, while others (e.g., phenolic, pKa>12) will tend to be protonated and neutral (Thurman, 1985). Overall, the NOM should have a net anionic nature. These results can be understood due the counter trends regarding the solubility of STX versus NOM with pH. Specifically, STX becomes less soluble, more hydrophobic, and with higher sorption potential, at higher pH (e.g., >8–9) due to depronotation of amino functionalities and their shifting to neutral forms (Figure 1). On the other hand, NOM becomes less soluble, more hydrophobic, and with higher sorption potential at lower pH (e.g., <4–5) due to shifting of acidic functionalities to non-ionized species.

At pH 5.7, while the NOM would be expected to sorb more strongly at this low pH, STX did not sorb well even in the absence of NOM. Thus, no decrease in the poor sorption of STX was noticeable.

At pH 8.2 and 10.7, NOM is strongly and predominantly anionic in nature. Thus, STX, with its significant neutral character and strong adsorption, is not significantly affected by the low sorption potential of the anionic NOM present.

At pH 7.05, however, STX is more ionic, more soluble and sorbs significantly less than at higher pH (Figure 2) as discussed above. At this same pH, NOM is more anionic, more soluble, and sorbs significantly less than at lower pH. At this pH, therefore, much more competition between STX and NOM can occur, and results in a net decrease in the removal of STX in the presence of NOM. These results suggest that the nature and concentration of NOM can play a significant role in the extent of removal of STX especially at more neutral pH levels. It should be noted that due to the wide range of NOM types and concentrations, very different effects of NOM may be observed depending on the water source. These effects warrant additional research to better understand the effects of NOM type and concentration on STX sorption.

The reduction in the removal of STX in D.I. and Bray Pond water as a function of pH is presented in Figure 4 for a PAC dosage of 20 mg/L and a contact time of 2 hours. For the reasons discussed above, the removal of STX is seen to be similar at pH 5.7, 8.2 and 10.7, while at pH 7.05 the removal is the presence of NOM is significantly less than in D.I. water.

3.3. Adsorption Isotherm

Adsorption rate and adsorption capacity at typical PAC contact times are two major considerations with respect to the efficiency of sorption processes in water treatment. Equilibrium adsorption experiments were also conducted to study the maximum adsorption capacity of PAC for STX at different pH levels in both D.I. and surface water systems.

In our study, the Freundlich adsorption model was used to interpret our adsorption isotherm results. The Freundlich adsorption equation is represented by:

$$q_e = KC_e^{1/n}$$

where K = Freundlich adsorption capacity parameter $((mg/g)(L/mg)^{1/n})$,

$1/n$ = Freundlich adsorption intensity parameter (unitless),

q_e = μg STX/g PAC at equilibrium, and

C_e = equilibrium concentration of STX (μg/L).

The linearized form of this equation is represented by:

$$\log q_e = \log K + (1/n) \log C_e$$

The resulting isotherms for STX in D.I. water and Bray Pond water are presented in Figure 5. The results show that as the pH increases from 5.7 to 10.7, the adsorption capacity of PAC for STX increased by approximately 1.5–2 orders of magnitude in both D.I. and Bray Pond water (Figure 5). This shows that speciation effects with changing pH affects not only the adsorption rate and the adsorption extent at typical PAC contact times, but it is also affects equilibrium (maximum) adsorption capacity of PAC for STX.

3.4. Effect of PAC type

Three different kinds of widely used PACs including WPH (coal base), HydroDarco B (HDB, ignite coal base), and Aqua Nuchar (AN, wood ash base) were examined and compared in this study. The experiments were performed at pH 8.2 with D.I. water system at 22°C.

Wood-based carbons (e.g., AN) tend to have greater surface area, and a macroporous nature (fewer micropores). Lignite coal-based carbons (e.g., HDB) tend to have less total surface area, with a highly microporous nature. Bituminous coal-based carbons (e.g., HDB) tend to have much lower total surface areas, and intermediate mix of macro- and micro-pores (compared with wood- and lignite-coal-based carbons). Table 1 presents data from Jain et al. (2004) on the BET surface areas (m^2/g), iodine number (mg/g), and active surface sites for the three PACs compared in this study. The iodine number is representative of the amount of micropores present in a carbon.

The experimental results showed that the sorption capacity for STX at pH 8.2 were (in decreasing order): HDB < WPH < AN (Figure 6). These results can be understood, first, due to the direct correlation with BET surface areas (Table 1), that is, greater surface areas are able to adsorb greater amount of STX.

4. CONCLUSIONS

This study shows a strong pH dependence of adsorption efficiency of PAC (WPH) for STX in water. For pH at or below 7, the removal efficiency was poor; while for pH 8.2 and 10.7, effective treatment of STX was achieved in both D.I. and natural waters. Specifically, when pH was increased from pH 5.7 to pH 10.7, the adsorption capacities of

PAC for STX in both D. I. water and natural water were increased by about 2 and 1.7 orders of magnitude, respectively.

NOM in natural water has strong adsorption competition with STX at pH 7.05 due to competition effects. At lower and higher pH levels, these competition effects do not manifest themselves in observable differences. Finally, the PAC type has a significant impact on adsorption efficiency for STX with the wood-based Aqua-Nuchar SA showing better removal (at pH 8.2) than coal-based carbons.

These results have significant implications for drinking water treatment. Specifically, STX can be effectively controlled at the typical pH of 8.2 with 10–20 mg/L of PAC with any of the wood-, lignite-coal- and/or bituminous-base-coals studied.

pH was shown to have a highly significant impact on the sorption of STX on bituminous-coal-based WPH, While the effect of pH was not explicitly examined in this study for the wood-based and lignite-coal-based, it is expected that a similar pH dependency would be observed.

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Fig. 1. Molecule structure and possible species of STX in aqueous solution of different pH.

Saxitoxin Species	Net Charge	Position on Saxitoxin Molecule					
		(1)	(2)	(3)	(4)	(5)	(6)
Sp.1	0	$-NH_2$	$-N=$	$-NH_2$	$-NH-$	$-NH_2$	$-N=$
Sp.2	0	$-NH^-$	$-N=$	$-NH_2$	$-NH-$	$-NH_2$	$-N=$
Sp.3	+1	$-NH_2$	$-N=$	$-NH_3$	$-NH-$	$-NH_3^+$	$-N=$
Sp.4	+1	$-NH_2$	$-N=$	$-NH_3^+$	$-NH-$	$-NH_2$	$-N=$
Sp.5	+2	$-NH_2$	$-N=$	$-NH_3^+$	$-NH-$	$-NH_3^+$	$-N=$
Sp.6	+3	$-NH_2$	$-NH^+=$	$-NH_2$	$-NH-$	$-NH_3^+$	$-NH^+=$
Sp.7	+3	$-NH_2$	$-NH^+=$	$-NH_3^+$	$-NH-$	$-NH_3^+$	$-N=$
Sp.8	+3	$-NH_2$	$-N=$	$-NH_3^+$	$-NH-$	$-NH_3^+$	$-NH^+=$
Sp.9	+4	$-NH_2$	$-NH^+=$	$-NH_3^+$	$-NH-$	$-NH_3^+$	$-NH^+=$
Sp.10	+4	$-NH_2$	$-NH^+=$	$-NH_3^+$	$-NH^+-$	$-NH_3^+$	$-N=$

Fig. 2. Removal efficiency of STX using PAC at different pH in D.I. water within 24 hours adsorption. (a) pH 5.7, (b) pH 7.05, (c) pH 8.2, (d) pH 10.7.

Fig. 3. Removal efficiency of STX using PAC at different pH in natural water within 24 hours adsorption. (a) pH 5.7, (b) pH 7.05, (c) pH 8.2, (d) pH 10.7.

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Fig. 4. pH effect on removal efficiency using 20 mg/L PAC for treatment of 2 hours.

Fig. 5. Adsorption isotherm of STX in D.I. water and natural water. (a) lD.I. water system, (b) natural water system.

Fig. 6. Comparison of different types of PAC's adsorption efficiency for STX. (a) 2-hour treatment, (b) 24-hour treatment.

Table 1. Characteristics of three PAC types compared in this study (from Jain et al., 2004).

PAC	Source	BET Surface Area (m²/g)	Iodine Number (mg/g)	Site (meg/g)				
				Total Basic	Total Acidic	Phenolic	Lactonic	Carboxylic
WPH	Bituminous Coal	1027±90	993±84	0.40	0.14	0.04	0.09	0.01
HDB	Lignite Coal	510±33	525±128	1.35	0.21	0.00	0.12	0.09
AN	Wood	1567±254	966±427	0.39	0.56	0.28	0.17	0.11

SECTION

3. CONCLUSIONS

Based on the experimental results of this project, following conclusions can be made:

1. This study showed a strong dependence of adsorption rate of PAC (WPH) for saxitoxin on water pH. The removal efficiency of saxitoxin in either laboratory or natural water was greatly increased by raising water pH. For pH equal to or below 7, the removal efficiency was poor. Less than 70 % saxitoxin was removed in laboratory water with a PAC dose of 20 mg/L for 2 hours and less than 30 % saxitoxin was removed in natural water with a PAC dose of 20 mg/L for 2 hours. For water pH 8.2 and pH 10.7, the removal efficiency with a PAC dose of 20 mg/L for 2 hours reached 80 % and 93 %, respectively.

2. This study also showed a strong dependence of adsorption capacity of PAC (WPH) for saxitoxin on water pH. When pH was increased from pH 5.7 to pH 10.7, the adsorption capacity of PAC for saxitoxin in both laboratory and natural water had been increased by about 2 and 1.7 orders of magnitude, respectively.

3. NOM in natural water has significant adsorption competition with saxitoxin at pH 7.05 for short-term treatment. At pH 7.05, the amount of saxitoxin removed in laboratory water was 2.5 times of the result in natural water, with a PAC dose of 20 mg/L for 2 hours.

4. PAC type has impact on adsorption efficiency for saxitoxin as well. Wood ash base PAC Aqua Nuchar showed a much better adsorption efficiency for saxitoxin compared with PACs WPH and HydroDarco B.

Since the drinking water treating process in industry usually applies a PAC treating period of around 2 hours which can be considered as short-term treatment, it would be very effective and efficient to remove saxitoxin from drinking water by raising pH to between pH 8.2 and pH 10.7, which can increase PAC's adsorption rate for saxitoxin and reduce the adsorption competition from NOM in drinking water. And this study also suggests using wood base PAC Aqua Nuchar for the removal of saxitoxin from drinking water.

APPENDIX A.

PHYSICOCHEMICAL PROPERTIES OF SAXITOXIN

Compound	Saxitoxin
Molecular Structure	
Molecular Formula	$C_{10}H_{17}N_7O_4$
CAS Number	35523-89-8
Molecular Weight	299.29
Solubility	Freely soluble in water
pKa in Water[*]	8.2, 11.3

[*]Australian Research Network for Algal Toxins. http://www.aims.gov.au/arnat/

arnat-0008.htm

APPENDIX B.

CHARACTERISTICS OF WPH ACTIVATED CARBON

WPH is a type of powdered activated carbon produced by CALGON Carbon Corp.. It is specifically designed for the treatment of drinking water, targeting to the removal of taste and odor causing constituents, herbicides, pesticides and other organics in the water. The characteristics of WPH PAC are as below (E. W. Flick, 1991):

PAC Type	WPH
Iodine No., mg/g	800 min
AWWA Modified Phenol Value, g/L	3.5 max
Moisture, As Packed, %	5.0 max
Apparent Density, lbs/cu ft	46 max
Sieve Analysis:Through 100 Mesh	99 min
200 Mesh	95 min
325 Mesh	90 min

APPENDIX C.

HPLC-FLD CONDITIONS FOR SAXITOXIN ANALYSIS

The HPLC-FLD conditions for saxitoxin analysis are as following:

- Column: Keystone Betabasic-C8, 250×4.6 mm, particle size 5 μ.

- Mobile phase: 30 mM ammonium phosphate with 2 mM sodium heptane sulphonate, pH 7.1, plus 5% acetonitrile.

- Mobile phase flow rate: 1 mL/min.

- Post column oxidation reagent: 7 mM periodic acid in 50 mM sodium phosphate buffer, pH 9.0.

- Oxidation reagent flow rate: 0.4 mL/min.

- Post column acidifying reagent: 0.5 M acetic acid.

- Acidifying reagent flow rate: 0.4 mL/min.

- Post column reaction: 10m Teflon tubing, 0.5mm i.d., 80°C in water bath.

- FLD detection: Excitation wavelength 330nm, emission wavelength 390nm.

- Toxin standards were diluted in 0.05M acetic acid aqueous solution.

APPENDIX D.

CHROMATOGRAM OF SAXITOXIN

The chromatogram of saxitoxin is as following. The retention time of saxitoxin is about 19 minutes. For Bray pond water, there are two peaks before 10 minutes, which should be from other organic compounds in the surface water.

APPENDIX E.

STANDARD CALIBRATION CURVES

The response for saxitoxin of same concentration could be different at different water pH, so separate calibration curves were made for each pH of both D.I. water and Bray pond water.

1) D.I. water, pH 5.7:

STX Concentration µg/L	Response Peak Area
1	35077
2	124145
5	446158
10	934674
25	2486733

$y = 102282x - 74264$

$R^2 = 0.9999$

2) D.I. water, pH 7.05:

STX Concentration µg/L	Response Peak Area
1	57434
2	127404
5	392484
10	804294
25	2157236

$y = 87784x - 47172$

$R^2 = 0.9996$

3) D.I. water, pH 8.2:

STX Concentration μg/L	Response Peak Area
1	92339
2	196449
5	564397
10	1150824
25	3156952

$y = 128128x - 69711$

$R^2 = 0.9991$

4) D.I. water, pH 10.7:

STX Concentration μg/L	Response Peak Area
1	81630
2	174521
5	570282
10	1254848
25	3181380

$y = 130078x - 66142$

$R^2 = 0.9998$

5) Bray pond water, pH 5.7:

STX Concentration μg/L	Response Peak Area
1	56445
2	132187
5	452742
10	958592
25	2593147

$y = 106278x - 75370$

$R^2 = 0.9996$

6) Bray pond water, pH 7.05:

STX Concentration µg/L	Response Peak Area
1	110729
2	212332
5	629159
10	1306588
25	2819534.5

$y = 113088x + 43108$

$R^2 = 0.9948$

7) Bray pond water, pH 8.2:

STX Concentration μg/L	Response Peak Area
1	138333
2	280771
5	654798
10	1439474
25	3705430.5

$y = 149332x - 40493$

$R^2 = 0.9995$

8) Bray pond water, pH 10.7:

STX Concentration µg/L	Response Peak Area
1	158639
2	223194
5	776756
10	1563975
25	3954944

$y = 159854x - 39239$

$R^2 = 0.9995$

APPENDIX F.

PROCEDURES FOR ADSORPTION EXPERIMENT

Starting experiment:

1. Prepare PAC stock solution.

    a. PAC was dried in the oven at 105°C over night.

    b. 800 mg/L and 200 mg/L PAC stock solution was made using MQ water in 500 mL glass bottle for later use.

2. PAC stock solution was homogenized with stir–bar on a mixing plate for at least 20 minutes.

3. 200 μL 1.25 mg/L saxitoxin solution and 8.8 mL buffered water was added into 12ml clear glass vials.

Following steps 4-7 were done quickly.

4. PAC stock solution and MQ water with total volume of 1 mL was added to each vial quickly and the vials were capped tightly.

    a. The total volume of reaction vessel after PAC and water addition was 10 ml in order to minimize head space.

5. The vials were placed in tumbler clips and tumbler was turned on.

Taking samples:

6. When 3-5 minutes before the time is up, the tumbler was turned off.

Following steps 10-13 should be done quickly.

7. Vials were taken off the clip and were shaken for 2 seconds before taking samples.

8. 1.5 mL sample from each vial was transferred to 12×75mm clear centrifuge tubes using an Eppendorff pipette.

9. Vials were re-capped tightly and put back onto the tumbler.

10. After all the samples had been collected, tumbler was turned on.

11. Samples were centrifuged for 2 minutes at high speed in the Sero-Fuge II centrifuge.

12. 800 μL supernatant liquid of each sample was transferred to the HPLC autosampler vials.

13. The HPLC autosampler vials were capped and stored at 4°C for later analysis.

Finishing Experiment:

14. The tumbler was turned off and the residual liquid in the tumbling vial and centrifuge tubes was transferred to a glass waste bottle.

APPENDIX G.

ADSORPTION ISOTHERM OF SAXITOXIN

The linear form of Freundlich adsorption isotherm was used to demonstrate the adsorption isotherm results of saxitoxin.

$$\log(q_e) = \log(K) + (1/n)\log(C_e)$$

where   K = Freundlich adsorption capacity parameter, $(mg/g)(L/mg)^{1/n}$

   $1/n$ = Freundlich adsorption intensity parameter, unitless

   q_e = µg STX/g PAC at equilibrium

   C_e = equilibrium concentration of STX, µg/L

1) D. I water, pH 5.7:

Plot of $\log q_e$ vs $\log C_e$ with fit $y = 0.5586x + 2.0665$, $R^2 = 0.9905$.

2) D. I. water, pH 7.05:

Plot of $\log q_e$ vs $\log C_e$ with fit $y = 0.468x + 3.1656$, $R^2 = 0.9921$.

3) D. I. water, pH 8.2:

y = 0.3852x + 3.5902
R^2 = 0.9902

4) D. I. water, pH 10.7:

y = 0.1893x + 4.3258
R^2 = 0.9976

5) Bray pond water, pH 5.7:

The graph shows data points and a linear trend line with:
$$y = 1.0576x + 1.626$$
$$R^2 = 0.9207$$
(x-axis: log Ce, y-axis: log qe)

6) Bray pond water, pH 7.05:

The graph shows data points and a linear trend line with:
$$y = 0.396x + 2.8388$$
$$R^2 = 0.9053$$
(x-axis: log Ce, y-axis: log qe)

7) Bray pond water, pH 8.2:

y = 0.3701x + 3.3687
$R^2 = 0.9745$

8) Bray pond water, pH 10.7:

y = 0.2565x + 3.8348
$R^2 = 0.9512$

In the Freundlich adsorption isotherm equation, 1/n is the Freundlich adsorption intensity parameter, and K is Freundlich adsorption capacity parameter, which can be used as an indicator of adsorption capacity of powered activated carbon. Below is a table of these two parameters derived from the adsorption isotherm results.

Water	D. I. water			
pH	5.7	7.05	8.2	10.7
1/n	0.56	0.47	0.39	0.19
log K	2.01	3.17	3.59	4.33
K	7.46	23.81	36.23	75.94

Water	Bray pond water			
pH	5.7	7.05	8.2	10.7
1/n	1.06	0.40	0.37	0.26
log K	1.63	2.84	3.37	3.83
K	5.10	17.12	29.08	46.06

APPENDIX H.

RESULTS OF NOM REMOVAL BY PAC

During the adsorption isotherm experiment for Bray pond water, TOC concentration of sample was also measured after 2 hours and 5 days treatment. This information can help us understand the adsorption competition of NOM in the natural water. The TOC results are as following:

1) pH 5.7:

2) pH 7.05:

3) pH 8.2:

4) pH 10.7:

APPENDIX I.

RESULTS OF UV_{254} ABSORBANCE

During the adsorption isotherm experiment for Bray pond water, UV_{254} absorbance of sample was also measured after 2 hours and 5 days treatment. The result of this analysis indicates the changing of humic acid concentration in the sample after treatment with PAC. The results of UV_{254} absorbance are as following:

1) pH 5.7:

2) pH 7.05:

3) pH 8.2:

4) pH 10.7:

APPENDIX J.

RESULTS OF SPECIFIC UV ABSORBANCE (SUVA)

SUVA value was calculated as the ratio of UV_{254} absorbance to the TOC. It can be correlated to the hydrophobic fraction of NOM in water. The SUVA results are as following:

1) pH 5.7:

2) pH 7.05:

3) pH 8.2:

4) pH 10.7:

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