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(81) ADSORPTION CAPACITY OF BACTERIOPHAGE Qβ ONTO ACTIVATED CARBONS

Shinta INDAH[1], Fusheng LI[2*], Keita TANIOKA[1], Kayako HIROOKA[2], Toshiro YAMADA[3]

[1]Graduate School of Engineering, Gifu University
(1-1 Yanagido, Gifu 501-1193, Japan)
[2]River Basin Research Center, Gifu University.
(1-1 Yanagido, Gifu 501-1193, Japan)
[3]Department of Civil Engineering, Gifu University.
(1-1 Yanagido, Gifu 501-1193, Japan)
* E-mail: *lifs@gifu-u.ac.jp*

Activated carbon (AC) adsorption is commonly applied in drinking water treatment to deal with natural organic matter and micropollutants. Based on the pore distribution of ACs, viruses present in drinking water sources can probably be admitted into pores of ACs. This research was performed to examine the adsorption capacity of viruses onto ACs in the presence of organic matters. Adsorption isotherms of bacteriophage Qβ, as a model virus, in coexistence with organic matters were analyzed to evaluate the impacts of pore size distribution of ACs and the likely impacts of organic matters. Single solute and simultaneous adsorption experiments with organic matters contained in a peat water and a wastewater after biodegradation were performed for four coal-based ACs having different pore distributions. The observed isotherm data indicated that the presence of organic matters adversely affected the uptake for bacteriophage Qβ. The competition for the adsorption site might cause the adsorption capacity reduction in the presence of organic matters. ACs with different pore size distributions revealed different adsorption capacity for bacteriophage Qβ.

Key Words: *activated carbon, virus, organic matter, adsorption, pore distributions*

1. INTRODUCTION

Viruses have been reported to be responsible for about 80% of waterborne disease outbreaks for which causative agents were identified[1]. Major contamination sources of viruses include septic tanks, sewage sludges, and wastewater. There is evidence that viruses can travel long distances in the subsurface environment to enter drinking water wells[2,3]. Current technologies (coagulation–flocculation, sedimentation, filtration and chlorination) for drinking water treatment are unfortunately not as effective as needed for complete virus removal. About 9% of conventionally treated drinking water has been tested positive for enteric viruses[4]. Additionally, viruses pose a public health threat at a very low level. The USEPA states a limit of two virus particles per 10^7 L of water to achieve an annual infection risk of less than 10^{-4} per individual [5].

So far, there are some researches conducted to investigate the adsorption of viruses onto solid surfaces. Most of them used non-porous materials as adsorbent, such as cellulose, kaolin, carbon black and river sediment as well as soil and sand[6-11]. For porous materials, Powell *et al.* (2000) reported that activated carbon (AC) might be used for removal of viruses at the heart of point-of-use devices for individual water treatment[12].

AC adsorption is one of the best available technologies for advanced drinking water treatment[13,14]. The application of AC for the removal of organic matters and micropollutants for public health and aesthetic significance (e.g. pesticides, taste and odors) in drinking water treatment is widespread. The porous nature of this adsorbent material and its high internal surface area are favorable properties for adsorption[15]. In the case of adsorption of organic matters, viruses present in drinking water sources can be simultaneously adsorbed. Based on the pore distribution of AC, viruses in water can probably be admitted into pores of AC when AC is used for the removal of organic matters. However, little is known about the uptake of viruses on AC in the presence of organic matters and the fate of viruses after adsorption.

Taking into consideration the apparent difference in particle size of organic matters and viruses, and the pore size of ACs, it is reasonable to infer that AC pore size distributions may affect the competition between these two types of adsorbates, such that some micropores that can be accessed by organic matters may not be able to admit viruses and that, in simultaneous adsorption system, organic matters will compete with viruses for adsorption sites where viruses can also be admitted. This can adversely affect the adsorption capacity of viruses. For the small organic compounds, a good correlation between the adsorption capacity and the pore volume was obtained for pores of size below 1.5 nm. However, for the natural organic matter (NOM), a good correlation was noticed for pores with sizes of 3-10 nm[16,17], while the size of viruses is in the range of 5-300 nm.

Pore size distribution (PSD) of ACs is reported as an important factor that affects the removal of organic matters[13]. The PSD's marked influence on the adsorption capacity was well demonstrated using humic matters and ACs of variable materials and types[16,17]. The effect of PSD was also confirmed in simultaneous adsorption of synthetic organic chemicals (SOCs) and naturally-occurring dissolved organic matters (DOMs), as results showed that the extent of DOMs' adversary competitive effects on SOCs' adsorption capacity changed with the PSD of ACs [17-20].

This study was performed in order to examine the adsorption capacity of viruses onto ACs with different PSDs as well as to determine if organic matters adversely affect the adsorption capacity of viruses onto ACs. This study was a preliminary one in order to achieve the final goal of clarifying the fate of viruses after admitted into the AC pores (i.e., the infectivity after adsorption). For the purposes of the present study, bacteriophage Qβ was used as a model virus and batch adsorption experiments were conducted by using four coal-based ACs with different PSDs in the presence of organic matters from a peat water and a wastewater after biodegradation as well as in organic free solutions. The adsorption capacity was then quantitatively analyzed.

2. MATERIALS AND METHODS

(1) Activated carbons

Four coal-based ACs possessing similar surface characteristics but different PSDs, referred hereafter as Carbons A, B, C and D, were used as the adsorbents. As illustrated in **Table 1**, Carbon B is highly micro porous which has more pores with sizes below 2 nm than Carbon A, Carbon C and Carbon D. At sizes more than 10 nm, Carbon D shows the largest pore volume, but unfortunately, more detailed size classification data are not available.

The representative sample of each AC was pulverized and sieved to a size below 45 μm. The carbon was then washed and rinsed with distilled water to remove fines, dried at 105 °C overnight and finally stored in a desiccator until use.

Table 1 Pore volumes and surface areas of ACs used.

Pore size (nm)	Pore volume (cm^3/g)			
	Carbon A	Carbon B	Carbon C	Carbon D
0-2	0.35	0.46	0.18	0.18
2-10	0.08	0.09	0.2	0.26
>10			0.02	0.11
Surface area (m^2/g)	1,290	1,047	1,060	1,080

(2) Background solutions

Three different background solutions were used: Milli Q that is free of organic matter for single solute adsorption, a peat water (Kitamura, Hokkaido, Japan) with humic substances comprising the majority of the organic matter and a wastewater after biodegradation process (Gifu, Japan) that contains humic-like organic matter for simultaneous adsorption with natural organic matter (NOM) and background organic matter (BOM), respectively. Characteristics of the background solutions are displayed in **Table 2**. All of solutions were filtered by 0.2 μm sterilized membrane filters (Toyo Roshi, Japan) before use.

Table 2 Characteristics of background solutions.

Solutions	UV_{260} (m^{-1})	DOC (mg/L)
Milli Q	0	0
Peat Water (containing NOM)	20.1	8.2
Wastewater after biodegradation (containing BOM)	52.5	28.5

(3) Bacteriophage Qβ

The bacteriophage Qβ (NBRC 20012) was obtained from the NITE Biological Research Center (NBRC, Chiba, Japan). Qβ is the prototype member of the genus *Allolevivirus* in the virus family *Leviviridae*. The genomes of the bacteriophage Qβ contain a single molecule of linear positive-sense, single-stranded RNA, which is encapsulated in an icosahedral protein capsid with a diameter of 20–30 nm[21]. Bacteriophage Qβ was propagated for 22–24 h at 37°C in *Escherichia coli* (NBRC 13965) obtained from NBRC. The bacteriophage culture solution was centrifuged (6000 g, 10 min) and then passed through

a membrane filter (pore size 0.2 μm, hydrophilic cellulose acetate; Dismic-25cs, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) to separate the bacteriophage Qβ from host cells. The concentration of the Qβ stock solution was approximately 10^{10} PFU/mL.

(4) Adsorption experiment

Batch adsorption experiments were conducted using the bottle-point method with 50 ml of each background solution containing variable masses of the ACs. Qβ stock solution was added to obtain an initial concentration of 10^8 PFU/ml. The bottles were covered with Teflon-lined rubber septa and shaked by a horizontal shaker in a temperature controlled room (20°C) for 12 hours. The solutions were then centrifuged by 12,000 g for 10 min to separate AC particles and subjected to virus quantification. For the data obtained, statistical analysis by *t test* at the confidence level 95% (p = 0.05) was conducted to evaluate whether the data were statistically different or not.

(5) Bacteriophage assay

Viral RNA of bacteriophages was quantified by the real-time reverse transcription polymerase chain reactions (RT-PCR) method, which detects viruses regardless of their infectivity. The concentration measured by the real-time RT-PCR method was defined as total bacteriophage concentration. For quantification of bacteriophage Qβ in the solutions, viral RNA was extracted from 10 μL of sample by the boiling method (heating at 90°C for 10 min and cooling 4°C for 1 min) in a thermal cycler (Thermal Cycler Dice Model TP800, Takara Bio Inc., Shiga, Japan). The extracted RNA solution was added to PrimeScript™ RT reagent Kit (Takara Bio Inc., Shiga, Japan) for the reverse transcription (RT) reaction according to the manufacturer's protocol, which included heating at 37°C for 15 min and 85°C for 5 s, followed by cooling to 4°C in the thermal cycler. RNA extraction and RT reaction were performed once for each sample since preliminary investigation using replicates in both these processes showed only slight differences in the virus concentrations. The cDNA obtained after RT reaction was then amplified by the TaqMan probe detection, using Premix Ex Taq ™ (Perfect Real Time) (Takara Bio Inc., Shiga, Japan). Two sets of specific Qβ primers including forward and reverse primers (5`-TCA AGC CGT GAT AGT CGT TCC TC-3` and 5`-AAT CGT TGG CAA TGG AAA GTG C-3` starting at base 49 and 187, respectively) and the probe (5`-CGA GCC GCG AAC ACA AGA ATT GA-3` starting at base 147) were used for real-time RT-PCR in the quantification of Qβ[21]. Amplification was conducted at 95°C for 30 s and 40 cycles of 95°C for 5 s and 60°C for 30 s in the Thermal Cycler Dice Model TP800 (Takara Bio Inc., Shiga, Japan). Amplification and quantification were made in duplicate for each sample. The PCR-based viral concentration was expressed as PFU equivalent/mL, which was determined by converting the number of cycles (Ct value) in the real time RT-PCR amplification of the sample to the PFU concentration according to the standard curve plotting the relationship between the Ct values and the PFU values of known-concentration of freshly-prepared bacteriophage Qβ stock solutions. In order to perform quantification as accurately as possible, it is desirable that PCR efficiency be in the range of 80-120%. The coexistence of PCR inhibitor which may come from water samples or reagents used in RNA extraction can be confirmed from the PCR efficiency if it exceeds the theoretical figure[22]. In this study, the PCR efficiencies were in the range of 105-118% suggesting no presence of reaction inhibitory substances and high accuracy of quantification. The detection limit of real-time RT-PCR for Qβ quantification was determined using the method documented[23,24]. A 10-fold dilution series of bacteriophage Qβ in the range of $2.8x10^9$-2.8 PFU equivalent/mL was used and analyzed in duplicate by real-time RT-PCR. The result of measurements showed that the detection limit was $2.8x10^2$ PFU/mL.

(6) Chemical analysis

The organic matter concentration was quantified using the index of UV-absorbance at the wavelength of 260 nm (UV260) by UV-visible spectrophotometer (model UV-1600, Shimazu Co., Japan). The molecular weight distributions of the NOM and BOM were measured by SE-HPLC (Model LC-10AD, Shimadzu Co., Kyoto, Japan) with a UV-visible detector (Model LC-10AV, Shimadzu Co., Kyoto, Japan) and a Hitachi column (Packed column: GL-W520-X 10.7×450 mm; eluent: 0.02 M-Na_2HPO_4+0.02 M-KH_2PO_4; flow rate: 0.5 ml/min). Particle size distributions of the solutions were determined by dynamic light scattering using Zetasizer Nano ZS (Model ZEN 3600, Malvern Instrument, London, England).

3. RESULTS AND DISCUSSION

(1) Particle size and molecular weight distributions of background solutions

Particle size distributions of all background solutions as well as bacteriophage Qβ solution is shown in **Fig.1**. Dynamic light scattering revealed a single size distribution peak with z-averaged

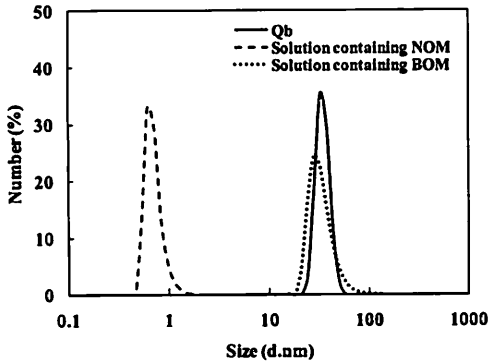


Fig.1 Particle size distributions of Qβ and background solutions measured by Zetasizer Nano ZS.

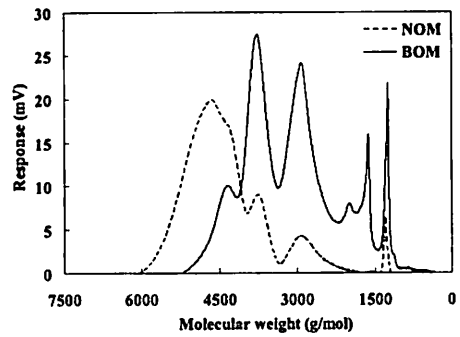


Fig.2 Molecular weight distributions of organic matters measured by SE-HPLC.

hydrodynamic diameters of 0.7 nm, 28 nm and 30 nm for each solution that contains NOM, BOM and bacteriophage Qβ, respectively. For bacteriophage Qβ, the value corresponded with the particle diameter previously reported: the particles sizes were distributed over the range of 20–30 nm[21].

Fig.2 displays the molecular weight (MW) distribution of NOM and BOM used in this study. The distributions of MW are in the ranges of 6000-1500 and 5250-1500 for NOM and BOM, respectively. Corresponding to the observed number of peaks of each MW distribution curve, the NOM was classified into four fractions and the BOM into six fractions.

(2) Residual concentrations of bacteriophage Qβ

The results of adsorption of bacteriophage Qβ onto the ACs in different background solutions, presented as the residual concentration ratio (C/C_0), are shown in **Fig.3**. The concentrations of bacteriophage Qβ decreased by increasing AC doses used for all of ACs, with the decreasing trend becoming less apparent after 1 g/L of AC dose. The results also revealed that the residual concentration ratios in single solute adsorption were lower than in simultaneous adsorption using solutions containing NOM and BOM, respectively.

At 5 g/L of AC dose, in single solute adsorption, the residual concentration ratios of bacteriophage Qβ were in the range of 8.3×10^{-5}-1.5×10^{-4} for all ACs used, while the residual concentration ratios in solutions containing NOM and BOM were 4.6×10^{-4}-1.4×10^{-3} and 9.7×10^{-4}-1.1×10^{-3}, respectively. Slight differences were shown between the residual concentration ratios of bacteriophage Qβ in single

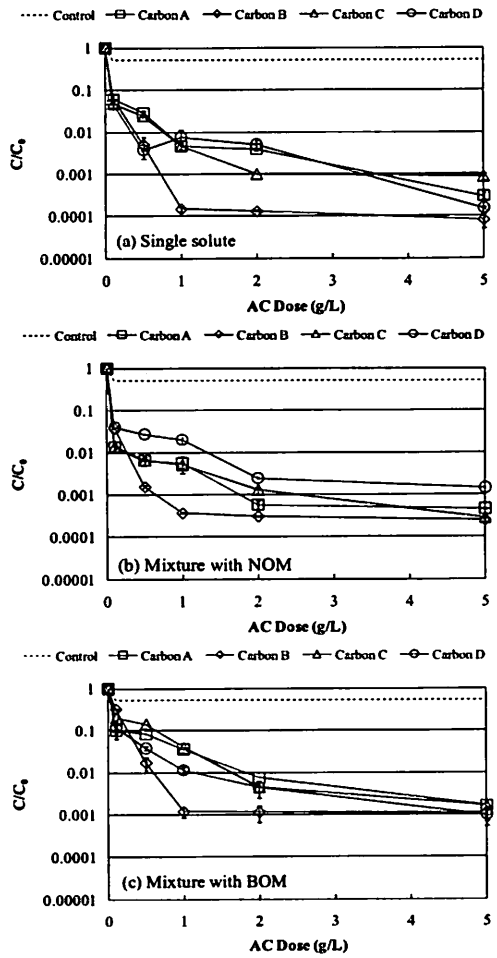


Fig.3 Residual concentration ratios of bacteriophage Qβ with carbon dose in different background solutions: (a) single solute, (b) mixture with NOM and (c) mixture with BOM. Error bars represent the standard deviation computed based on duplicates in measurement of Qβ concentration.

solute adsorption and in simultaneous adsorption with NOM. However, no statistical difference in residual concentration ratios of bacteriophage Qβ among three kinds of water samples was obtained judging through the *t test* at the confidence level 95% (p = 0.05). This was probably due to the small number of data sets in this study. More variations and data sets should be needed in order to get convinced results in statistical analysis.

Taking into consideration of the particle size as displayed in **Fig.1**, a big difference in size of bacteriophage Qβ with NOM (0.7 nm and 30 nm for NOM and Qβ, respectively) and a slight difference with BOM (28 nm for BOM) were observed. These probably caused the slight differences between the residual concentration ratios of bacteriophage Qβ in single solute adsorption and in simultaneous adsorption with NOM. The size exclusion effect probably prevented Qβ molecules from entering pores with diameters less than 20-30 nm, while as smaller molecules, NOM and BOM can be admitted into the small pores as well as large pores where Qβ was preferably adsorbed, leading to the competition among them.

Ebie *et al.* (2001) evaluated the impact of the AC pore size distributions on the competitive adsorption of micropollutants in the presence of NOM. Batch adsorption experiments were conducted for several agricultural organic chemicals in the presence of NOM remaining in a coagulation-pretreated surface water and for three coal-based ACs with different pore distributions. The results showed that, for all the carbons used, the adsorption capacity of the chemicals was reduced distinctly in the presence of NOM, resulting in increases of residual concentrations of the agricultural organic chemicals in the simultaneous adsorptions with NOM[17].

(3) Adsorption isotherm of bacteriophage Qβ

For aqueous adsorption with porous materials, Langmuir and Freundlich are two models well used to describe the adorption isotherm. These two models were also used to describe virus adsorption on solid surfaces, but Freundlich isotherm allows the heterogeneity in adsorption[12]. In the present study, based on the experimental data obtained (**Fig.3**), isotherm analysis with both Freundlich and Langmuir models were conducted. Comparison of the determination coefficients of Langmuir (0.09-0.90) and with those of Freundlich (0.82-0.97) indicated that the Freundlich model described the bacteriophage Qβ adsorption better than the Langmuir model. To further evaluate the suitability of using Freundlich isotherm, besides the correlation analysis, ANOVA was also conducted to test the fitting results with both models. By comparing the F test statistic, statistical significance was obtained for the Freundlich model ($p < 0.05$). Therefore, in the following discussions, results based on the Freundlich model will be given.

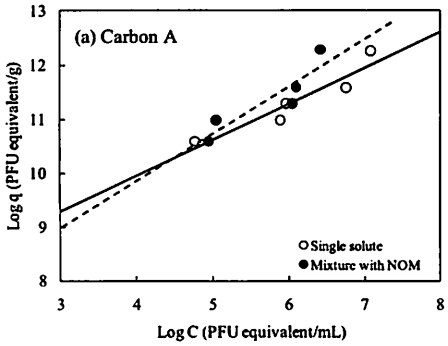
Adsorption isotherms of the bacteriophage Qβ onto Carbons A, B, C and D are displayed in **Fig. 4(a)-(d)** and **Fig. 5(a)-(d)**, respectively. Lines shown in these figures represent the model calculations with the Freundlich isotherm expression,

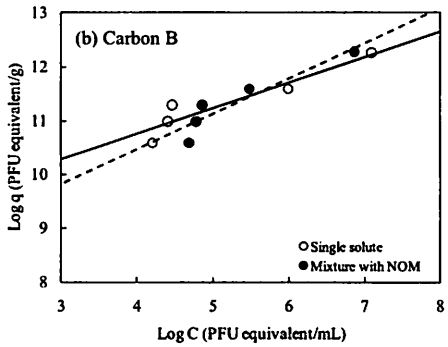
$$q = KC^{1/n} \quad (1)$$

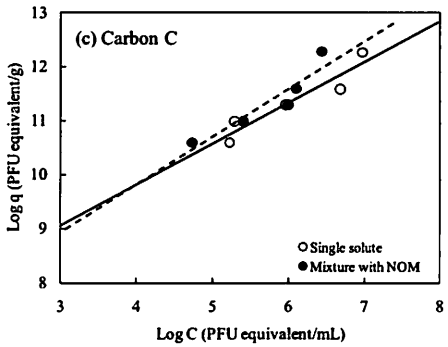
where q is the amount of adsorbate per unit adsorbent and C is the concentration of adsorbate. K and $1/n$ are Freundlich constants. The constant K characterizes the adsorption capacity and the constant $1/n$ is related to the strength of the adsorption forces between the adsorbate and the adsorbent. The determined values of K and $1/n$ with their lower and upper limits of the 95% confidence interval are summarized in **Table 3**. The results showed that the estimated values of both K and $1/n$ fell in the respective ranges of limits, even if the ranges are unexpectedly broader.

Comparing the K values of each AC in single solute adsorption, Carbon B had the highest K value among the ACs, with the values following the order of Carbon B > D > A > C. Larger pore volume in the size above 10 nm of Carbon B and D that is probably suitable for adsorption of bacteriophage Qβ (size = 20-30 nm) might be the reason for higher K values. For simultaneous adsorption, the highest K value was also obtained for Carbon B, followed by Carbon A, C and D in the solution containing NOM, and by Carbon C, D and A in the solution containing BOM, respectively. These orders differed from the order shown earlier for single solute adsorption (Carbon B > D > A > C). Compared to Carbon D, Carbon C has smaller volume in pores of >10 nm, but its adsorption capacity was higher in both solutions containing NOM and BOM. All these suggested that, in addition to the pore size, interactions among carbon surface, bacteriophage Qβ and coexisting organic matter may also affect the adsorption of bacteriophage Qβ in either single solute or simultaneous adsorption systems, which needs to be investigated in coming studies. Moreover, detailed classification of ACs to different size ranges, at least containing one that has sizes above the bacteriophage Qβ size of 20-30 nm, is also needed for better interpretation of the pore effect, which is also undertaken now.

For all ACs used in this study, generally, the K values for simultaneous adsorption were lower than those for single solute adsorption; similarly, the $1/n$ values for simultaneous adsorption were higher than those for single solute adsorption as well. However,







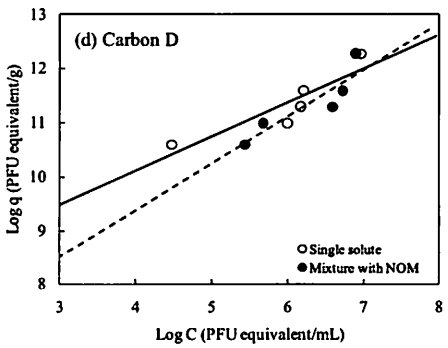
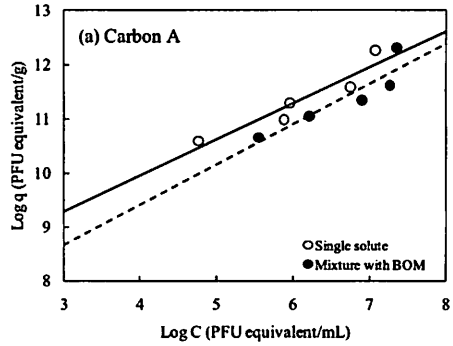
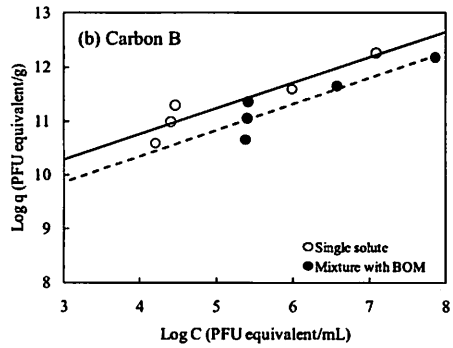
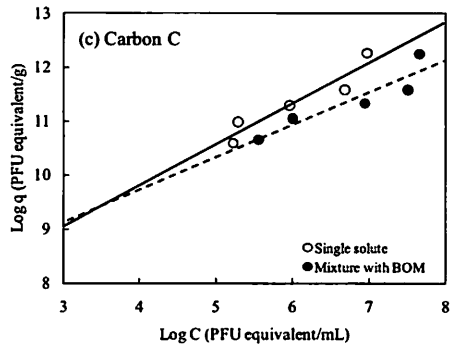


Fig. 4 Adsorption isotherms of bacteriophage Qβ in single solute solution and the solution containing NOM.







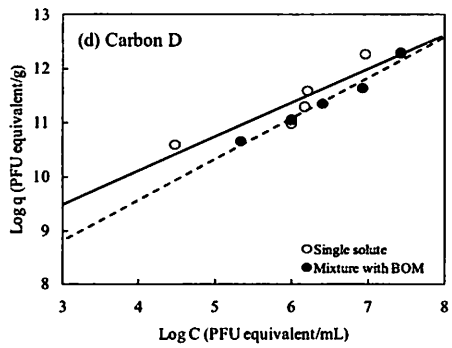


Fig. 5 Adsorption isotherms of bacteriophage Qβ in single solute solution and the solution containing BOM.

Table 3 Freundlich isotherm parameters for bacteriophage Qβ on Carbons A, B, C and D.

AC type	Single solute			Mixture with NOM			Mixture with BOM		
	K	*1/n*	R^2	*K*	*1/n*	R^2	*K*	*1/n*	R^2
A	$2.0x10^7$	0.66	0.899	$2.3x10^6$	0.87	0.824	$3.0x10^6$	0.74	0.825
	($4.5x10^4$-$8.8x10^9$)	(0.23-1.10)		($1.3x10^2$-$4.2x10^{10}$)	(0.13-1.61)		($2.0x10^2$-$4.6x10^{10}$)	(0.11-1.37)	
B	$7.0x10^8$	0.47	0.886	$7.0x10^7$	0.65	0.876	$2.0x10^7$	0.60	0.899
	($1.6x10^7$-$3.5x10^{10}$)	(0.16-0.78)		($2.6x10^5$-$8.8x10^{10}$)	(0.20-1.11)		($7.6x10^5$-$8.9x10^{10}$)	(0.08-0.89)	
C	$6.0x10^6$	0.76	0.893	$8.9x10^5$	0.89	0.870	$2.0x10^7$	0.60	0.855
	($7.0x10^3$-$5.1x10^9$)	(0.27-1.24)		($4.3x10^2$-$7.9x10^9$)	(0.26-1.52)		($2.0x10^4$-$2.6x10^{10}$)	(0.15-1.01)	
D	$4.0x10^7$	0.63	0.810	$4.7x10^5$	0.87	0.802	$4.0x10^6$	0.75	0.969
	($1.7x10^4$-$9.2x10^{10}$)	(0.07-1.19)		($8.9x10^1$-$7.8x10^{10}$)	(0.08-1.65)		($9.1x10^4$-$1.4x10^8$)	(0.51-1.00)	

K = [(PFU/g)/(PFU/mL)]. R^2= coefficient of determination.
Values within parentheses are the lower and upper limits of the 95% confidence interval.

Table 4 Comparisons of Freundlich isotherm parameters with porous adsorbents (this study) and non-porous ones (literatures).

Virus	Adsorbent	Surface area (m^2/g)	*K*	*1/n*	References
MS2	Ferriudic Cambosols soil	9	0.228	1.04	Zhao et. al (2008)[10]
MS2	Red soil	31.3	1,760.80	1.08	Zhang et.al (2010)[11]
Qβ	Carbon Black	7	$5.93x10^5$	0.76	Sakoda et.al (1997)[6]
Qβ	Kaolin	3.7	$8.02x10^4$	0.81	Sakoda et.al (1997)[6]
Qβ	Sediment (from river)	6.2	10.348	0.94	Sakoda et.al (1997)[6]
Qβ	Carbon A	1,290	$2.0x10^7$	0.66	This study
Qβ	Carbon B	1,047	$7.0x10^8$	0.47	This study
Qβ	Carbon C	1,060	$6.0x10^6$	0.76	This study
Qβ	Carbon D	1,080	$4.0x10^7$	0.63	This study

an exception was noticed for Carbon C in the mixture with BOM, which showed higher *K* and lower *1/n* values than in single solute adsorption. Difference in the interaction among carbons, bacteriophage Qβ and coexisting organic matters from the system with this carbon might be the reason behind, which will be clarified in undergoing studies.

With respect to the adsorption of the bacteriophage Qβ in the presence of organic matters, including NOM and BOM, generally, distinct reductions in *K* values and increases in the slope of isotherms (*1/n* values) were observed as compared to corresponding single solute adsorption results. These suggested that organic matters that contained in solutions might adversely affect the adsorption capacity of ACs for uptake of bacteriophage Qβ. The isotherm slope is generally considered indicative of the adsorption energy reflecting the interaction of the adsorbate molecules with the internal pore increases in the slope indicated some changes in the surface[16,17]. Therefore, it can be inferred that the site heterogeneity of pores that could admit both the bacteriophage Qβ and the organic matters, and is thus indicative of the involvement of direct site competition in the simultaneous adsorption systems. The results coincide with the previous results on AC adsorption of four types of halogenated organic compounds[16], four agricultural organic chemicals[17] and chloroform[18] in the presence of NOM.

Comparing the Freundlich isotherm parameters of this study and previous studies that used non-porous materials, such as soil, kaolin and river sediment as the adsorbent revealed that the *K* and *1/n* values of this study were higher and lower than those of the previous ones, respectively, as shown in **Table 4**. This may indicate that higher adsorption capacity and stronger affinity between AC and bacteriophage Qβ were obtained by using AC as the adsorbent.

(4) Estimation of AC dose needed for achieving objective Qβ levels after pre-coagulation

Based on the results shown above, it is reasonable to consider that viruses, if presented in water sources, can be simultaneously removed to certain extent together with NOM and micropollutants when AC is applied to deal with these adsorbates commonly targeted for advanced drinking water treatment. To better demonstrate the effect of ACs, the dose of ACs needed for decreasing the concentration of virus was simply estimated using the Freundlich isotherm and the mass balance equation shown below, based on the single solute adsorption isotherm parameters shown in **Table 3.**

$$q = \frac{C_0 - C}{C_{AC}} \qquad (2)$$

where C_0 is the initial concentrations of the adsorbate and C_{AC} is the AC dose.

The estimation was made by considering the following conditions and assumptions: (1) in the raw water, the concentration of virus is 1000 PFU/mL and by coagulation process in drinking water treatment, it can be removed to 100 PFU/mL (90 % removed); (2) equilibrium is reached within the contact time during coagulation and sedimentation stages of the conventional drinking water treatment system; (3) the adversary effect of NOM is not considered because, compared to the solution used in this experiment, for most natural water sources, TOC concentrations are lower; and (4) even in low concentrations, Freundlich isotherm model still applies.

Table 5 Estimation of AC doses needed.

AC type	For 1 PFU/2 L		For 1 PFU in 10 L	
	q (PFU/g)	AC needed (mg/L)	q (PFU/g)	AC needed (mg/L)
A	1.3×10^5	0.776	4.4×10^4	2.258
B	1.9×10^7	0.005	9.1×10^6	0.011
C	1.9×10^4	5.265	5.6×10^3	17.809
D	3.4×10^5	0.295	1.2×10^5	0.810

The concentration of Qβ subjected to estimation of AC adsorption is 100 PFU/mL after coagulation treatment of water containing 1000 PFU/mL of Qβ.

As shown in **Table 5**, if the objective concentration of Qβ is 1 PFU/2L, the doses of carbon A, carbon B, carbon C and carbon D were 0.776, 0.005, 5.265 and 0.295 mg/L, respectively and if the objective concentration of Qβ is 1 PFU in 10 L, the doses were 2.258, 0.011, 17.809 and 0.810 mg/L, respectively, indicating marked differences in the doses based on the carbon applied.

4. CONCLUSIONS

Batch adsorption experiments on AC adsorption of bacteriophage Qβ in organic free solution, peat water containing NOM and wastewater after degradation containing BOM were conducted for four coal-based ACs possessing different pore size distributions. The results showed that bacteriophage Qβ can be simultaneously adsorbed, no matter if targeted for removal or not. Adsorption capacity of bacteriophage Qβ changes with ACs used. Adversary effect of coexisting organic matter in water onto the adsorption capacity of bacteriophage Qβ seems to be existed.

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REFERENCES

1) Ryan, J.N., Harvey, R.W., Metge, D., Elimelech, M., Navigato, T. and Pieper, A.P. : Field and laboratory investigations of inactivation of viruses (PRD1 and MS2) attached to iron oxide-coated quartz sand, *Environ. Sci. and Technol.*, Vol. 36, pp.2403–2413, 2002.
2) Gerba, C.P., Goyal, S.M., Cech, I. and Bogdan, G.F.: Quantitative assessment of the adsorptive behavior of viruses to soils, *Environ. Sci. and Technol.*, Vol.15, 940–944, 1981.
3) Gerba, C.P.: Applied and theoretical aspects of virus adsorption to surfaces, *Advances in Applied Microbiology*, Vol. 30, pp. 133–168, 1984.
4) Gerba, C.P., Rose, J.B. and Haas, C.N: Waterborne rotarivirus : a risk assessment, *Water Research*, Vol. 30, No. 12., pp.2929-2940, 1996.
5) US Environmental Protection Agency: National primary drinking water regulations: ground water rule; proposed rule. Federal Register 65, 30194-30274, USEPA, Washington, DC. 2000.
6) Sakoda, A., Sakai, Y., Hayakawa, K. and Suzuki, M.: Adsorption of viruses in water environment onto solid surfaces. Water Science and Technology 35 (7), pp. 107–114, 1997.
7) Schijven, J.F. and Hassanizadeh, S.M.: Removal of viruses by soil passage: overview of modeling, processes, and parameters, *Critical Reviews in Environ. Sci. and Tech.ogy*, Vol. 30, pp 49–127, 2002.
8) Jin, Y. and Flury, M.: Fate and transport of viruses in porous media, *Advances in Agronomy*, Vol.77, pp. 39–102, 2002.
9) Jin, Y., Chu, Y. and Li, Y.: Virus removal and transport in saturated and unsaturated sand columns, *J. Contaminant Hydrology*, Vol. 43, pp.111–128, 2000.
10) Zhao B., Zhang H., Zhang J. and Jin Y.: Virus adsorption and inactivation in soil as influenced by autochthonous microorganisms and water content, *Soil Biology & Biochemistry* Vol. 40, pp 649–659, 2008.
11) Zhang H., Zhang J., Zhao, B. and Zhang, C. : Removal of bacteriophages MS2 and phiX174 from aqueous solutions using a red soil, *J. Hazardous Materials*, Vol. 180, pp. 640–647, 2010.

12) Powell T., Brion, G.M., Jagtoyen, M. and Derbyshire, F.: Investigating the effect of carbon shape on virus sorption, *Environ. Sci. Technol.*, Vol. 34, pp 2779–2783, (2000).
13) Li, F.S., Yuasa, A., Ebie, K., Azuma, Y., Hagishita, T. and Matsui, Y.: Factors affecting the adsorption capacity of dissolved organic matter onto activated carbon: modified isotherm analysis, *Water Research*, No. 36, pp 4592–4604, (2002).
14) Hijnen, W.A.M., Suylen, G.M.H., Bahlman, J.A., Brouwer-Hanzens A. and Medema, G.J.: GAC adsorption filters as barriers for viruses, bacteria and protozoan (oo)cysts in water treatment, *Water Research*, Vol. 44, No. 4, pp. 1-11, 2010.
15) Pelekani, C. and Snoeyink, V. L.: Competitive adsorption between atrazine and methylene blue on activated carbon: the importance of pore size distribution, *Carbon*, Vol. 38, pp. 1423-1436, 2000.
16) Ebie, K, Li, F.S. and Hagishita, T.: Effect of pore size distribution of activated carbon on the adsorption of humic substances and trace organic compounds, *Water Supply*; Vol.13 (3–4), pp. 65–70, 1995.
17) Ebie, K, Li, F.S., Azuma, Y, Yuasa, A. and Hagishita, T.: Pore distribution effect of activated carbon in adsorbing organic micropollutants from natural water, *Water Research*, Vol. 35(1) 67–79. 2001.
18) Sakoda, A,. Suzuki, K., Hirai, R. and Kawazoe, K.: Trihalomethane adsorption on activated carbon fibers, *Water Research*,Vol. 25(2), pp. 219–225, 1991.
19) Pelekani, C. and Snoeyink, V.L.: Competitive adsorption in natural water: role of activated carbon pore size, *Water Research*, Vol. 33(5), pp. 1209–1219, 1999.
20) Kilduff, J.E., Karanfil, T., Chin, Y. and Weber, W.J.: Adsorption of natural organic polyelectrolytes by activated carbon: a size-exclusion chromatography study. *Environ Sci. Technol.*, Vol. 30(4): pp. 1336–43. 1996.
21) Shirasaki, N., Matsushita, T., Matsui, Y., Urasaki, T. and Ohno, K.: Comparison of behaviors of two surrogates for pathogenic waterborne viruses, bacteriophages Qb and MS2, during the aluminum coagulation process, *Water Research*, Vol. 43: pp. 605-612, 2009.
22) Kennedy, S. and Oswald, N.: PCR Troubleshooting and Optimization: The Essential Guide. ISBN 978-1-904455-72-1. Caister Academic Press, 2011.
23) Huber, I., Campe, H., Sebah, D., Hartberger, C., Konrad, R., Bayer, M., Busch, U. and Sing, A.: A multiplex one-step real-time RT-PCR assay for influenza surveillance, *Eurosurveillance*, Vol. 16 (7), article 4, 2011.
24) Suzuki, Y., Saito, R., Zaraket, H., Dapat, C., Caperig-Dapat, I. and Suzuki, H.: Rapid and specific detection of amantadine-resistant influenza A viruses with a Ser31Asn mutation by cycling probe method, *J. Clinical Microbiology*, Vol. 48 (1), p. 57-63., 2010.

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