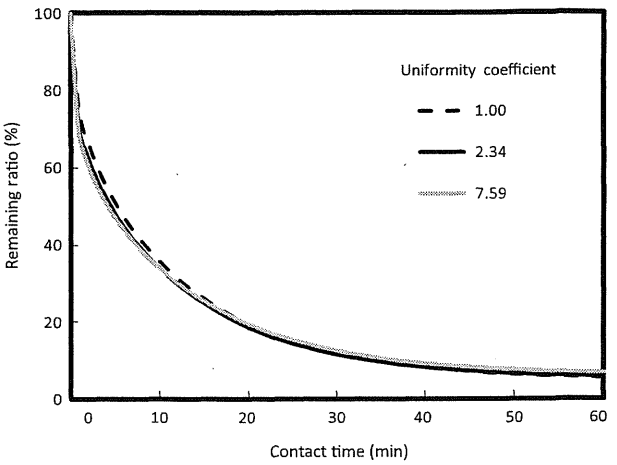

Fig. 4 – Effect of particle size distribution on adsorption kinetics of geosmin: BKAM-SAM simulations (D_{40} = 1.0 μm, activated carbon dose = 0.5 mg/L, initial geosmin concentration: 1000 ng/L, NOM water, D_S: 2.1 × 10^{-7} cm^2/s, K_S: 3.8 × 10^{-4} s^{-1}, ϕ: 0.29).

case, one would expect that the most representative characteristic diameter would be less than the median diameter D_{50}. Because geosmin has a slower intraparticle mass transfer rate than MIB (as is indicated by the different curvatures of the concentration decay curves in Fig. 2 and by the comparison of the D_P and K_S values between Panels B and C and between Panels D and E for the same carbons in the figure caption), it is reasonable that the most representative diameter for geosmin removal (D_{30}) was more heavily weighted to the smaller size fraction than that of MIB (D_{40}). There was no single best representative size to describe the adsorption kinetics, but the RMS values of D_{30} and D_{40} were not so different. Overall, D_{40} was the best, as it minimized the total RMS (Fig. 5C). This representative diameter is expected to characterize adsorption kinetics well regardless of whether the size distribution is wide or narrow. Variations in size dispersity might result from variations in the type of grinder used, or the grinding time, but then this diameter could be used as an index to show whether the size was sufficiently small after grinding. In the present BPKM-SAM simulations, the particle size distribution of the adsorbent was taken into consideration, but most other research and practical applications of adsorption kinetics models assume uniform particle size to simplify calculations. We propose that D_{40} be used as a representative particle diameter in model simulations when a uniform particle size is assumed.

3.3. *Optimum diameter for efficient absorption*

The relationship between D_{40} and required dose was calculated through BPKM-SAM simulations. For a given contact time, the carbon dose required to effect a given removal percentage could be reduced by decreasing D_{40} (Fig. 6). Between D_{40} values of 5 and 50 μm, the relationship was roughly linear; for example, the required dose was reduced by one fifth when D_{40} was decreased by one fifth. Matsui et al. (2007) compared the SPAC and PAC doses required for 60–98% geosmin removal in a system using flow-through PAC adsorption followed by microfiltration separation, and reported that the SPAC (D_{50} = 0.65 μm) dose required for a 4-min PAC–water contact time was 6–25% that of the PAC (D_{50} = 7.6 μm) dose for the same removal and contact time. In our analysis of geosmin removal, the corresponding dose ratio was 10% for a 90% removal (10% remaining ratio) over a 10-min contact time (Fig. 6C and E). Although removal efficiencies may differ between flow-through and batch reactors, the results of the current study are generally consistent with the previous study.

In the scenarios modeled, the required dose initially decreased linearly with the particle size, but this trend leveled off as D_{40} reached a critical range (Fig. 6). That is to say, for a given contact time and removal ratio, reducing the particle size to a certain degree effectively reduced the required carbon dose, but eventually further size reduction was not worthwhile. It is convenient to define a critical D_{40} value below which further grinding was not useful; we define critical D_{40} at the intersection between the line extrapolated from the linear dose decline and the constant line representing the lowest dose (for an example, see Fig. 6A). As shown in Fig. 7, critical D_{40} was larger for longer contact times, reflecting the fact that adsorption capacity becomes progressively more important than kinetics as contact time is increased. The critical D_{40} value for MIB removal was ~1 μm for a 10-min contact time, but the values were 2–2.5 and 3–4 μm for contact times of 60 and 180 min, respectively; critical D_{40} values for geosmin removal were ~0.2, 0.4–0.5, and 0.5–0.8 μm for contact times of 10, 60, and 180 min, respectively. As just mentioned, adsorption capacity becomes relatively more important than kinetics as contact time increases. When contact times are long, only the large particles will fail to reach adsorption equilibrium, so only these large particles will have

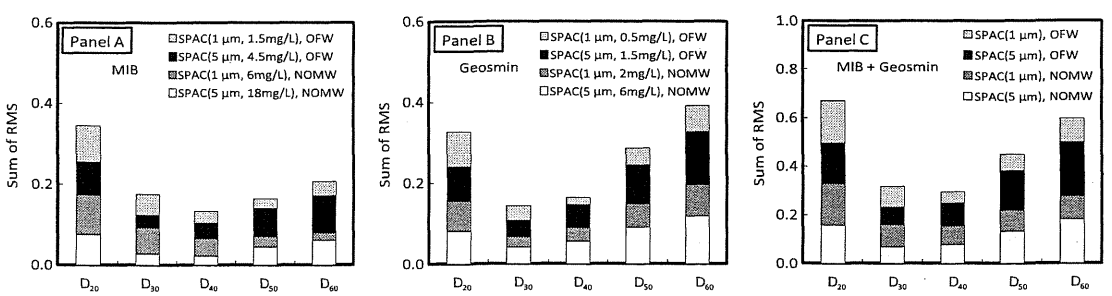

Fig. 5 – Sums of root mean square (RMS) values to select the best characteristic size that represents a group of adsorbent particles with the same size distribution (Initial MIB or geosmin concentration is 1000 ng/L).

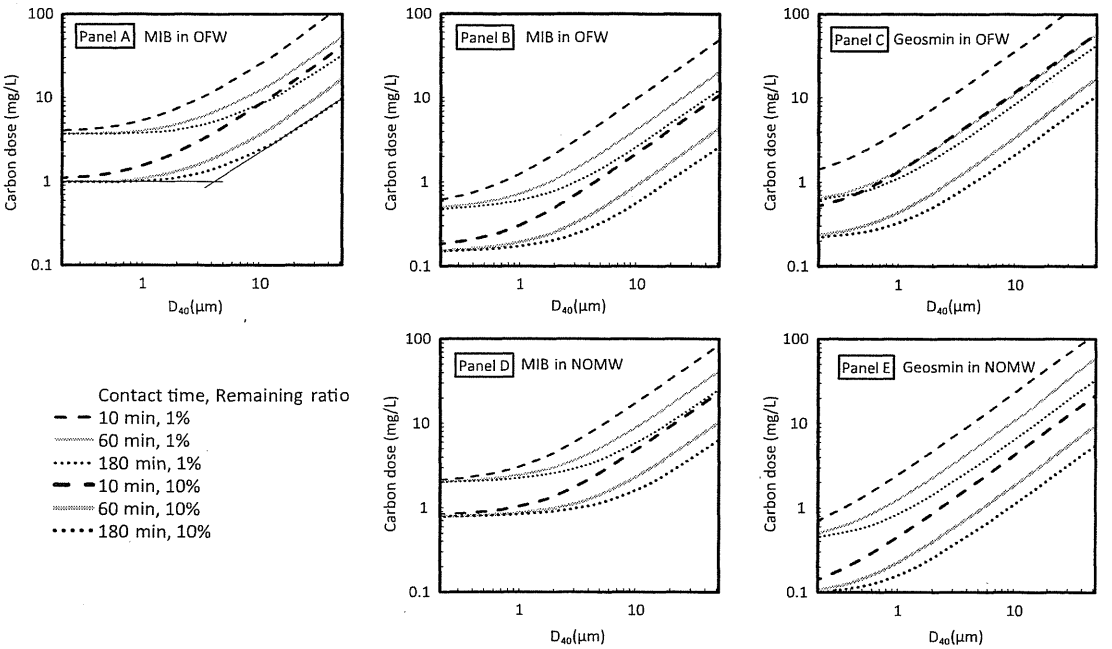

Fig. 6 – Dose required for various removal percentages and contact times vs. particle size of activated carbon for MIB (Panels A, B, and D) and geosmin (Panels D and E) removal: PBKM-SAM simulations. Initial concentration is 1000 ng/L. PBKM-SAM parameter values are the same as those of Figs. 1 and 2. Panel A: Carbon sample from the year 2007. Panels B–E: Carbon sample from the year 2010.

any potential for improvement of kinetics through particle size reduction. For these large particles, therefore, adsorption kinetics does still play a role even for longer contact times. Importance of adsorption kinetics only for large particles means that the critical D_{40} increases for longer contact times.

Critical D_{40} was smaller for geosmin than for MIB. As stated earlier, geosmin has slower intraparticle mass transfer within a carbon particle, meaning that adsorption kinetics plays a more important role in determining the necessary dose. Accordingly, within a size range below the critical D_{40} for MIB removal, continued reduction in size was irrelevant to MIB removal but continued to reduce the necessary dose for the equivalent geosmin removal. Using small adsorbent particles brought about fast adsorptive removal, but this effect became less important as contact time was increased. Overall, grinding activated carbon until its representative diameter D_{40} was a few microns was found to be an effective method for enhancing its adsorptivity and thereby enabling a reduction in its dose.

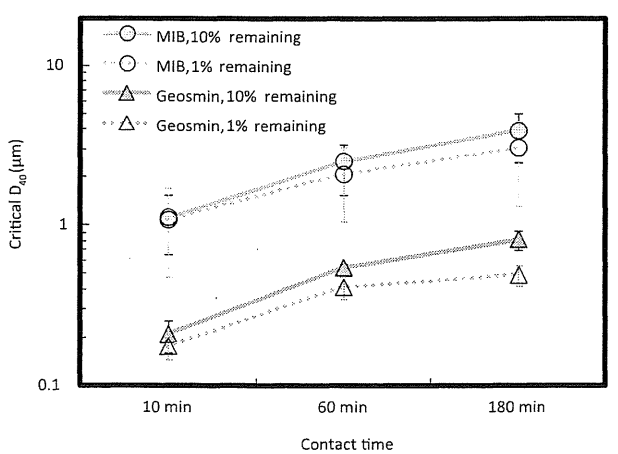

Fig. 7 – Effect of carbon–water contact time on the critical D_{40} value.

4. Conclusions

1. Owing to decreased carbon particle size, removal of MIB and geosmin over a given contact time was greatly enhanced. The change in adsorption isotherms with decreased particle size was explained by SAM. Irrespective of the adsorbent particle size, adsorption kinetics was well simulated by BPKM combined with SAM, using a given set of model parameter values.
2. BPKM-SAM simulations suggested that D_{40}, the diameter that 40% by volume of all the particles are finer than, was a suitable characteristic diameter that represented the adsorption kinetics of adsorbent particles well regardless of their size dispersity. Therefore, if a model simulation is simplified by assuming uniform adsorbent particle size, we propose that D_{40} be used as the representative diameter.
3. The activated carbon dose required to effect a certain removal percentage was reduced in proportion to carbon particle size represented by D_{40}, but this effect leveled off as D_{40} fell below a certain size. This critical D_{40} value depended on the carbon–water contact time and the intraparticle mass transfer rate of adsorbate in a carbon particle; these

factors determine the relative importance of adsorption capacity and kinetics on adsorptive removal.

4. Because of the kinetics enhancements that smaller particles provide, the merit of reducing particle size through grinding continued into a smaller size domain for systems that used shorter carbon–water contact times. Similarly, because geosmin was adsorbed more slowly than MIB, further reductions in particle size beyond MIB's leveling-off point continued to have greater merit for geosmin removal. The critical D_{40} values of MIB were ~1 and 3–4 μm for 10- and 180-min contact times, respectively, whereas those of geosmin were ~0.2 and 0.5–0.8 μm, respectively. Overall, grinding activated carbon until its representative diameter D_{40} was a few microns improved the speed of MIB and geosmin removal and/or reduced the needed dose over a wide range of conditions.

Acknowledgments

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REFERENCES

Ando, N., Matsui, Y., Kurotobi, R., Nakano, Y., Matsushita, T., Ohno, K., 2010. Comparison of natural organic matter adsorption capacities of super-powdered activated carbon and powdered activated carbon. Water Research 44 (14), 4127–4136.

Considine, R., Denoyel, R., Pendleton, P., Schumann, R., Wong, S.-H., 2001. The influence of surface chemistry on activated carbon adsorption of 2-methylisoborneol from aqueous solution. Colloids and Surfaces A: Physicochemical and Engineering Aspects 179 (2), 271–280.

Cook, D., Newcombe, G., Sztajnbok, P., 2001. The application of powdered activated carbon for MIB and geosmin removal: predicting PAC doses in four raw waters. Water Research 35 (5), 1325–1333.

Gillogly, T.E.T., Snoeyink, V.L., Elarde, J.R., Wilson, C.M., Royal, E.P., 1998. ^{14}C-MIB adsorption on PAC in natural water. Journal of the American Water Works Association 90 (1), 98–108.

Heijman, S.G.J., Hamad, J.Z., Kennedy, M.D., Schippers, J., Amy, G., 2009. Submicron powdered activated carbon used as a pre-coat in ceramic micro-filtration. Desalination & Water Treatment 9, 86–91.

Huang, C., VanBenschoten, J.E., Jensen, J.N., 1996. Adsorption kinetics of MIB and geosmin. Journal of the American Water Works Association 88 (4), 116–128.

Huang, H., Schwab, K., Jacangelo, J.G., 2009. Pretreatment for low pressure membranes in water treatment: a review. Environmental Science & Technology 43 (9), 3011–3019.

Leenheer, J.A., 2007. Progression from model structures to molecular structures of natural organic matter components. Annals of Environmental Science 1, 57–68.

Letterman, R.D., Quon, J.E., Gemmell, R.S., 1974. Film transport coefficient in agitated suspensions of activated carbon. Journal of the Water Pollution Control Federation 46 (11), 2536–2547.

Matsui, Y., Fukuda, Y., Murase, R., Aoki, N., Mima, S., Inoue, T., Matsushita, T., 2004. Micro-ground powdered activated carbon for effective removal of natural organic matter during water treatment. Water Science and Technology: Water Supply 4 (4), 155–163.

Matsui, Y., Murase, R., Sanogawa, T., Aoki, N., Mima, S., Inoue, T., Matsushita, T., 2005. Rapid adsorption pretreatment with submicrometre powdered activated carbon particles before microfiltration. Water Science and Technology 51 (6–7), 249–256.

Matsui, Y., Aizawa, T., Kanda, F., Nigorikawa, N., Mima, S., Kawase, Y., 2007. Adsorptive removal of geosmin by ceramic membrane filtration with super-powdered activated carbon. Journal of Water Supply: Research and Technology-AQUA 56 (6–7), 411–418.

Matsui, Y., Ando, N., Sasaki, H., Matsushita, T., Ohno, K., 2009a. Branched pore kinetic model analysis of geosmin adsorption on super-powdered activated carbon. Water Research 43 (12), 3095–3103.

Matsui, Y., Hasegawa, H., Ohno, K., Matsushita, T., Mima, S., Kawase, Y., Aizawa, T., 2009b. Effects of super-powdered activated carbon pretreatment on coagulation and transmembrane pressure buildup during microfiltration. Water Research 43 (20), 5160–5170.

Matsui, Y., Ando, N., Yoshida, T., Kurotobi, R., Matsushita, T., Ohno, K., 2011. Modeling high adsorption capacity and kinetics of organic macromolecules on super-powdered activated carbon. Water Research 45 (4), 1720–1728.

Matsui, Y., Yoshida, T., Nakao, S., Knappe, D.R.U., Matsushita, T., 2012. Characteristics of competitive adsorption between 2-methylisoborneol and natural organic matter on superfine and conventionally sized powdered activated carbons. Water Research 46 (15), 4741–4749.

Najm, I., Snoeyink, V.L., Suidan, M.T., Lee, C.H., Richard, Y., 1990. Effect of particle size and background natural organics on the adsorption efficiency of PAC. Journal of the American Water Works Association 82 (1), 65–72.

Newcombe, G., Cook, D., 2002. Influences on the removal of tastes and odours by PAC. Journal of Water Supply Research and Technology-AQUA 51 (8), 463–474.

Nowack, K.O., Cannon, F.S., Mazyck, D.W., 2004. Enhancing activated carbon adsorption of 2-Methylisoborneol: methane and steam treatments. Environmental Science & Technology 38 (1), 276–284.

Peel, R.G., Benedek, A., 1980. Attainment of equilibrium in activated carbon isotherm studies. Environmental Science and Technology 14 (1), 66–71.

Pendleton, P., Wong, S.H., Schumann, R., Levay, G., Denoyel, R., Rouquero, J., 1997. Properties of activated carbon controlling 2-Methylisoborneol adsorption. Carbon 35 (8), 1141–1149.

Sontheimer, H., Crittenden, J.C., Summers, R.S., 1988. Activated Carbon for Water Treatment, second ed. DVGW-Forschungsstelle, Karlsruhe, Germany.

Srinivasan, R., Sorial, G.A., 2011. Treatment of taste and odor causing compounds 2-methyl isoborneol and geosmin in drinking water: a critical review. Journal of Environmental Sciences 23 (1), 1–13.

Tennant, M.F., Mazyck, D.W., 2007. The role of surface acidity and pore size distribution in the adsorption of 2-methylisoborneol via powdered activated carbon. Carbon 45 (4), 858–864.

Traegner, U.K., Suidan, M.K., Kim, B.R., 1996. Considering age and size distributions of activated-carbon particles in a completely-mixed adsorber at steady state. Water Research 30 (6), 1495–1501.

Yu, J., Yang, M., Lin, T.-F., Guo, Z., Zhang, Y., Gu, J., Zhang, S., 2007. Effects of surface characteristics of activated carbon on the adsorption of 2-methylisobornel (MIB) and geosmin from natural water. Separation and Purification Technology 56 (3), 363–370.

ウイルス処理に有効な新規アルミニウム系凝集剤の開発

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本研究では，凝集剤の塩基度，凝集剤中の硫酸およびアルミニウム形態がウイルスの処理性に与える影響を詳細に評価し，ウイルス処理に有効な新規アルミニウム系凝集剤を開発した．開発した新規凝集剤を凝集沈澱処理に用いた場合，弱酸性および中性のpH領域のみならず，弱アルカリ性のpH領域においても，約6 logの高い除去率が得られ，従来のアルミニウム系凝集剤を用いた場合に比べ，除去率が飛躍的に向上した．また，ESI-FT-MS法および^{27}Al-NMR法による分析の結果，新規凝集剤には，アルミニウム13量体や30量体が含まれていることが明らかとなったことから，これらのアルミニウム種がウイルスの処理性の向上に大きく影響している可能性が示唆された．

Key Words : *aluminum species, basicity, novel aluminum-based coagulant, sulfate ion, virus removal*

1. はじめに

分子生物学的なウイルス検出法および水中からのウイルス回収・濃縮法の発展に伴い，水環境におけるウイルスの実態調査が世界的に広く行われるようになり，我が国においても，水道水源と成り得る水環境中にノロウイルスに代表される水系感染症を引き起こすウイルス（水系感染症ウイルス）が存在していることは周知の事実となってきている[1-4]．従って，水系感染症ウイルスに汚染された環境水を水道水源として取水する場合，仮に浄水処理が不十分であれば，水道水を媒体としたウイルスによる大規模な水系感染症が突発的に発生する可能性は十分に考えられる．実際に，水道水を媒体としたウイルスによる水系感染症の報告もなされている[5-7]．また，気候変動や人口増加に伴う世界的な水不足の顕在化により，これまで使用されてこなかったウイルス汚染レベルの高い低水質の環境水や排水をも水道水源として利用（再利用）する必要が生じてきている[8]．その一方で，トリハロメタンに代表される消毒副生成物による発癌性等の健康影響が指摘され[9]，水系感染症制御のために塩素等の消毒剤の注入量を容易に増加させることが困難な状況となってきている．このような状況から，消毒副生成物の生成を最小限に抑え，ウイルスを含む広範な原水水質に柔軟に対応可能な，低コスト・省エネルギー型の新たな浄水処理技術の開発が求められている[10]．

一方，現行の浄水処理においては，水道水源中に含まれる懸濁質やコロイド粒子，溶解性有機物の除去を目的として，凝集剤の添加による凝集沈澱処理が広く用いられている．凝集沈澱処理におけるウイルスの処理性評価はこれまでに数多くなされており，最適処理条件下では，ノロウイルス，A型肝炎ウイルス，ポリオウイルス等の効果的な除去も期待できることが示されている[11-13]．従って，凝集沈澱処理は，水系感染症発生リスク低減のためのマルチバリアの一つとしても位置付けられている[10]．また，凝集剤の多量注入とpH制御による強化凝集沈澱処理を実施することにより，水系感染症ウイルスのみならず，消毒副生成物の前駆物質である自然由来有機物（NOM）を含む溶解性有機物を効果的に除去できることが報告されている[14,15]．従って，水系感染症ウイルスおよび消毒副生成物による健康被害を低減化する観点から，凝集沈澱処理の重要性が再認識されてきている．

凝集沈澱処理に用いられる凝集剤としては，ポリ塩化アルミニウム（PACl）や硫酸バンドといったアルミニウム系凝集剤が一般的であり，国内/国外を問わず広く普及している．しかしながら，近年の水道水源の富栄養化に伴うpH上昇により，従来のアルミニウム系凝集剤では最適pH条件下（中性付近）での処理が困難な状況が生じてきている．このような弱アルカリ性の原水にお

表-1. プライマーとプローブの塩基配列

Coliphage		Oligonucleotide sequences	Positions	Reference
MS2	Forward primer	5'-GTC GCG GTA ATT GGC GC-3'	632-648	22)
	Reverse primer	5'-GGC CAC GTG TTT TGA TCG A-3'	690-708	
	TaqMan probe	5'-AGG CGC TCC GCT ACC TTG CCC T-3'	650-671	

いては，ウイルスの処理性も著しく低下することが知られており[16]，最適pH条件下での処理のために凝集剤の多量注入や酸注入によるpH制御を実施せざるを得ない場合も多く，結果として，薬品の大量消費，注入設備の増設，管理の煩雑性，更には処理水中の残留アルミニウム濃度の増加といった問題が生じている[17,18]．これに対し，PACIの塩基度（アルミニウムと結合したOHの当量数/アルミニウムの当量数）を高めた高塩基度PACI（塩基度70%）を凝集沈澱処理に用いた場合，従来のPACI（塩基度50%）や硫酸バンドに比べ，中性付近の原水のみならず，弱アルカリ性の原水においても溶解性有機物を効果的に除去可能であり，また，処理水中の残留アルミニウム濃度をも低減可能であることが報告されている[19]．

そこで，本研究では，組成の異なる複数のアルミニウム系凝集剤を実験に使用し，凝集剤の塩基度がウイルスの処理性に与える影響を評価した．また，従来のPACIには，フロック形成速度の促進等を目的に，加水分解しない範囲であらかじめ硫酸が適当量混合されるが，凝集剤中の硫酸がウイルスの処理性に与える影響についてはこれまでに知見が得られていないことから，凝集剤に混合される硫酸がウイルスの処理性に与える影響についても評価した．加えて，凝集剤中のアルミニウム形態等の特性を詳細に分析し，これらの結果を踏まえた上で，ウイルス処理に有効な新規アルミニウム系凝集剤を開発した．

これまで，凝集沈澱処理におけるpHや凝集剤添加濃度等の凝集条件を最適化することによりウイルスの処理性を向上させようとした例は数多くあるが[13-15]，凝集剤自体を根本から開発し，ウイルスの処理性を向上させようとした例はない．従って，本研究は，ウイルス処理に影響を与え得る凝集剤の特性を詳細に把握した上で，ウイルス処理に有効な新規アルミニウム系凝集剤の開発を目指した世界で初めての試みである．

2. 実験方法

(1) 使用したウイルスの培養，精製，定量法

本研究では，（独）製品評価技術基盤機構（NITE）バイオテクノロジー分野 生物遺伝資源部門（NBRC）から分譲された大腸菌ファージMS2（NBRC 102619）を使用した．レビウイルス科に属する大腸菌ファージMS2は，直径約24 nmの正20面体構造を有しており，一本鎖RNAを遺伝子として持つ．この構造がA型肝炎ウイルスやポリオウイルスと類似しているため，水系感染症ウイルスの代替指標ウイルスとして広く用いられている[12-15,20]．

MS2は，F繊毛大腸菌（NBRC 13965）を用いて37℃のシェイキングバス内にて22-24時間振とう培養した後，2,000 × gにて10分間遠心分離し，上清をメンブレンフィルター（膜孔径 0.45 μm，酢酸セルロース，Advantec）にて滅菌ろ過することにより高濃度保存液を得た．得られた高濃度保存液中の有機物の持ち込みを低減させるため，実験に先立ち，遠心式フィルターユニットAmicon Ultra-15（分画分子量 100,000，再生セルロース，Millipore）を用いて，12 mLの高濃度保存液を5,000 × gにて20分間遠心濃縮し，得られた約100 μLの濃縮液に12 mLのMilli-Q水を加えることでバッファー置換した．

MS2の定量には，プラック形成法およびリアルタイム定量RT-PCR法を用いた．なお，プラック形成法は，Adams[21]の方法に従って行った．一方，リアルタイム定量RT-PCR法においては，QIAamp MinElute Virus Spin Kit（Qiagen）を用いてMS2のRNAを抽出し，これをHigh Capacity cDNA Reverse Transcription Kit with RNase Inhibitor（Applied Biosystems）を用いて逆転写させ，cDNAを合成した．このcDNAをTaqMan Universal PCR Master Mix with UNG（Applied Biosystems），プライマー（最終濃度 400 nM，タカラバイオ），プローブ（最終濃度 250 nM，Applied Biosystems），Distilled waterと混合した後，リアルタイム定量PCR装置（Applied Biosystems 7,300，Applied Biosystems）に供した．本研究で使用したプライマーおよびプローブの塩基配列を表-1に示す．なお，PCR反応は，50℃で2分間および95℃で10分間の加熱を行った後，95℃で15秒間と60℃で1分間から成るサイクルを40回繰り返した．

(2) 使用した凝集剤

a) ポリ塩化アルミニウム（PACI）

本研究では，我が国の浄水処理場で従来から広く用いられているPACI（PACI-B50s: 塩基度 50%，硫酸 2-3%，多木化学）に加え，凝集剤の塩基度がウイルスの処理性に与える影響を評価するため，PACIの塩基度を高めた高塩基度PACI（PACI-B70s: 塩基度 70%，硫酸 2-3%，多木化

学）を実験に使用した．また，凝集剤中の硫酸がウイルスの処理性に与える影響を評価するため，硫酸無添加の高塩基度PACl（PACl-B70ns: 塩基度 70%，硫酸 0%，多木化学）を実験に使用した．なお，各凝集剤は，使用まで4℃にて冷蔵保存した．

b) 高塩基性塩化アルミニウム（HPA）

本研究では，凝集剤中のアルミニウム形態の大きく異なる複数のHPAを作製し，実験に使用した．HPAは，加熱撹拌下で塩化アルミニウム溶液にNaOHを添加し，室温下で静置させることにより作製した．なお，塩基度，塩化アルミニウム溶液濃度，NaOH濃度，加熱撹拌時間を変化させることにより，凝集剤中のアルミニウム形態の大きく異なる複数のHPAを作製した．なお，各凝集剤は，作製後3日以上常温で静置することにより安定化させた後，使用まで4℃にて冷蔵保存した．

(3) 使用した凝集剤の分析

a) フェロン法

各凝集剤を0.1 mol-Al/LになるようにMilli-Q水にて希釈した後，フェロン（8-ヒドロキシ-7-ヨードキノリン-5-スルホン酸，$C_9H_6INO_4S$，和光純薬工業），酢酸ナトリウム，HClおよびMilli-Q水により作製したフェロン混合溶液25 mLに20 μL添加した．これをマグネティックスターラーを用いて10秒間撹拌した後，凝集剤添加1分後および120分後の吸光度（波長366 nm）を分光光度計（UV-1700，島津製作所）にて測定した（分析時のpHは4-5程度）．フェロン法は，フェロンと凝集剤に含まれるアルミニウム種の反応速度の違いから，アルミニウム種の形態存在割合を分析する手法であることから[23,24]，フェロンと1分以内に反応したアルミニウム種をモノマー状のアルミニウム種（Alモノマー），1分から120分の間に反応したアルミニウム種をポリマー状態のアルミニウム種（Alポリマー），120分以降もフェロンと反応しなかったアルミニウム種をコロイド状のアルミニウム種（Alコロイド）とした．なお，希釈した0.1 mol-Al/Lの各凝集剤のpHを0.5になるように硝酸にて調整し，これをドライオーブンを用いて85℃にて3時間加熱することにより，凝集剤に含まれるAlポリマーおよびAlコロイドをAlモノマーまで分解させた後，上述した手順で凝集剤添加1分後の吸光度を測定したものを全アルミニウムとし，凝集剤中のアルミニウム種の形態存在割合を計算した．

b) ESI-FT-MS法

各凝集剤を20 mg-Al/LになるようにMilli-Q水にて希釈したものを試料とし，ESI-FT-MS装置（Exactive，Thermo Scientific）に供した．分析はシリンジポンプを用いたインフュージョン法で実施し，ポジティブイオンモード，分解能 50,000，質量範囲 50-1,000 m/z，スプレーボルテージ 3.0 kV，キャピラリーボルテージ 25 V，チューブレンズボルテージ 55 V，スキマーボルテージ 15 V，流速 200 μL/minとした（分析時のpHは4-5程度）．分析により得られたマスススペクトルから，凝集剤中のアルミニウム種を特定した．

c) ^{27}Al-NMR法

各凝集剤を0.1 mol-Al/LになるようにMilli-Q水にて希釈した後，重水を75%（v/v）となるよう添加したものを試料とした．これをNMR分析用石英製サンプルチューブ（外径 5.0 mm，内径 4.2 mm，シゲミ）に注入した．また，アルミン酸ナトリウムを0.01 mol-Al/LになるようにMilli-Q水にて希釈した後，重水を75%（v/v）となるよう添加したものを内部標準物質として使用した．内部標準物質は，NMR分析用ガラス製細型チューブ（外径 3.0 mm，内径 2.5 mm，シゲミ）に注入した後，試料を含むサンプルチューブ内に挿入し，二重管構造とした．この二重管にチューブキャップ（ポリエチレン，和光純薬工業）をした後，NMR装置（ECA-600，JEOL）に供した．なお，塩化アルミニウムを上述した手順で調整したものを基準物質とし，試料の化学シフトを計算した．分析はシングルパルス法で実施し，磁場強度 14.09 T，共鳴周波数 156.39 MHz，パルス幅 5.0 μs，パルス繰り返し時間 1.13 s，観測範囲 78,247，ポイント数 65,536，積算回数 8,000回とした（分析時のpHは4-5程度）．分析により得られたNMRスペクトルから，凝集剤中のアルミニウム種を特定した．

d) コロイド滴定法

Milli-Q水150 mLに滴定終点検出指示薬としてトルイジンブルー指示薬溶液（$C_{15}H_{16}ClN_3S$，和光純薬工業）を300 μL添加し，マグネティックスターラーを用いて撹拌した．ここに，凝集剤を1.0，1.5，1.6，2.0 mg-Al/Lになるように添加した後，直ちにアニオンポリマーであるポリビニル硫酸カリウムによる滴定を開始し，トルイジンブルーの色調が変化した点を滴定終点とした．滴定操作および滴定終点の検出は，自動滴定装置（COM-555，平沼産業）を用いて行い，ポリビニル硫酸カリウムの滴定量から凝集剤のコロイド荷電量を計算した．

(4) 凝集沈澱処理実験

本研究では，回分式凝集沈澱処理による大腸菌ファージMS2の処理性を評価した．精製したMS2を10^8 PFU/mLになるように添加した北海道札幌市豊平川河川水（札幌市水道局藻岩浄水場原水: pH 7.4，DOC 0.8 mg/L，UV260

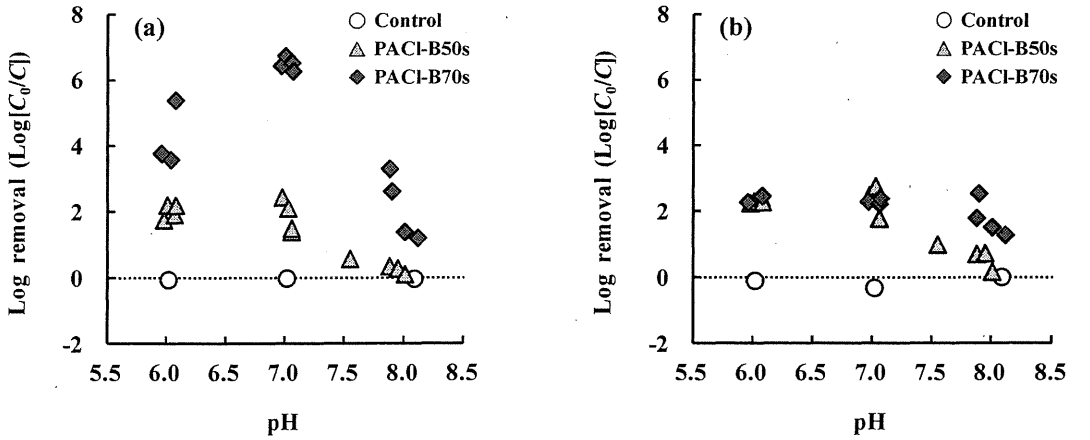

図-1. 凝集剤の塩基度がMS2の処理性に与える影響: MS2濃度はプラック形成法（a）およびリアルタイム定量RT-PCR法（b）にて定量

0.029 cm^{-1}，濁度 7.2 NTU，アルカリ度 17.1 mg-$CaCO_3$/L）を原水とし，角型ビーカーに1,000 mL添加した．ここに，凝集剤を1.89 mg-Al/L（河川水採水時の藻岩浄水場における凝集剤添加濃度）になるように添加し，直ちにHClあるいはNaOHにてpHを6，7あるいは8に調整した．これを攪拌翼を用いてG値200 s^{-1}にて1分間急速攪拌，20 s^{-1}にて10分間緩速攪拌し，60分間静置した．原水および静置後の上澄水約600 mLを採取し，それぞれのMS2濃度をプラック形成法およびリアルタイム定量RT-PCR法にて定量することにより，MS2の凝集沈澱処理性を評価した．また，原水および上澄水をメンブレンフルター（膜孔径 0.45 μm，PTFE，Advantec）にてろ過し，それぞれの試料の吸光度（波長260 nm）を分光光度計（UV-1700，島津製作所）にて測定することにより，NOMの処理性を評価した．加えて，ろ過後の試料に硝酸を1%（v/v）となるよう添加した後，ICP-MS（Agilent 7,700， Agilent Technologies）にてアルミニウム濃度を測定することにより，凝集剤の残留アルミニウム性を評価した．

(5) 粒径分布および電気移動度測定

河川水中における大腸菌ファージMS2の粒径および表面電位特性を把握するために，粒径分布および電気移動度の測定を行った．分画分子量100,000のUF膜（YM-100，再生セルロース，Millipore）にてろ過した豊平川河川水を，HClを用いてpH 7に調整した後，精製したMS2を10^{10} PFU/mLになるように添加した．この試料の粒径分布および電気移動度をゼータ電位・粒子径・分子量測定装置（Zetasizer Nano ZS，Malvern）にて測定した．

3. 結果と考察

(1) 凝集剤の塩基度がウイルスの処理性に与える影響

従来PACl（PACl-B50s）とPAClの塩基度を高めた高塩基度PACl（PACl-B70s）を用いた場合の凝集沈澱処理後（静置後）のMS2の除去率を図-1に示す．なお，図の縦軸はLog[C_0/C]（C_0: 原水のMS2濃度，C: 処理水のMS2濃度）にて表記し，MS2濃度はプラック形成法（a）およびリアルタイム定量RT-PCR法（b）にて定量した．図より，凝集剤を添加しない場合は，いずれのpH領域においてもMS2は全く除去されなかった．河川水中におけるMS2の粒径分布を測定したところ，MS2の粒径は，20-30 nmの範囲となり，電子顕微鏡観察により確認された直径と同程度であった[25]．また，河川水中におけるMS2の電気移動度を測定したところ，-1.734 (μm/s)/(V/cm)となり，負に帯電していることが明らかとなった．従って，本研究で使用した河川水中においては，負に帯電した粒子間の電気的反発力により，MS2が凝集塊を形成せず，安定的に単分散した状態で存在していたことから，凝集剤を添加しない場合は，いずれのpH領域においてもMS2は全く除去されなかったものと考えられた．これに対し，PACl-B50sを用いた場合，pH 6付近の弱酸性領域およびpH 7付近の中性領域において約2 logの除去率が得られた．これは，凝集剤の添加により，負に帯電したMS2および共存する懸濁質の表面電位が中和され，MS2間あるいはMS2と懸濁質間の引力（ファンデルワールス力）が電気的反発力に比べて大きくなることにより，自重沈降可能な大きさまで凝集粗大化し，静置により水相から沈澱除去されたためであると考えられた．なお，Hijnenらは，既往の凝集沈澱処理によるウイルス除去研究を実験スケール等を考慮した上でReviewしており，凝集沈澱処理により1.8 logの除去率が得られると推定している[26]．このことから，従来PAClであるPACl-B50sを用いた場合に得られた約2 logの除去率は，妥当な値であると考えられる．一方，pH 8付近の弱アルカリ性領域では，弱酸性および中性領域に比べて除去率が著しく低下した．

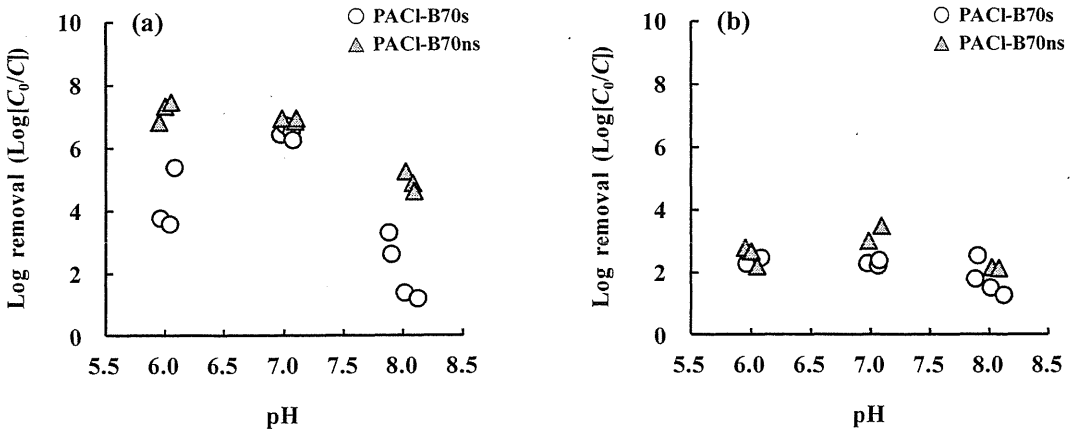

図-2. 凝集剤中の硫酸がMS2の処理性に与える影響：MS2濃度はプラック形成法（a）およびリアルタイム定量RT-PCR法（b）にて定量

従って，従来PACIを用いた凝集沈澱処理においては，弱アルカリ性のpH領域ではウイルスの除去率がほとんど期待できないことが明らかとなった．

これに対し，PACIの塩基度を高めたPACl-B70sを用いた場合，プラック形成法にて評価した除去率は，pH 6付近では4-5 log程度，pH 7付近では更に向上し，6-7 log程度となった．また，pH 8付近においても，1-2 log程度の除去率が得られ，いずれのpH領域においても，PACl-B50sを用いた場合に比べて高い除去率が得られた．一方，リアルタイム定量RT-PCR法にて評価した除去率は，pH 6付近およびpH 7付近においては，PACl-B50sを用いた場合と同程度であり，pH 8付近においては，PACl-B50sを用いた場合に比べて高い除去率が得られた．加えて，PACl-B70sを用いた場合，pH 6付近およびpH 7付近においては，プラック形成法にて評価した除去率とリアルタイム定量RT-PCR法にて評価した除去率の間に2-5 log程度の差が見られ，プラック形成法にて評価した除去率の方がリアルタイム定量RT-PCR法にて評価した除去率に比べて高くなった．2つの定量法によって得られた結果に差が生じた原因として，感染力を失ったウイルス，すなわち，不活化したウイルスの存在と，幾つかの感染性のあるウイルスによって形成される凝集塊をプラック形成法にて評価した場合に生じる除去率の過大評価の2つが考えられた．なお，Matsushitaらは，PACIを用いた凝集沈澱処理によってウイルスが除去されるのみならず不活化されることを報告している[27]．このことから，PACl-B70sを用いた凝集沈澱処理においては，ウイルスの物理的な除去のみならず，不活化効果も期待できる可能性が示唆された．以上の結果から，凝集剤の塩基度はウイルスの処理性に影響し，塩基度の高い凝集剤の方がウイルス処理に有効であることが示された．

(2) 凝集剤中の硫酸がウイルスの処理性に与える影響

硫酸無添加の高塩基度PACl（PACl-B70ns）を用いた場合の凝集沈澱処理後のMS2の除去率を図-2に示す．なお，MS2濃度はプラック形成法（a）およびリアルタイム定量RT-PCR法（b）にて定量した．図より，硫酸無添加のPACl-B70nsを用いた場合，プラック形成法にて評価した除去率は，pH 6付近およびpH 7付近において約7 logとなった．また，pH 8付近においても，約5 logの除去率が得られ，いずれのpH領域においても，硫酸を含む高塩基度PACl（PACl-B70s）を用いた場合に比べて高い除去率が得られた．一方，リアルタイム定量RT-PCR法にて評価した除去率は，いずれのpH領域においても，PACl-B70sを用いた場合と同程度であった．なお，PACl-B70nsを用いた場合，pH 6付近およびpH 7付近のみならず，pH 8付近においても，プラック形成法にて評価した除去率とリアルタイム定量RT-PCR法にて評価した除去率の間に約3 logの差が見られたことから，PACl-B70nsを用いた凝集沈澱処理においては，弱酸性および中性のpH領域のみならず，弱アルカリ性のpH領域においてもウイルスの不活化効果が期待できる可能性が示唆された．以上の結果から，凝集剤中の硫酸はウイルスの処理性に影響し，硫酸を含まない凝集剤の方がウイルス処理に有効であることが示された．

(3) フェロン法，コロイド滴定法による凝集剤の分析

フェロン法により得られた各凝集剤中のアルミニウム種の形態存在割合を図-3に示す．図より，ウイルスの処理性が高かったPACl-B70sおよびPACl-B70nsは，硫酸の有無に関わらずPACl-B50sに比べてAlモノマーの存在割合が小さく，Alコロイドの存在割合が大きかった．従って，凝集剤の塩基度がアルミニウム種の形態存在割合に与える影響は大きいことが明らかとなった．既往研究に

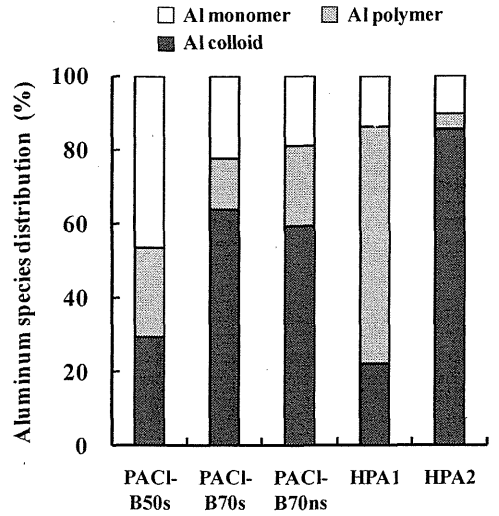

図-3. フェロン法により得られた各凝集剤中のアルミニウム種の形態存在割合

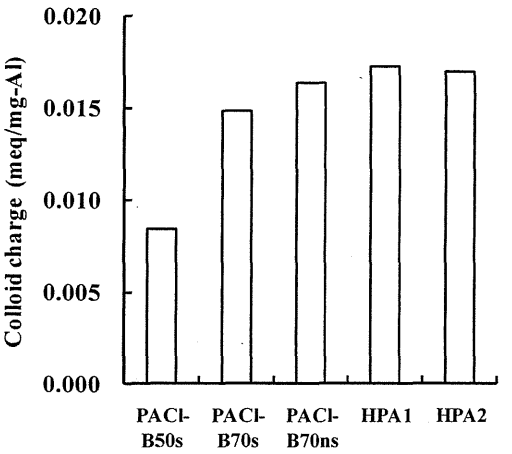

図-4. コロイド滴定法により得られた各凝集剤のコロイド荷電量

おいても，凝集剤の塩基度を高めることにより，凝集剤中のAlモノマーの存在割合が減少し，Alコロイドの存在割合が増加することが報告されている[23,28,29]．一方，高塩基度PAClにおいては，硫酸の有無に関わらず，いずれのアルミニウム種の形態存在割合も同程度であった．従って，凝集剤中の硫酸がアルミニウム種の形態存在割合に与える影響は小さいものと考えられた．

コロイド滴定法により得られた各凝集剤のコロイド荷電量を図-4に示す．PACl-B70sは，PACl-B50sに比べてコロイド荷電量が大きかった．従って，凝集剤の塩基度を高めることにより，凝集剤中のAlコロイドの存在割合が増加し，結果として荷電中和力が増加したことによりウイルスの処理性が向上した可能性が示唆された．また，硫酸無添加のPACl-B70nsは，硫酸を含むPACl-B70sに比べてコロイド荷電量が統計学的に有意に大きかった（t検定，$n = 3$，$P < 0.05$）．PAClに硫酸を混合させることにより，弱酸性のpH領域における濁度の処理性が向上することが報告されているが[28]，コロイド荷電量については減少することが明らかとなった．従って，凝集剤中に硫酸を含まないことにより，荷電中和力が増加し，結果としてウイルスの処理性が向上した可能性が示唆された．

(4) 新規アルミニウム系凝集剤の作製

上述したように，塩基度が高く，硫酸を含まない凝集剤を凝集沈澱処理に用いることにより，弱酸性，中性，弱アルカリ性のいずれのpH領域においても，高いウイルスの処理性が得られることが明らかとなった．また，塩基度の増加に伴う凝集剤中のAlコロイドの存在割合の増加がウイルスの処理性の向上に影響している可能性が考えられた．そこで，凝集剤中のアルミニウム形態がウイルスの処理性に与える影響をより詳細に評価するため，凝集剤中のアルミニウム形態の大きく異なる複数のHPAを作製した．HPAの作製においては，塩基度を高めることによりAlポリマーの存在割合が増加し，塩化アルミニウム溶液濃度およびNaOH濃度を高め，且つ加熱撹拌時間を長くすることによりAlコロイドの存在割合が増加する傾向が見られた．これらの傾向を考慮し，Alポリマーの存在割合が大きいHPA1（塩基度 70%，硫酸 0%）およびAlコロイドの存在割合が大きいHPA2（塩基度 70%，硫酸 0%）を作製した（図-3）．なお，これら2種類のHPAのコロイド荷電量は，凝集剤中のアルミニウム形態が大きく異なるにも関わらず同程度であった（図-4）．

(5) 新規アルミニウム系凝集剤によるウイルスの処理

作製したHPA1およびHPA2を用いた場合の凝集沈澱処理後のMS2の除去率を図-5に示す．なお，MS2濃度はプラック形成法（a）およびリアルタイム定量RT-PCR法（b）にて定量した．図より，Alポリマーの存在割合が大きいHPA1を用いた場合，プラック形成法にて評価した除去率は，pH 6付近およびpH 7付近において，7-8 log程度となった．一方，pH 8付近においては，弱酸性および中性のpH領域に比べて除去率が低下したものの，3-5 log程度の除去率が得られた．これに対し，Alコロイドの存在割合が大きいHPA2を用いた場合，pH 6付近およびpH 7付近においては，HPA1を用いた場合と同程度の除去率であったのに対し，pH 8付近では，HPA1を用いた場合に比べて約2 log高い6-7 log程度の除去率が得られた．また，弱酸性および弱アルカリ性のpH領域において，硫酸無添加のPACl-B70nsを用いた場合に比べて高い

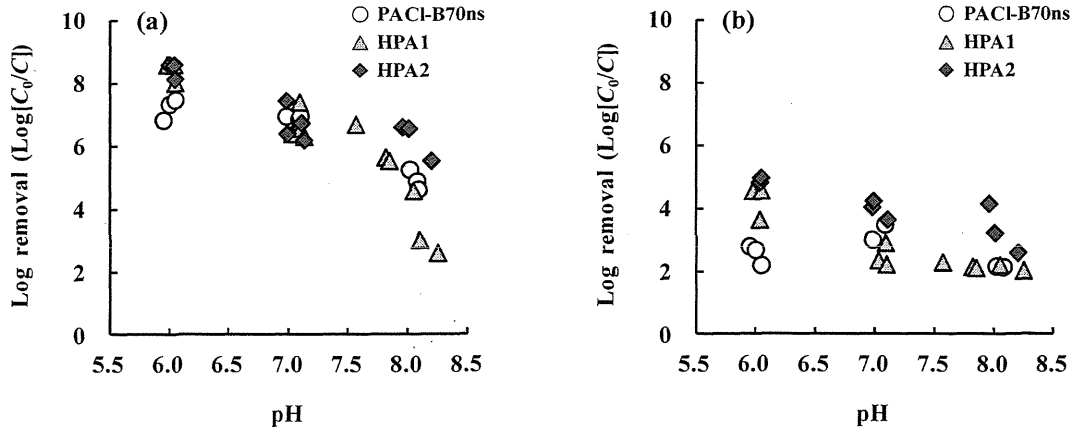

図-5. 新規アルミニウム系凝集剤によるMS2の処理: MS2濃度はプラック形成法（a）およびリアルタイム定量RT-PCR法（b）にて定量

除去率が得られた．リアルタイム定量RT-PCR法にて評価した除去率についても，HPA1およびPACl-B70nsを用いた場合に比べて高い除去率が得られた．以上の結果から，凝集剤中のアルミニウム形態はウイルスの処理性に影響し，Alコロイドの存在割合が大きい凝集剤の方がウイルス処理に有効であることが示された．

(6) ESI-FT-MS法，^{27}Al-NMR法による凝集剤の分析

作製したHPA1とHPA2に含まれるアルミニウム種を特定するために，ESI-FT-MS法および^{27}Al-NMR法による分析を行った．ESI-FT-MS法により得られたHPA1およびHPA2のマススペクトルを図-6に示す．図より，いずれの凝集剤においても，アルミニウム単量体（m/z = 97, $[Al(OH)_2(H_2O)_2]^+$）[30]のピークの相対強度が最も大きく，相対強度80%程度のアルミニウム2量体（m/z = 157, $[Al_2H_7O_6]^+$）[30]のピークも検出された．加えて，HPA1においては，相対強度20%以上において，m/z = 309, 315, 321, 445のピークが，また，HPA2においては，m/z = 315, 321, 436, 445, 454のピークが検出された．これらのピークは，アルミニウム13量体のフラグメントイオンであることが報告されていることから（m/z = 309, $[Al_{13}O_4(OH)_{28}(H_2O)_2]^{3+}$, m/z = 315, $[Al_{13}O_4(OH)_{28}(H_2O)_3]^{3+}$, m/z = 321, $[Al_{13}O_4(OH)_{28}(H_2O)_4]^{3+}$, m/z = 436, $[Al_{13}O_6(OH)_{25}]^{2+}$, m/z = 445, $[Al_{13}O_5(OH)_{27}]^{2+}$, m/z = 454, $[Al_{13}O_4(OH)_{29}]^{2+}$）[31]，作製したHPAにはアルミニウム13量体が含まれていることが明らかとなった．

^{27}Al-NMR法により得られたHPA1およびHPA2のNMRスペクトルを図-7に示す．図より，いずれの凝集剤においても，アルミニウム単量体（δ = 0 ppm），アルミニウム13量体（δ = 63 ppm），内部標準物質として使用したアルミン酸ナトリウム（δ = 80 ppm）[32,33]のピークが検出された．また，HPA1のアルミニウム13量体のピーク

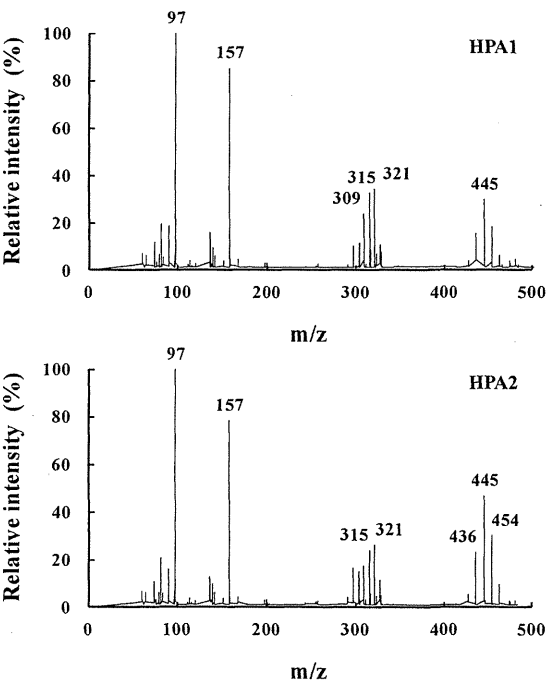

図-6. ESI-FT-MS法により得られたHPA1およびHPA2のマススペクトル

は，HPA2のピークに比べて大きかった．一方，HPA2においては，HPA1には見られなかったδ = 10-12 ppmおよび70 ppmにおいてブロード状のピークが検出された．δ = 10-12 ppmのピークは，アルミニウム13量体およびアルミニウム30量体の外殻部分の存在を，また，δ = 70 ppmのピークは，アルミニウム30量体の核部分の存在を示すことが報告されていることから[32]，作製したHPA2にはアルミニウム13量体のみならずアルミニウム30量体が含まれていることが明らかとなった．これに対し，従来PAClであるPACl-B50sにおいては，ESI-FT-MS法および^{27}Al-NMR法のいずれの分析においても，アルミニウム13

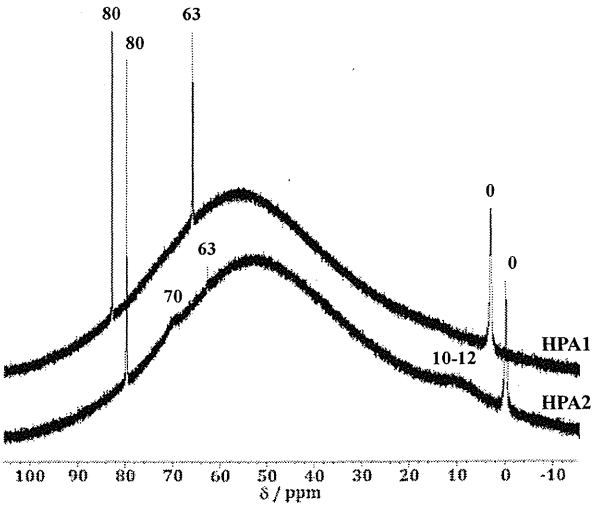

図-7. ^{27}Al-NMR法により得られたHPA1およびHPA2のNMRスペクトル

量体および30量体のピークは検出されなかった．以上の結果から，アルミニウム13量体および30量体の存在がウイルスの処理性に大きく影響し，中でも，アルミニウム30量体を含むAlコロイドを主成分とする凝集剤を凝集沈澱処理に用いることにより，弱酸性，中性，弱アルカリ性のいずれのpH領域においても，従来PACl（PACl-B50s）に比べてウイルスの除去率を飛躍的に向上できる可能性が示唆された．

(7) 開発した新規アルミニウム系凝集剤の適用可能性

本研究で開発した塩基度70%，硫酸無添加のAlコロイドを主成分とする新規アルミニウム系凝集剤（HPA2）を凝集沈澱処理に用いた場合，弱酸性，中性，弱アルカリ性のいずれのpH領域においても，約6 logあるいはそれ以上の高いウイルスの除去率が得られたことから，従来のアルミニウム系凝集剤（PACl-B50s）を用いた場合に比べ，後段の消毒処理への負荷低減が可能であり，凝集剤使用量の削減にも繋がるものと考えられる．また，本研究で使用した凝集剤の中で，消毒副生成物の前駆物質であるNOMの除去率が最も高く（pH 8付近においても60%以上，PACl-B50sを用いた場合は20-30%程度），処理水中の残留アルミニウム濃度も最も低かったことから（pH 8付近においても0.06 mg-Al/L未満，PACl-B50sを用いた場合は0.3-0.4 mg-Al/L程度），水系感染症発生リスクの低減のみならず，消毒副生成物の生成抑制や残留アルミニウム濃度の低減にも繋がるものと考えられる．以上のことから，本研究で開発した新規アルミニウム系凝集剤を凝集沈澱処理に適用することにより，凝集剤の多量注入や酸注入によるpH制御に頼ることなく，広範なpH領域の原水のウイルス処理に対応可能であると考えられる．

4. 結論

本研究では，凝集剤の塩基度，凝集剤中の硫酸およびアルミニウム形態がウイルスの処理性に与える影響を詳細に評価し，これらの結果を踏まえた上で，ウイルス処理に有効な新規アルミニウム系凝集剤を開発した．

本研究で得られた知見を以下にまとめる．

(1) 凝集剤の塩基度および凝集剤中の硫酸はウイルスの処理性に影響し，塩基度が高く，硫酸を含まない凝集剤を用いることにより，従来PAClに比べて高い除去率が得られた．

(2) 凝集剤中のアルミニウム形態はウイルスの処理性に影響し，Alコロイドの存在割合が大きい凝集剤は，Alポリマーの存在割合が大きい凝集剤に比べてウイルス処理に有効であった．

(3) Alコロイドの存在割合が大きい凝集剤には，アルミニウム13量体のみならずアルミニウム30量体が含まれていたことから，これらのアルミニウム種がウイルスの処理性の向上に大きく影響している可能性が示唆された．

(4) 本研究で開発したAlコロイドを主成分とする新規アルミニウム系凝集剤を凝集沈澱処理に用いることにより，弱酸性，中性，弱アルカリ性のいずれのpH領域においても，約6 logあるいはそれ以上の高いウイルス除去率が得られた．

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参考文献

1) Haramoto E., Katayama H., Oguma K. and Ohgaki S.: Application of cation-coated filter method to detection of noroviruses, enteroviruses, adenoviruses, and torque teno viruses in the Tamagawa River in Japan, *Applied and Environmental Microbiology*, Vol. 71, No. 5, pp. 2403-2411, 2005.

2) Sano D., Ueki Y., Watanabe T. and Omura T.: Genetic variation in the conservative gene region of norovirus genogroup II strains in environmental and stool samples, *Environmental Science and Technology*,

Vol. 40, No. 23, pp. 7423-7427, 2006.

3) Kitajima M., Oka T., Haramoto E., Katayama H., Takeda N., Katayama K. and Ohgaki S.: Detection and genetic analysis of human sapoviruses in river water in Japan, *Applied and Environmental Microbiology*, Vol. 76, No. 8, pp. 2461-2467, 2010.
4) Kishida N., Morita H., Haramoto E., Asami M. and Akiba M.: One-year weekly survey of noroviruses and enteric adenoviruses in the Tone River water in Tokyo metropolitan area, Japan, *Water Research*, Vol. 46, No. 9, pp. 2905-2910, 2012.
5) Kukkula M., Maunula L., Silvennoinen E. and von Bonsdorff C.H.: Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses, *Journal of Infectious Diseases*, Vol. 180, No. 6, pp. 1771-1776, 1999.
6) Nygard K., Torven M., Ancker C., Knauth S.B., Hedlund K.O., Giesecke J., Andersson Y. and Svensson, L.: Emerging genotype (GGIIb) of norovirus in drinking water, Sweden, *Emerging Infectious Diseases* Vol. 9, No. 12, pp. 1548-1552, 2003.
7) Maunula L., Miettinen I. T. and von Bonsdorff C.H.: Norovirus outbreaks from drinking water, *Emerging Infectious Diseases*, Vol. 11, No. 11, pp. 1716-1721, 2005.
8) Bosch A.: Human Viruses in Water, pp. 109-110, Elsevier, Amsterdam, NL, 2007.
9) Dunnick J.K. and Melnick R.L.: Assessment of the carcinogenic potential of chlorinated water−experimental stsudies of chlorine, chloramine, and trihalomethanes, *Journal of the National Cancer Institute*, Vol. 85, No. 10, pp. 817-822, 1993.
10) Shannon M.A., Bohn P.W., Elimelech M., Georgiadis J.G., Marinas B.J. and Mayes A.M.: Science and technology for water purification in the coming decades, *Nature*, Vol. 452, No. 7185, pp. 301-310, 2008.
11) Guy M.D., Mciver J.D. and Lewis M.J.: Removal of virus by a pilot treatment-plant, *Water Research*, Vol. 11, No. 5, pp. 421-428, 1977.
12) Nasser A., Weinberg D., Dinoor N., Fattal B. and Adin A.: Removal of hepatitis A virus (HAV), poliovirus and MS2 coliphage by coagulation and high-rate filtration, *Water Science and Technology*, Vol. 31, No. 5-6, pp. 63-68, 1995.
13) 白崎伸隆，松下拓，松井佳彦，大芝淳，浦崎稔史，大野浩一：遺伝子組換えノロウイルス外套タンパク粒子（rNV-VLPs）を用いたヒトノロウイルスの浄水処理性評価，環境工学研究論文集，Vol. 46, pp. 415-422, 2009.
14) Abbaszadegan M., Mayer B.K., Ryu H. and Nwachuku N.: Efficacy of removal of CCL viruses under enhanced coagulation conditions, *Environmental Science and Technology*, Vol. 41, No. 3, pp. 971-977, 2007.
15) Mayer B.K., Ryu H. and Abbaszadegan M.: Treatability of U.S. Environmental Protection Agency contaminant candidate list viruses: removal of coxsackievirus and echovirus using enhanced coagulation, *Environmental Science and Technology*, Vol. 42, No. 18, pp. 6890-6896, 2008.
16) Shirasaki N., Matsushita T., Matsui Y. and Sato S.: Effective removal of virus by high-basicity polyaluminum coagulation treatment, Proc. of IWA International Conference on Particle Separation 2012, pp. 91-98, 2012.
17) 宮崎達彦，佐藤文彦，斎藤薫，中島史貴，喜多俊之：実施設における新規高塩基度 PAC 適用による浄水中残留アルミニウムの低減，第 62 回全国水道研究発表会講演集，pp. 240-241, 2011.
18) 梶原聡，古田純一，宮崎達彦，佐藤文彦：日田市上野浄水場における新規高塩基度 PAC の適用評価，第 63 回全国水道研究発表会講演集，pp. 146-147, 2012.
19) Kimura M., Matsui Y., Oshiba A., Ishikawa T., Machida K., Kondo K., Shirasaki N. and Matsushita T.: Basicity-optimized polyaluminum chloride to minimize dissolved residual aluminum concentration, Proc. of IWA International Conference on Particle Separation 2012, pp. 276-283, 2012.
20) Shirasaki N., Matsushita T., Matsui Y., Urasaki T. and Ohno K.: Comparison of behaviors of two surrogates for pathogenic waterborne viruses, bacteriophages Qβ and MS2, during the aluminum coagulation process, *Water Research*, Vol. 43, No. 3, pp. 605-612, 2009.
21) Adams M.H.: Bacteriophages, pp. 450-454, Interscience Publishers, New York, NY, USA, 1959.
22) O'Connell K.P., Bucher J.R., Anderson P.E., Cao C.J., Khan A.S., Gostomski M.V. and Valdes J.J.: Real-time fluorogenic reverse transcription-PCR assays for detection of bacteriophage MS2, *Applied and Environmental Microbiology*, Vol. 72, No. 1, pp. 478-483, 2006.
23) Wang D.S., Sun W., Xu Y., Tang H.X. and Gregory, J.: Speciation stability of inorganic polymer flocculant-PACl, *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, Vol. 243, No. 1-3, pp. 1-10, 2004.
24) Chen Z.Y., Fan B., Peng X.J., Zhang Z.G., Fan, J.H. and Luan Z.K.: Evaluation of Al_{30} polynuclear species in polyaluminum solutions as coagulant for water treatment, *Chemosphere*, Vol. 64, No. 6, pp. 912-918, 2006.
25) Shirasaki N., Matsushita T., Matsui Y., Kobuke M. and Ohno K.: Comparison of removal performance of two surrogates for pathogenic waterborne viruses, bacteriophage Qβ and MS2, in a coagulation-ceramic microfiltration system, *Journal of Membrane Science*, Vol. 326, No. 2, pp. 564-571, 2009.
26) Hijnen W.A.M. and Medema G.J.: Elimination of Micro-organisms by Water Treatment Processes, pp. 23-34, IWA Publishing, London, UK, 2010.
27) Matsushita T., Shirasaki N., Matsui Y. and Ohno K.: Virus inactivation during coagulation with aluminum coagulants, *Chemosphere*, Vol. 85, No. 4, pp. 571-576, 2011.
28) Wang D.S., Tang H.X. and Gregory J.: Relative importance of charge neutralization and precipitation on coagulation of kaolin with PACl: effect

of sulfate ion, *Environmental Science and Technology*, Vol. 36, No. 8, pp. 1815-1820, 2002.

29) Yang Z.L., Gao B.Y., Cao B.C., Xu W.Y. and Yue Q.Y.: Effect of OH^-/Al^{3+} ratio on the coagulation behavior and residual aluminum speciation of polyaluminum chloride (PAC) in surface water treatment, *Separation and Purification Technology*, Vol. 80, No. 1, pp. 59-66, 2011.

30) Urabe T., Tanaka M., Kumakura S. and Tsugoshi T.: Study on chemical speciation in aluminum chloride solution by ESI-Q-MS, *Journal of Mass Spectrometry*, Vol. 42, No. 5, pp. 591-597, 2007.

31) Stewart T.A., Trudell D.E., Alam T.M., Ohlin C.A., Lawler C., Casey W.H., Jett S. and Nyman M.: Enhanced water purification: a single atom makes a difference, *Environmental Science and Technology*, Vol. 43, No. 14, pp. 5416-5422, 2009.

32) Chen Z.Y., Luan Z.K., Fan J.H., Zhang Z.G., Peng X.J. and Fan B.: Effect of thermal treatment on the formation and transformation of Keggin Al_{13} and Al_{30} species in hydrolytic polymeric aluminum solutions, *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, Vol. 292, No. 2-3, pp. 110-118, 2007.

33) Liu H.J., Hu C.Z., Zhao H. and Qu J.H.: Coagulation of humic acid by PACl with high content of Al_{13}: the role of aluminum speciation, *Separation and Purification Technology*, Vol. 70, No. 2, pp. 225-230, 2009.

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Development of Novel Aluminum-Based Coagulant for Effective Virus Removal

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A novel aluminum-based coagulant for effective virus removal was developed in the present study based on the investigation of roles of the basicity, sulfate ion and aluminum speciation in coagulation of virus. The coagulation process with the novel aluminum-based coagulant effectively removed viruses compared with other aluminum-based coagulants, and achieved apporimately 6-log removals not only in the weakly acidic and neutral pH conditions but also weakly alkaline pH conditions. In addition, Al_{13} and Al_{30} polymers were detecteded by electrospray ionization mass spectrometry and ^{27}Al-NMR spectrometry in the novel aluminum-based coagulant. Accordingly, Al_{13} and Al_{30} species are probably dominant species to control the virus removal performance, and that lead effective removals of viruses in the coagulation process with novel aluminum-based coagulant even in the weakly alkaline pH condition.

Difference in behaviors of F-specific DNA and RNA bacteriophages during coagulation–rapid sand filtration and coagulation–microfiltration processes

N. Shirasaki, T. Matsushita, Y. Matsui, T. Urasaki and K. Ohno

ABSTRACT

Difference in behaviors of F-specific DNA and RNA bacteriophages during coagulation–rapid sand filtration and coagulation–microfiltration (MF) processes were investigated by using river water spiked with F-specific DNA bacteriophage f1 and RNA bacteriophage f2. Because the particle characteristics of f1 (filamentous) and f2 (spherical) are quite different and the surface charge of f1 in the river water was slightly more negative than that of f2, the removal ratios of f1 were approximately 1-log lower than the removal ratio of f2 after any treatment process used in the present study. This result indicates that the behaviors of the two bacteriophages during the treatment processes were different, and that the removal of f1 by the combination of coagulation and filtration processes was more difficult than that of f2. The removal ratios for f1 and f2 were approximately 3-log and 4-log, respectively, in the coagulation–rapid sand filtration process, and 6-log and 7-log, respectively, in the coagulation–MF filtration process. Therefore, as expected, the coagulation–MF process appears to be more effective than the coagulation–rapid sand filtration process for the removal of not only spherical viruses but also filamentous viruses.

Key words | coagulation, F-specific DNA bacteriophage, F-specific RNA bacteriophage, microfiltration, rapid sand filtration

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INTRODUCTION

Microbial safety of drinking water has been of primary interest for public health protection. Most waterborne pathogens are introduced into drinking water supplies by human or animal feces (Guillot & Loret 2010). Because fecal coliform bacteria are consistently present and often abundant in human and animal feces (Nappier *et al.* 2006), these bacteria have traditionally been used as surrogates for fecal contamination in source and drinking water. However, some researchers have demonstrated that fecal coliform bacteria may not be appropriate surrogates for waterborne enteric viruses, owing to the differences in their resistance to drinking water treatment processes (Payment *et al.* 1985; Havelaar *et al.* 1993). In other words, fecal coliform bacteria are less resistant than enteric viruses to physicochemical treatments such as filtration and disinfection processes. Hence, other reliable surrogates are required for enteric viruses so as to guarantee the microbial safety of drinking water.

Bacteriophages, which are viruses that infect bacteria, have been proposed as surrogate candidates for enteric viruses. This is based on the greater similarity of bacteriophages to enteric viruses than to fecal coliform bacteria, in terms of their environmental persistence and resistance to drinking water treatment processes as well as lack of pathogenicity to humans (Stetler 1984; Havelaar *et al.* 1993). Among bacteriophages, especially the F-specific bacteriophages, viruses that infect F+ male *Escherichia coli* bacteria through the F sex pilus, are considered to be better surrogates for enteric viruses (World Health Organization 2008).

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F-specific bacteriophages are categorized into DNA and RNA bacteriophages, and belong to the two families, Inoviridae and Leviviridae (Cole *et al.* 2003). Virions in the Inoviridae family are rods or filaments containing a single molecule of circular, positive-sense, single-stranded DNA, whereas virions in the Leviviridae family are spherical and of icosahedral symmetry and contain a single molecule of linear, positive-sense, single-stranded RNA (Fauquet *et al.* 2005). Because these F-specific DNA and RNA bacteriophages are widely present in fecal waste (Cole *et al.* 2003), the presence, prevalence and population of F-specific DNA and RNA bacteriophages in surface water have been investigated to identify fecal contamination sources (Cole *et al.* 2003; Haramoto *et al.* 2009). In addition, because F-specific RNA bacteriophages are morphologically similar to hepatitis A viruses and polioviruses, the bacteriophages are used worldwide as surrogates for enteric viruses to estimate the removal of enteric viruses during drinking water treatment processes (Shelton & Drewry 1973; Matsushita *et al.* 2005; Zhu *et al.* 2005; Fiksdal & Leiknes 2006; Shirasaki *et al.* 2009; Pierre *et al.* 2010). F-specific DNA bacteriophages are not used as surrogates for enteric viruses because of their morphological differences. However, the F-specific DNA bacteriophage of *Vibrio cholerae* has been implicated in the lysogenic conversion of *V. cholerae* to its toxic form, suggesting that at least some F-specific DNA bacteriophages are indirectly involved in waterborne disease transmission (Waldor & Mekalanos 1996; Redman *et al.* 1999). Accordingly, removal of not only enteric viruses but also F-specific DNA bacteriophages by drinking water treatment processes is important to demonstrate the microbial safety of drinking water. However, removal of F-specific DNA bacteriophages has not been fully investigated in drinking water treatment processes, although the environmental persistence of F-specific DNA bacteriophages has been investigated and compared with that of F-specific RNA bacteriophages (Long & Sobsey 2004).

Our objective in the present study was to investigate the difference in behaviors of F-specific DNA and RNA bacteriophages during the coagulation–rapid sand filtration process, which is commonly used in drinking water treatment facilities, and during the coagulation–microfiltration (MF) process, which is becoming an important technology in this century for drinking water treatment.

MATERIALS AND METHODS

Source water, coagulant, filter media and MF membrane

River water was sampled from the Toyohira River (Sapporo, Japan; water quality shown in Table 1) on 12 October 2007. The coagulant used was a commercial aluminium coagulant, polyaluminium chloride (PACl) (PACl 250A; 10.5% Al_2O_3, relative density 1.2 at 20 °C; Taki Chemical Co., Ltd, Hyogo, Japan). Silica sand (effective size 0.6 mm, uniformity coefficient <3; Nihon Genryo Co., Ltd, Kanagawa, Japan) was used as a filter medium for rapid sand filtration. A flat type of ceramic MF membrane (nominal pore size 0.1 μm, effective filtration area 0.0007 m^2; NGK Insulators, Ltd, Nagoya, Japan), which was installed in an acrylic-resin casing, was used for the MF process.

Bacteriophages

The F-specific DNA bacteriophage, f1 (NBRC 20015), and the F-specific RNA bacteriophage, f2 (NBRC 20011), were obtained from the NITE Biological Research Center (NBRC, Chiba, Japan). f1 is a filamentous particle that has a diameter of 6 nm and length of 800 nm (Dotto *et al.* 1981). In contrast, f2 is an icosahedral particle that has a diameter of 22 nm (Shelton & Drewry 1973). Each bacteriophage was propagated for 22–24 h at 37 °C in *E. coli* (NBRC 13965) obtained from NBRC. The bacteriophage culture solution was centrifuged (2,000×*g*, 10 min) and then passed through a membrane filter (pore size 0.45 μm, hydrophilic cellulose acetate; Dismic-25cs, Toyo Roshi Kaisha, Ltd, Tokyo, Japan). The filtrate was purified by using a centrifugal filter device (molecular weight cutoff 100,000, regenerated cellulose; Centriplus-100, Millipore Corp.,

Table 1 | Water quality of the Toyohira River

pH	7.5
DOC (mg/L)	0.90
OD260 (cm^{-1})	0.027
Turbidity (NTU)	0.50
Alkalinity (mg-$CaCO_3$/L)	19.1

Billerica, MA, USA) to prepare the bacteriophage stock solution. The concentration of each bacteriophage stock solution was approximately 10^{12} PFU/mL.

Coagulation experiments

Batch coagulation experiments were conducted with 200 mL of bacteriophage-spiked river water in glass beakers at 20 °C. The bacteriophage stock solution (see section on Bacteriophages) was added to the beaker at approximately 10^6 or 10^8 PFU/mL, and the spiked water was mixed with an impeller stirrer. Because around 1 mg-Al/L of PACl is usually dosed for the treatment of Toyohira River water, which is the source water in the present study, in the actual drinking water treatment plant (Moiwa drinking water treatment plant, Sapporo, Japan), PACl was injected into the water at a coagulant dose of 0.54, 1.08 or 1.62 mg-Al/L. The pH of the water was immediately adjusted to, and maintained at, 6.8 with HCl. The water was stirred rapidly for 2 min ($G = 200\ s^{-1}$, 61 rpm) and then slowly for 28 min ($G = 20\ s^{-1}$, 13 rpm). The water was then left at rest for 20 min to settle the generated aluminium floc particles. Samples were taken from the beaker before coagulant dosing (C_{c0}) and after settling (C_{cs}) for quantification of the bacteriophage concentrations.

The suspended aluminium floc particles that had not settled by gravity were separated from the floc mixture by centrifugation (2,000×g, 10 min) to quantify the bacteriophage concentration in the liquid phase of the floc mixture.

Rapid sand filtration experiments

After the coagulation experiments (without centrifugal separation), rapid sand filtration experiments were carried out with a glass column (diameter 0.8 cm, length 20 cm) packed with silica sand washed with Milli-Q water and dried at 105 °C for 1 h. The cleaned silica sand was gradually added into the glass column to achieve a filter depth of 10 cm. This column was connected to another such column to achieve a total filter depth of 20 cm. Subsequently, Milli-Q water was pumped through the column with the help of a peristaltic pump for 15 min to saturate the filter medium, and the excess Milli-Q water was drained from the column just before the filtration experiment. Approximately 170 mL of the supernatant of the settling sample (see section on Coagulation experiments) was withdrawn from the beaker by the peristaltic pump and transferred to another glass beaker to be considered as raw water for rapid sand filtration experiments. During the filtration experiments, the raw water was continuously mixed with a magnetic stirrer at 200 rpm and fed into the column at a constant flow rate (120 m/day) by the peristaltic pump. Samples were taken from the beaker (C_{r0}) and from the first (10 cm) and second (20 cm) column filtrates (C_{rf}) at 5, 15 and 30 min after the initiation of filtration for the quantification of bacteriophage concentrations.

MF experiments

After the coagulation experiments (without centrifugal separation), MF filtration experiments were carried out with a flat type of ceramic MF membrane. Approximately 170 mL of the supernatant of the settling sample (see section on Coagulation experiments) was withdrawn from the beaker by the peristaltic pump and transferred to another glass beaker to be considered as raw water for MF filtration experiments. During the filtration experiment, the raw water was continuously mixed with a magnetic stirrer at 200 rpm and fed into a ceramic MF membrane at a constant flux (83 L/(m^2 h)) by the peristaltic pump. Samples were taken from the beaker (C_{m0}) and from the MF permeate (C_{mf}) at 15, 30, 60 and 120 min after the initiation of filtration for the quantification of bacteriophage concentrations.

Bacteriophage assay

The infectious bacteriophages were enumerated by the determination of the number of plaque-forming units (PFU) according to the double-layer method (Adams 1959) with the bacterial host *E. coli* (NBRC 13965). The average of the plaque counts of triplicate plates prepared from one sample was considered as the infectious bacteriophage concentration for that sample.

Electron micrograph

Negative-stain electron microscopy was used to analyze the morphology of the bacteriophages. Ten microliters of f1 or

f2 stock solution (see section on Bacteriophages) was placed on a 400-mesh copper grid with collodion membrane (Nissin EM Corp., Tokyo, Japan) and adsorbed onto the grid for 1 min. Excess solution was drained from the side of the grid with filter paper, and f1 or f2 was negatively stained with 10 μL of 2% phosphotungstic acid (pH 7.0) for 45 s. After the excess strain was drained off, the grid was examined with a transmission electron microscope (TEM, JEM-1210, Jeol Ltd, Tokyo, Japan).

Electrophoretic mobility

The electrophoretic mobility of bacteriophages was measured in prepared Milli-Q water and in filtered river water. Alkalinity was adjusted to 20 mg-$CaCO_3$/L with 0.4 mM $NaHCO_3$, and HCl was used to adjust the pH to 6.8. River water was filtered through a stirred ultrafiltration cell (Model 8400, Millipore Corp.) with an ultrafiltration membrane (molecular weight cutoff 100,000, regenerated cellulose; Ultrafiltration Disks, YM-100, Millipore Corp.) to exclude the large particles, and the pH was adjusted to 6.8 with HCl. The Milli-Q and river water samples were kept for 1 day at 20 °C to stabilize the pH. Just before the measurement of the electrophoretic mobility, bacteriophage stock solutions (see section on Bacteriophages) were used to suspend each bacteriophage at approximately 10^{10} PFU/mL in the prepared Milli-Q water or filtered river water. The electrophoretic mobility of the bacteriophages was measured 25 times for each sample at 25 °C and at a 15° measurement angle with an electrophoretic light-scattering spectrophotometer (ELS-6000, Otsuka Electronics Co., Ltd).

RESULTS AND DISCUSSION

Particle diameter and electrophoretic mobility

Figure 1 shows the electron micrographs of f1 and f2. Filamentous particles that were approximately 6–10 nm in diameter and 600–1,000 nm in length were observed for f1, whereas icosahedral particles approximately 25 nm in diameter were observed for f2. These particle characteristics are in agreement with previous reports (Shelton & Drewry 1973; Dotto *et al.* 1981). As seen in sandy aquifer treatment processes (Dowd *et al.* 1998), the differences in the particle sizes of f1 and f2 also probably influence their behavior during drinking water treatment processes.

Figure 2 shows a comparison in electrophoretic mobility (i.e. surface charge) between f1 and f2 in prepared Milli-Q water and filtered river water. When an electric field is applied to a suspension of charged particles, the particles move toward one of the electrodes. Negatively charged particles move toward the positive electrode and vice versa. The velocity of the particles is directly proportional to the applied field strength, and the ratio of these two quantities is known as the electrophoretic mobility (Gregory 2006). Accordingly, surface charge of particles can be evaluated by electrophoretic mobility measurements. Both bacteriophages were negatively charged in the prepared Milli-Q water, with f1 having a slightly more negative surface charge than f2. This difference in electrophoretic mobility is probably due to the differences in the types of amino acids (Weber & Konigsberg 1967; Beck & Zink 1981) on the surfaces of f1

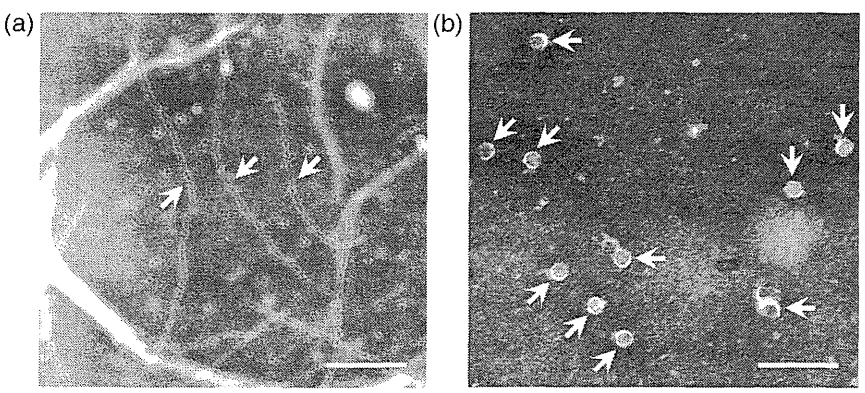

Figure 1 | Negative-stain electron micrographs of (a) f1 and (b) f2. The scale bar represents 100 nm.

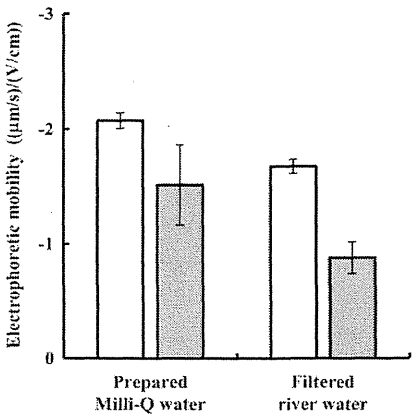

Figure 2 | Electrophoretic mobility of f1 (white) and f2 (gray) in prepared Milli-Q water and filtered river water. Values represent the mean and standard deviation of 25 measurements. The bacteriophage concentration in each sample was approximately 10^{10} PFU/mL.

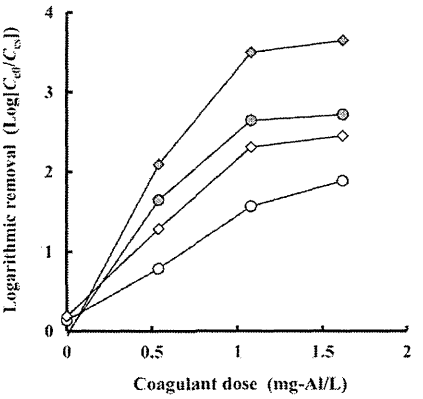

Figure 3 | Effects of coagulant dose (0.54, 1.08, or 1.62 mg-Al/L) on f1 (white) and f2 (gray) removal after settling, without (circles) or with (diamonds) centrifugal separation. The initial bacteriophage concentration in raw water was approximately 10^6 PFU/mL.

and f2. The same trend in electrophoretic mobility seen in prepared Milli-Q water was observed in filtered river water, although the electrophoretic mobility of both bacteriophages was shifted to the neutral owing to the differences in the ionic strength of the river water or to the influence of multivalent cations, such as calcium and magnesium ions. In general, the surface charge on virus particles strongly affects their behavior during various physicochemical water treatment processes, including coagulation processes (Matsushita *et al.* 2004). The more negatively charged viruses may resist aggregation, making it more difficult to destabilize and aggregate them by charge neutralization during coagulation in comparison to less negatively charged viruses. Thus, removal of f1 from river water by coagulation is expected to be more difficult than removal of f2.

Effects of coagulant dose on bacteriophage removal

Figure 3 shows the removal ratios, ($\log[C_{c0}/C_{cs}]$), for f1 and f2 after settling, with and without centrifugal separation, during coagulation. Because the diameters of f1 and f2 were small (as seen by electron microscopy) and they were stabilized by electrical repulsion in the river water, neither bacteriophage was removed (<0.2-log) from river water in the absence of coagulant. In contrast, both bacteriophages were removed by coagulation by the addition of any dose of the coagulant. The addition of coagulant destabilized the stably monodispersed bacteriophages in the river water, which got adsorbed onto/entrapped in the aluminium floc particles generated during coagulation. The aluminium floc particles were settled from suspension by gravity along with the adsorbed/entrapped bacteriophages during the settling process. Removal ratios of f1 and f2 were only approximately 1-log at 0.54 mg-Al/L of the coagulant, and the ratios increased to approximately 2-log with 1.08 mg-Al/L or more. Hijnen & Medema (2010) reviewed literature for viral removal performance in different coagulation processes (full-scale systems, pilot-plant studies and laboratory experiments) and estimated that the micro-organism elimination credit (MEC) of coagulation was 1.8±0.7-log for viruses (Hijnen & Medema 2010). Although the efficacy of coagulation for the removal of viruses is influenced by several conditions, such as raw water quality, the nature and concentrations of chemicals, pH, temperature and the type of mixing (Hijnen & Medema 2010), a similar removal performance (approximately 2-log) was observed for both bacteriophages in the present coagulation process.

The removal ratios of both bacteriophages increased after centrifugal separation. This indicates that centrifugal separation was able to remove the bacteriophages that were adsorbed onto/entrapped in the floc particles that were unable to settle by gravity.

However, regardless of centrifugal separation, the removal ratios of f1 were approximately 1-log lower than the ratio of f2 at any dose of the coagulant. This difference could be partly due to the difference in the electrophoretic

mobility of f1 and f2 in river water, as described above. Thus the removal of f1 from river water by coagulation was more difficult than that of f2.

Comparison of removal ratios of f1 and f2 during the coagulation–rapid sand filtration process

Figure 4 shows the removal ratios, ($\log[C_{c0}/C_{cs}]+\log[C_{r0}/C_{rf}]$), for f1 and f2 in the coagulation–rapid sand filtration process. Based on the results of the coagulation experiments, 1.08 mg-Al/L of coagulant was used in the coagulation–rapid sand filtration experiments. Although the DOC concentration of bacteriophage-spiked river water increased with the bacteriophage feed concentration, owing to the unavoidable uptake of the residual component of the culture medium, the removal ratios did not differ between initial concentrations of 10^6 and 10^8 PFU/mL for both bacteriophages (data not shown). This finding suggests that the DOC component from the bacteriophage culture solution did not affect bacteriophage removal during the coagulation process. Approximately 1-log improvement was seen in the removal ratios for f1 and f2 by the addition of the rapid sand filtration process in comparison to the coagulation process alone (Figure 3). This means that the bacteriophages entrapped in the suspended aluminium floc particles were effectively removed by the rapid sand filtration process after coagulation. In addition, no differences were observed in the removal ratios for f1 or f2 for filter depths of 10 and 20 cm. Thus, the effect of filter depth on bacteriophage removal was negligible, and most of the suspended aluminium floc particles were retained in the surface layer of the silica sand filter in the coagulation–rapid sand filtration process. Moreover, the removal ratios for both bacteriophages increased slightly with filtration time, which was probably due to the surface clogging of the silica sand filter by the deposition of suspended aluminium floc particles on the silica sand. In general, virus removal performance in the filtration process is increased with filtration time by pore clogging. In other words, the virus removal performance in the beginning of filtration time or just after the hydraulic backwashing can be regarded as a worst case scenario. Accordingly, the virus removal performances observed in the present study are assumed as their worst cases for elimination of virus in the filtration process since filtration time applied here is shorter than that of actual cases.

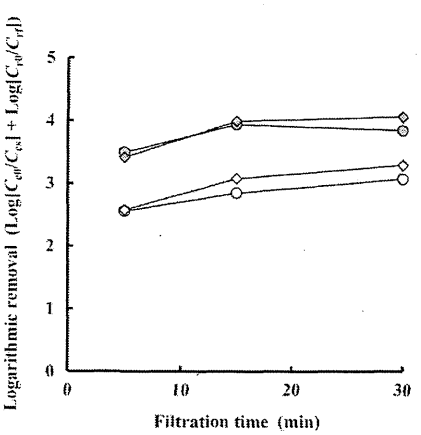

Figure 4 | Removal of f1 (white) and f2 (gray) by coagulation–rapid sand filtration with 10 cm (circles) or 20 cm (diamonds) of filter depth. The coagulant dose was 1.08 mg-Al/L. The initial bacteriophage concentration in raw water was approximately 10^8 PFU/mL.

Based on literature reports describing the viral removal performance by coagulation–rapid granular filtration processes, MEC was estimated to be 3.0±1.4-log for viruses (Hijnen & Medema 2010). A similar removal ratio of approximately 3–4-log was also observed for both bacteriophages after 30 min of filtration time in the present coagulation–rapid sand filtration process. However, the removal ratios of f1 were approximately 1-log lower than those of f2 for any duration of filtration. Thus, the behavior of both bacteriophages was different not only during the coagulation process but also in the coagulation–rapid sand filtration process.

Comparison of removal ratios of f1 and f2 during the coagulation–MF process

Figure 5 shows the removal ratios, ($\log[C_{c0}/C_{cs}]+\log[C_{m0}/C_{mf}]$), for f1 and f2 during the coagulation–MF process. Based on the results of the coagulation experiments, the coagulant dose used in the coagulation–MF experiments was 1.08 mg-Al/L. The coagulation–MF process effectively removed both bacteriophages: approximately 5-log increments were observed in the removal ratios for both bacteriophages by the addition of the MF process in comparison to the coagulation process alone (Figure 3), and time-averaged removal ratios of 5.6-log and 6.7-log were obtained for f1 and f2, respectively. This indicates that almost all suspended aluminium floc particles with adsorbed/entrapped bacteriophages exceeded the pore size of

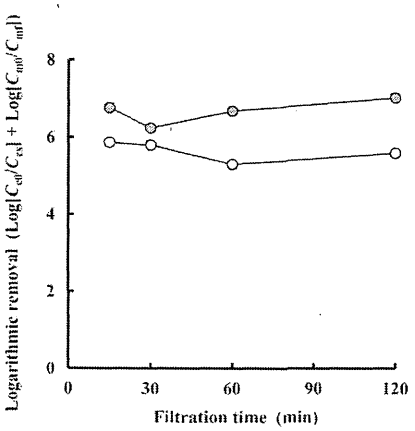

Figure 5 | Removal of f1 (white) and f2 (gray) by coagulation–MF. The coagulant dose was 1.08 mg-Al/L. The initial bacteriophage concentration in raw water was approximately 10^8 PFU/mL.

the MF membrane and were removed by the MF process. Other researchers have also demonstrated the effectiveness of the coagulation–MF process: virus removal ratios of >4-log were achieved by the combination of aluminium/iron-based coagulation and organic/inorganic MF processes (Matsushita *et al.* 2005; Zhu *et al.* 2005; Fiksdal & Leiknes 2006).

The removal ratios for both bacteriophages were almost constant during filtration. In contrast, removal ratios for the F-specific RNA bacteriophages Qβ and MS2 gradually increased with filtration time in an in-line coagulation–MF process, probably due to the accumulation of (i) a cake layer on the membrane surface and (ii) viruses as irreversible foulants in the internal structure of the membrane pores (Shirasaki *et al.* 2009). One reason for this discrepancy is the difference in the coagulation processes (batch coagulation vs. in-line coagulation): most of the aluminium floc particles generated during the coagulation process were settled out from the suspension by gravity, and the floc particles remaining in the supernatant were fed into the MF membrane in the batch coagulation process. On the other hand, almost all the floc particles generated were fed into the MF membrane in the in-line coagulation process. Thus, the effects of the cake layer and of the irreversible foulants on bacteriophage removal during batch and in-line coagulation were quite different, leading to the differences in the trends of the removal ratios for bacteriophages.

As seen in the coagulation and coagulation–rapid sand filtration processes, the removal ratios for f1 were approximately 1-log lower than those for f2 for any duration of filtration during the coagulation–MF process. Thus, the removal of f1 by the combination of coagulation and filtration processes is more difficult than that of f2 from river water.

The US Environmental Protection Agency (USEPA) National Primary Drinking Water Standards (US Environmental Protection Agency 2001) require 4-log removal or inactivation of enteric viruses from source water by filtration, disinfection or a combination of these technologies. This 4-log removal was achieved in the present study by the coagulation–MF process, regardless of bacteriophage characteristics (filamentous vs. spherical). Thus, the coagulation–MF process is effective for the removal of both filamentous and spherical viruses, and has the potential to effectively mitigate the public health risk posed by virus contamination in drinking water.

CONCLUSIONS

1. The removal performances of coagulation, coagulation–rapid sand filtration, and coagulation–MF processes were different for f1 and f2: the removal ratios for f1 were approximately 1-log lower than those for f2, probably due to the differences in the particle characteristics and the surface charges of f1 and f2.
2. The coagulation–MF process was more effective than the coagulation–rapid sand filtration process for the removal of both bacteriophages: the removal ratios for f1 and f2 in the coagulation–MF process were approximately 6-log and 7-log, respectively, and these values were approximately 3-log higher than those observed for the coagulation–rapid sand filtration process.
3. The public health risk posed by virus contamination in drinking water will be effectively mitigated by the replacement of the coagulation–rapid sand filtration process with the coagulation–MF process.

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