浄水システム選定表作成に当たっては、まず、各水質項目について、浄水レベル1を90%以上達成できる浄水システムにおける原水濃度の最大値あるいは99～90%値によって「原水レベル」を2～3段階に区分けした。図1にトリハロメタン生成能（THMFP）を例とした場合の原水レベルの設定概念を示す。

次いで、原水レベル毎に、浄水レベルに応じた基本浄水プロセスを当てはめ、水質項目毎に適応可能な浄水システム選定表を作成した。対象水質項目は濁度、TOC、THM、かび臭物質とした。

一例としてトリハロメタン（THM）に関する浄水システム選定表を表2に示す

4. 浄水処理システム選定方法

「原水水質および目標浄水水質に応じた浄水処理システム選定」の基本的な考え方を以下に示す。まず、浄水処理プロセスを「濁度除去プロセス」と「有機物除去プロセス」に分け、それぞれについて、浄水システム選定表より、原水水質および目標浄水水質レベルに応じた適切な処理プロセスを選定する。その組み合わせをベースに、必要に応じてマンガン除去、アンモニア除去などの設備を付加した「水質面での最適浄水システム」を提示し、さらに、コスト、スペース、ライフサイクルアセスメント（LCA）などの情報も併せて提供する。

図 2 に具体的な浄水処理システム選定方法についてフローを示す。

なお、ここで提示する浄水システムについては、以下の4つの前提条件により、表3に示す11の基本処理システムに集約される。

(1)システムの最終段階（消毒を除く）に固液分離プロセスを置く（生物漏出対策等）。(2)溶解性成分に対しては、粉末活性炭、粒状活性炭、オゾン・粒状活性炭の順でより高度の処理ができる。(3) 各プロセスは日本国内で稼動実績および実データがあるもので構成される。(4)各プロセスの処理性能を加算（除濁性能＋有機物除去性能）することにより、システムとしての除去性能を示すことが可能である。

最終的なシステムでは、選定されたシステム中に除マンガン設備などの、考慮すべき水質項目に応じたプロセスが必要に応じて付加される。

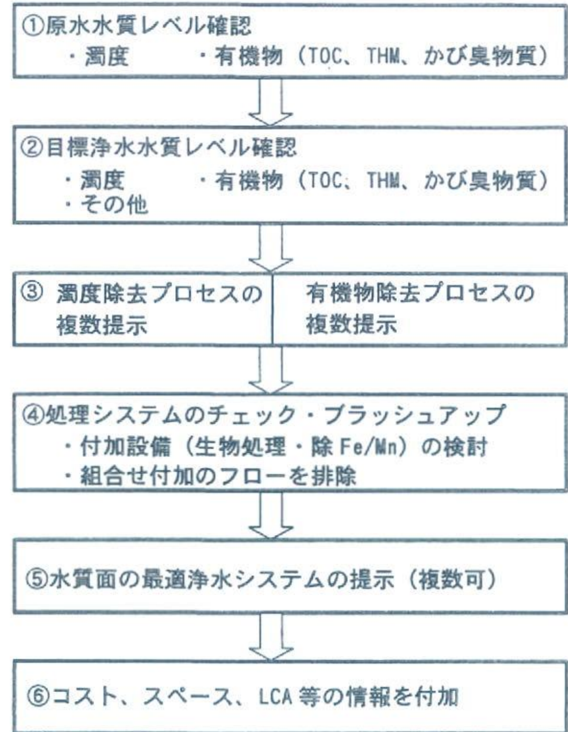

図 2　浄水処理システム選定フロー

表3　選定対象システム

1		(粉炭) + (凝集+) 膜ろ過
2	-1	(粉炭) + 凝集 + 沈澱 + 急速ろ過
	-2	(粉炭) + 凝集 + 沈澱 + 膜ろ過
3		凝集 + 急速ろ過
4		凝集 + 前ろ過 + 膜ろ過
5	-1	(粉炭) + 凝集 + 沈澱 + 粒状炭 + 急速ろ過
	-2	(粉炭) + 凝集 + 沈澱 + 粒状炭 + 膜ろ過
6	-1	凝集 + 沈澱 + O3 + 粒状炭 + 急速ろ過
	-2	凝集 + 沈澱 + O3 + 粒状炭 + 膜ろ過
7		凝集 + 粒状炭 + 膜ろ過
8		凝集 + 前ろ過 + 粒状炭 + 膜ろ過

※前塩、中塩、粉末活性炭の有無については、各システムの小分類として扱う
※必要に応じて除Mnなどのオプションが入る

謝辞

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第 59 回全国水道研究発表会　平成20.5

(4-39)ナノ沪過膜による浄水処理についての研究(Ⅱ)

－多段型 NF 装置の連続運転経過－

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1.はじめに

浄水処理においてナノろ過(NF)は、溶解性物質の除去に優れ、既存のオゾン・活性炭吸着処理の代替技術として注目されている。本研究では、NF を高度浄水処理方式として適用するための性能評価や運転管理方法等の開発を目的に、東京都の水源である荒川を原水として連続処理試験を実施した。昨年度は、鉄系凝集剤を用いた凝集沈澱と MF を前処理として、良好な NF 処理水質が得られたこと、小型 NF 装置を用い、大型 NF 装置の水質予測が可能であることを報告した[1)]。本報では、その後実施した連続処理試験の経過について報告する。

2.実験フロー及び運転条件

実験設備のフローを図-1 に示す。また、実験設備の仕様を表-1 に示す。

原水は東京都朝霞浄水場の原水接合井から取水した。設備は、前処理設備(凝集沈澱と MF)、NF 装置からなる。

前処理の凝集沈澱では、原水に塩化第二鉄($FeCl_3$)を 10～15mg as $FeCl_3$/L となるように添加した。その後、上澄水は MF 設備でろ過した。MF 処理水には重亜硫酸ナトリウム(SBS)を添加し、残留塩素を除去してから、NF 装置に供給した。

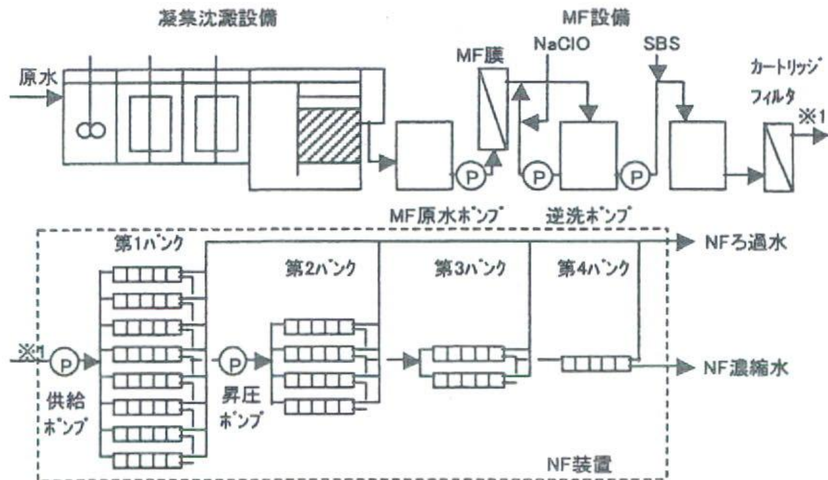

図-1　実験フロー

NF 装置は多段型(ベッセル配置;8-4-2-1、各ベッセルに 5 エレメント)とし、運転条件は平均膜透過流束 0.5m/日、回収率 90%に設定した。

3.実験結果

3.1.膜差圧の経過

NF 装置の膜差圧の経過と第 1 バンクと第 4 バンクの圧力損失の経過を図 2 に示す。膜差圧は、運転開始から約 300 日まで、全てのバンクで安定していた。この間、凝集剤の注入率を 15 から 10mg as $FeCl_3$/L に減らしたが、顕著な影響は確認されなかった。300 日以降、膜差圧は上昇したが、洗浄④(水酸化ナトリウム(NaOH)と塩酸)後は、膜差圧の上昇は抑制された。

3.2.圧力損失の経過

Run1 では第 1 バンクの圧力損失が運転開始直後から上昇したため、洗浄①(シュウ酸と NaOH)を実施した所、圧力損失は低下した。なお、シュウ酸洗浄廃液に鉄が多く含まれており、膜エレメントに鉄が蓄積して

いたと考えられた。

Run2 では第 1 バンクの圧力損失は安定したが、第 4 バンクの圧力損失が増加したため、洗浄②(シュウ酸と NaOH)を実施した所、圧力損失は低下した。洗浄①と比べるとシュウ酸洗浄廃液中の鉄は減少し、代わりにマンガンが増加していた。Run2 では $FeCl_3$ 注入率を 10mg/L に減らしたため、NF 供給水の鉄濃度は平均 0.012(Run1)→0.004(Run2)mg/L に減少した。このため、エレメントへの鉄の蓄積が抑制されたと考えられた。280 日以降も、第 4 バンクで圧力損失が上昇し、洗浄③(シュウ酸と NaOH)と洗浄④を実施した。洗浄③では洗浄②と同様にシュウ酸洗浄廃液にマンガン、洗浄④では塩酸洗浄廃液にカルシウムの溶出が確認されたが、これらの薬品洗浄では圧力損失の低下は限定的で洗浄効果は小さかった。このため 280 日目以降の圧力損失上昇の原因はそれまでと異なり、金属類よりも有機物の膜エレメントへの蓄積が考えられた。なお、第 2、第 3 バンクの圧力損失は第 1、第 4 バンクと比べて変化が小さかった。

表-1 実験設備の仕様

凝集沈澱	型式	傾斜管付凝集沈澱
	凝集剤	塩化第二鉄
	最大処理量	$600m^3$/日
MF 設備	膜型式	加圧型 MF 膜
	膜種類	外圧式中空糸膜(PVDF)
	公称孔径	0.05 μm
	膜面積	$72m^2 \times 6$ 本
NF 設備	膜材質	ポリアミド系複合膜
	塩排除率	55%
	膜面積	$7m^2$/エレメント
	平均 Flux	0.5m/日
	回収率	90%
	システム構成	5 エレメント/ベッセル 多段型(8-4-2-1 ベッセル

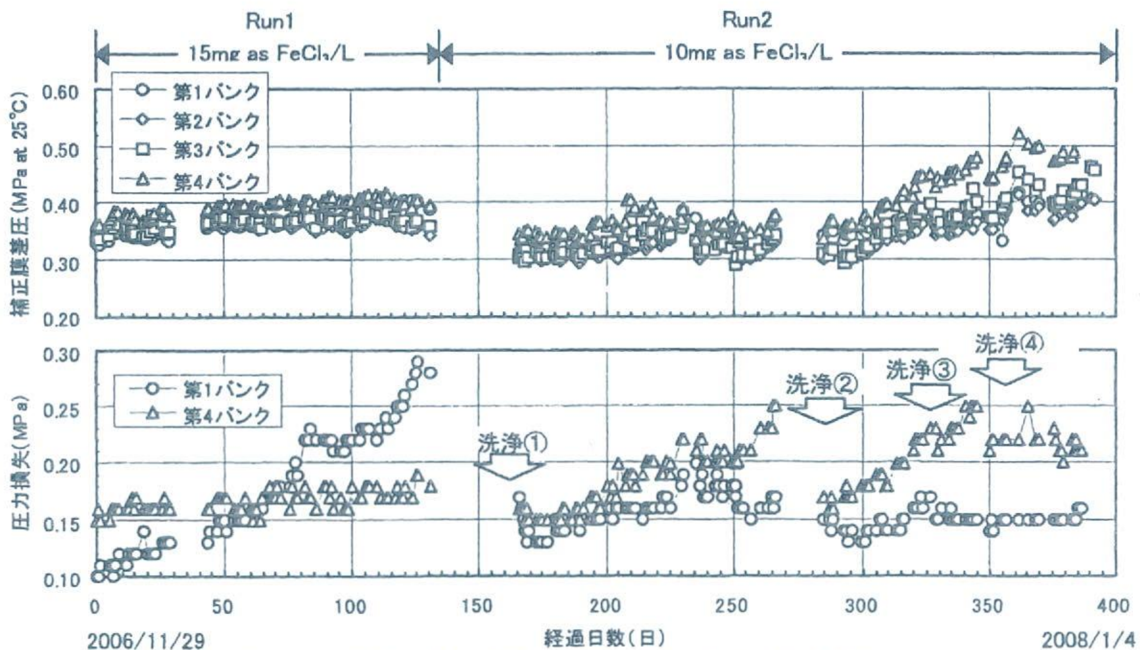

図-2 NF 装置の運転経過(上;補正膜差圧、下;圧力損失)

4.まとめ

河川表流水を原水として平均膜透過流束 0.5m/日、水回収率 90%の条件で、NF 装置を約 1 年間運転することができた。この間、薬品洗浄(シュウ酸、NaOH、塩酸)は 4 回実施した。また、前処理として塩化第二鉄を添加した凝集沈澱と MF が有効であった。NF 膜への有機物の蓄積への対策はさらに検討が必要であると考えられた。

5.参考文献

1)鹿島田他, ナノろ過膜による浄水処理についての研究(Ⅰ), 第 58 回水道研究発表会, 2007

(4 −40)ナノ沪過膜による浄水処理についての研究(Ⅲ)

－高度処理/通常処理との水質比較－

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1.はじめに

実験設備フロー

朝霞浄水場フロー

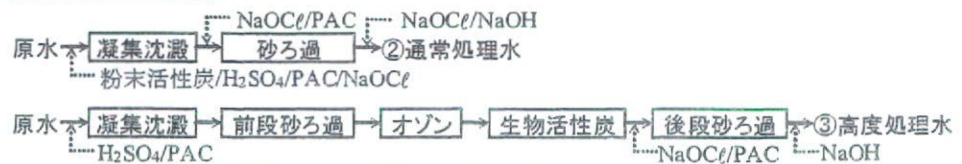

図 1　実験設備および朝霞浄水場の概略フローと採水位置

浄水処理においてナノろ過(NF)は、溶解性物質の除去に優れ、既存のオゾン・活性炭吸着処理の代替技術として注目されている。本研究では、NF 膜ろ過を高度浄水処理方式として適用するための性能評価や運転管理方法等の開発を目的に、東京都の水源である荒川を原水として NF 膜ろ過の連続処理試験を実施している[1]。本発表では、朝霞浄水場の通常処理水、高度処理水と、実験設備の NF 膜ろ過水の水質分析を行って得られた水質特性の違いについて報告する。

2.設備フロー及び採水位置

実験設備および朝霞浄水場の概略フローを図 1 に示す。NF 膜ろ過装置は多段型装置とし、運転条件は平均膜透過流束 0.5m/日、回収率 90%に設定した(詳細は前編(Ⅱ)を参照のこと)。添加薬品は、朝霞浄水場が凝集剤として PAC を添加しているのに対し、実験設備では $FeCl_3$ を添加し、また NF 膜ろ過供給水には MF 膜洗浄水由来の残留塩素除去を目的として重亜硫酸ナトリウム(SBS)を添加している。採水位置は、図 1 の①～③に示すとおり、実験設備の NF 膜ろ過水と、朝霞浄水場の通常処理水、高度処理水とした。

3.実験結果

(1) 水道水質基準項目および水質管理目標設定項目

水道水質基準項目および水質管理目標設定項目の測定結果の一例を表 1、2 に示す。

表 1　水道水質基準項目測定結果（平均値）

分析項目		単位	NF 膜ろ過実験 (n=4)				朝霞浄水場 (n=4)	
No	項目名		原水	供給水	濃縮水	①NF 膜ろ過水	②通常処理水	③高度処理水
11	フッ素及びその化合物	mg/ℓ	0.10	0.10	0.56	<0.08	0.09	0.09
25	臭素酸	mg/ℓ	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
32	アルミニウム及びその化合物	mg/ℓ	0.17	<0.01	<0.01	<0.01	0.02	0.01
33	鉄及びその化合物	mg/ℓ	0.33	<0.01	0.01	<0.01	<0.01	<0.01
36	マンガン及びその化合物	mg/ℓ	0.058	0.054	0.257	0.018	<0.005	<0.005
38	カルシウム、マグネシウム等(硬度)	mg/ℓ	75.1	75.1	438.0	29.9	75.5	74.2
39	蒸発残留物	mg/ℓ	168	161	741	98	179	167
45	有機物(全有機炭素(TOC)の量)	mg/ℓ	1.35	0.76	6.45	0.11	0.82	0.59
46	pH 値	－	7.5	7.0	7.5	6.9	7.4	7.4

※網掛け部は定量下限値以下

表 2　水質管理目標設定項目測定結果（平均値）

分析項目		単位	NF 膜ろ過実験 (n=2)				朝霞浄水場 (n=1)	
No	項目名		原水	供給水	濃縮水	①NF 膜ろ過水	②通常処理水	③高度処理水
1	アンチモン及びその化合物	mg/ℓ	0.0002	0.0002	0.0016	<0.0002	0.0002	0.0002
3	ニッケル及びその化合物	mg/ℓ	0.002	0.002	0.015	<0.001	0.001	0.001
27	腐食性(ランゲリア指数)	－	−1.3	−1.8	−0.1	−2.4	−1.3	−1.4

※網掛け部は定量下限値以下

NF 膜ろ過水は、後 pH 処理を行っていないことからランゲリア指数が−2.4 と低下する傾向が見られたが、水質基準を満たし、特に有機物の低減化に有効であった。また、原水や凝集剤由来のアルミニウム・鉄は定量下限値以下であったが、マンガンは水質基準値に対し約 4 割 の値であった。

(2)消毒副生成物(DBP)

消毒副生成物生成能測定結果を表2に示す。総トリハロメタン生成能は、通常処理水の0.024mg/ℓ、高度処理水の0.009mg/ℓに対し、NF膜ろ過水では定量下限値(0.001mg/ℓ)以下であった。またNF膜ろ過水の消毒副生成物生成能は測定した9項目全てにおいて定量下限値以下であった。

表3 消毒副生成物生成能測定結果 (平均値)

分析項目	単位	NF膜ろ過実験 (n=4)				朝霞浄水場 (n=3)	
		原水	供給水	濃縮水	①NF膜ろ過水	②通常処理水	③高度処理水
クロロホルム生成能	mg/ℓ	0.014	0.005	0.074	<0.001	0.010	0.002
クロロジブロモメタン生成能	mg/ℓ	0.003	0.003	0.003	<0.001	0.004	0.004
ジクロロブロモメタン生成能	mg/ℓ	0.008	0.005	0.020	<0.001	0.009	0.003
ブロモホルム生成能	mg/ℓ	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
総トリハロメタン生成能	mg/ℓ	0.026	0.013	0.097	<0.001	0.024	0.009
クロロ酢酸生成能	mg/ℓ	<0.002	<0.002	0.006	<0.002	<0.002	<0.002
ジクロロ酢酸生成能	mg/ℓ	0.008	0.003	0.038	<0.002	0.007	<0.002
トリクロロ酢酸生成能	mg/ℓ	0.014	0.004	0.068	<0.002	0.008	<0.002
ホルムアルデヒド生成能	mg/ℓ	0.004	0.003	0.024	<0.002	0.006	<0.002

※網掛け部は定量下限値以下

(3)かび臭物質

原水2-MIB濃度が上昇した際の工程別の測定結果を表4に示す。原水の2-MIB濃度が11.16ng/ℓの時、NF膜ろ過水は定量下限値以下となった。各バンクの2-MIBの除去率は、後段のバンクほど除去率が高くなる傾向が見られた。NF総ろ過水の除去率は85%(凝集沈澱+MF処理までを含めると92%)と、高度処理の100%には及ばないものの、通常処理系(粉末活性炭注入率5mg/ℓ)の60%より高かった。

表4 かび臭物質の工程別測定結果

分析項目	測定値(ng/L, n=1)					除去率(%)		
	原水	凝集沈澱水	NF供給水	NF濃縮水	NF膜ろ過水	①NF膜ろ過	②通常処理	③高度処理
2-MIB	11.16	10.40	5.80	40.25	0.87	前処理込 92% NF単独 85%	60%	100%
ジェオスミン	—	3.30	2.55	8.06	0.56	前処理込 83% NF単独 78%	73%	100%

※網掛け部は定量下限値以下。ただし処理傾向を見るため、定量下限値1ng/L以下の数値も表記した。

(4)農薬類

農薬類の測定を行ったうち、原水で検出されたものを表5に示す。検出された6物質について物質収支より求めた除去率(水回収率は90%なので、除去率100%だと濃縮水の濃度は供給水の10倍になる)は、27~100%であった。定量下限値付近の低濃度のため効果が明確ではないが、原水で定量下限値以下であった他の7物質でも濃縮水中の検出例があり、NF膜ろ過による農薬類の除去性が認められた。

表5 農薬類の測定結果 (最大値)

分析項目		単位	NF膜ろ過実験 (n=3)					朝霞浄水場 (n=2)		
No	項目名		原水	供給水	濃縮水	ろ過水	除去率	原水	通常ろ過	高度ろ過
12	イプロベンホス(IBP)	mg/ℓ	0.00020	0.00019	0.00140	<0.00008	74%	0.00012	0.00005	0.00001
32	メフェナセット	mg/ℓ	0.00026	0.00021	0.00110	0.00009	52%	0.00013	0.00007	0.00002
37	ブロモブチド	mg/ℓ	0.0008	0.0008	0.0066	<0.0004	83%	0.0006	0.0004	<0.0001
38	モリネート	mg/ℓ	0.00009	0.00007	0.00019	<0.00005	27%	0.00008	<0.00001	<0.00001
64	フィプロニル	mg/ℓ	0.000012	0.000010	0.000031	<0.000005	31%	<0.00001	<0.00001	<0.00001
68	イソキサチオン-OXON	mg/ℓ	0.0001	0.0001	0.0015	<0.0001	100%	<0.00001	<0.00001	<0.00001

※網掛け部は定量下限値以下(NF膜ろ過実験と朝霞浄水場のデータで定量下限値が異なるものもある)

4.まとめ

NFろ過実験と朝霞浄水場の水質比較を行い、NF膜ろ過水について以下の特徴があることがわかった。

・有機物の除去性が高度処理と比べて高く、消毒副生成物生成能を抑制できる

・アルミニウム・鉄は定量下限値以下であったが、マンガンは水質基準値の約4割の値であった

・ランゲリア指数が低下しやすい(後pH調整を行っていない影響もあり)

・2-MIB、ジェオスミン除去率は、高度処理に及ばずも通常処理(粉末活性炭注入率5mg/ℓ)より高かった

・農薬類は、原水中で検出された6物質について除去率27~100%であった

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(6－9)減圧式逆流防止器における弁差圧と流量の関係を利用した異常検知手法の開発に関する研究

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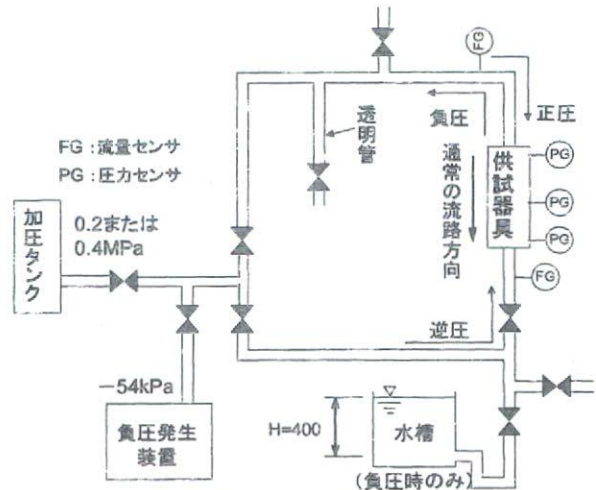
1. 本研究の目的

逆流防止装置の異常検知手法の開発を目的として研究を行い、バネ式の単式逆止弁、二重式逆止弁の各種条件における弁差圧と流量の挙動を基にした検知方法を一昨年度、昨年度に発表している[1)2)]。本年度の研究対象とした減圧式逆流防止器は、弁異常時には逃がし弁からの排水機能を有し、吐水口空間に次ぐ信頼性のある逆流防止器であり、さらに逃がし弁からの排水検知による弁異常の検知手法も確立されている。しかし、弁異常がない場合においても負圧時には逃がし弁からの排水があること、弁異常による逃がし弁からの排水を伴わない逆流の恐れがあることなど、さらなる検討の余地があり、本研究ではこれらを検証するとともに、これまでの研究において提案した弁差圧と流量による異常検知方法の有効性について検討した。

2. 実験方法

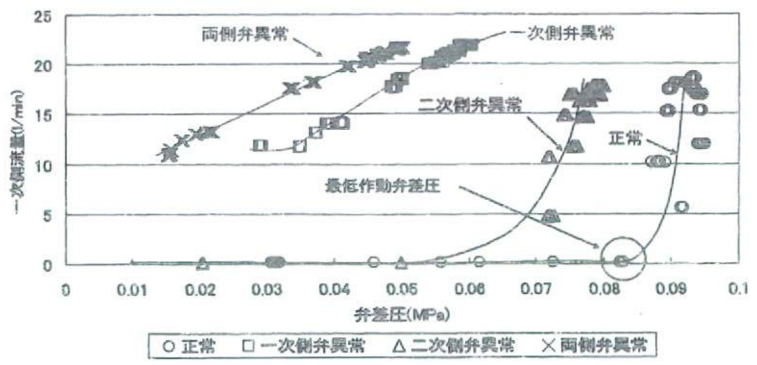
本研究では、図－1 に示す実験装置を用い、供試器具として JWWA 規格の口径 20mm の減圧式逆流防止器を設置し、各種条件下における圧力及び流量を計測した。弁異常状態の再現方法としては、主に径 1mm の針金（ピアノ線）を用い、一次側弁のみ、二次側弁のみ、一次側及び二次側弁の両側弁へ針金を噛ませて、比較を行った。供試器具への圧力条件としては正圧（加圧タンク圧力を 0.2 または 0.4MPa に設定）、逆圧（正圧状態後、二次側増圧、一次側減圧）及び負圧（正圧状態後、加圧停止、負圧－54kPa 発生）とした。また、負圧時における実験条件として実使用条件を想定し、供試器具二次側から水槽による 4kPa の逆圧を発生させた。



図－1　実験装置

3. 実験結果

図－2 に弁正常時（針金なし）及び弁異常時の正圧状態における弁差圧－一次側流量特性の一例を示す。一次側弁異常時、両側弁異常時の場合において常に正の流量が発生している。これは一次側弁異常により逃がし弁から連続的に排水されているためである。また、弁差圧－二次側流量特性では、これまでの他のバネ式逆止弁と同様の傾向を示し、異常時には、正常時の最低作動弁差圧以下で正の流量が発生している。



図－2　正圧状態における弁差圧－一次側流量特性の一例（加圧タンク 0.2MPa）

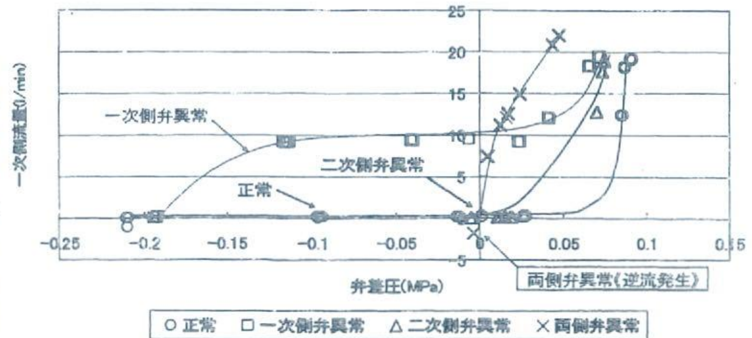
図－3 に逆圧状態における弁差圧－一次側流量特性の一例を示す。両側弁異常時には、一次側流量及び二次側流量の双方で逆流が発生した。特に二次側流量では、より多くの逆流量が発生しており、逃がし弁からの排水機構で大半は排水されるものの少量ではあるが逆流が起こりうることが明

らかとなった。

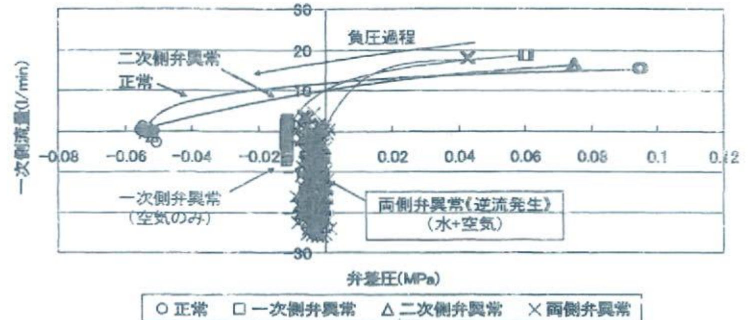
図－4 に負圧状態における弁差圧－一次側流量特性の一例を示す。両側弁異常時では、一次側流量及び二次側流量の双方において逆流が発生していた。このとき、逃がし弁からの排水は発生しなかった。但し、逃がし弁から空気吸入により、一次側の正確な逆流量が計測できなかったことから、現象を確認するために図―1 の実験装置において供試器具一次側の水抜きを行い、さらに一次側から負圧を発生させ、二次側に設置している水槽の水が透明管に流入するか確認を行った。この結果、やはり逃がし弁からの排水は発生せず、全量が一次側に逆流し、透明管に流入する結果となった。これらのことから、逃がし弁の排水検知による異常検知方法は基本的には有効であるが、まれなケースにおいては、必ずしも十分な手法ではないと考えられる。

4. 考察

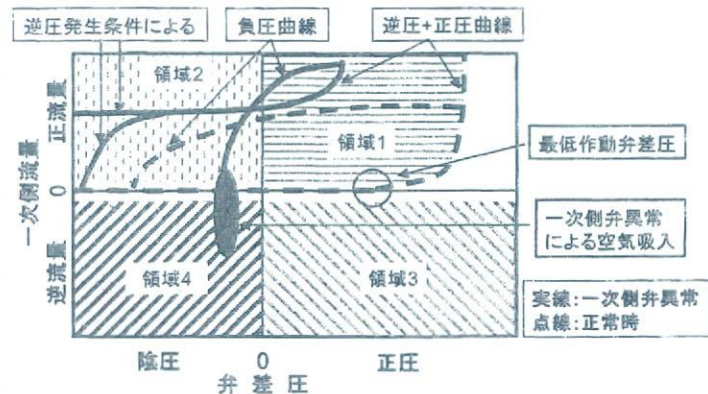
図－5 にこれまでの結果から弁差圧－一次側流量特性を模式化した一次側弁異常時の異常検知条件を示す。正常時の場合、正圧下では最低作動弁差圧以下で流量は 0 であり、それ以後正方向に上昇する。逆圧及び負圧による陰圧下では、それぞれ挙動は微妙に異なるものの最終的に 0 となる。一方、一次側に弁異常がある場合、正圧下では領域 1 に位置し、常に正流量が発生する。陰圧下では、逆圧時と負圧時で挙動が異なり、逆圧時には領域 2 に位置し、負圧時には主に領域 4 に位置する。このように弁差圧－一次側流量特性のいずれの領域に位置しているかを判別することで異常検知が可能である。しかし、どちらかの流量と弁差圧のみでは異常の発生場所の特定までは出来ないので、弁差圧－二次側流量特性においても同様な判定図を作成し、組み合わせて判断することにより、一次側の弁異常、二次側の弁異常等の異常の原因を特定することが可能である。



図－3 逆圧状態における弁差圧－一次側流量特性の一例（加圧タンク 0.2MPa）



図－4 負圧状態における弁差圧－一次側流量特性の一例（加圧タンク 0.2MPa）



図－5 弁差圧－一次側流量特性による一次側弁異常の異常検知条件

5. まとめ

減圧式逆流防止器は、吐水口空間に次ぐ逆流防止器であるが、最悪の場合には逆流が発生する危険性があり、異常監視システムの構築が必要であること、逃がし弁からの排水検知による異常検知方法も有効ではあるが必ずしも十分とはいえないことが分かった。そのため、これまで提案してきた弁差圧－流量特性を利用した異常検知方法を減圧式逆流防止器に用いることにより、一層の安全性を確保できる給水装置逆流防止システムが構築できると考えられる。

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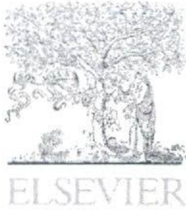

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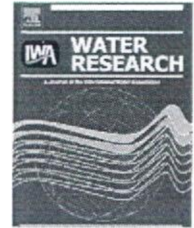

Comparison of behaviors of two surrogates for pathogenic waterborne viruses, bacteriophages Qβ and MS2, during the aluminum coagulation process

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ABSTRACT

Differences in the behaviors of two surrogates for pathogenic waterborne viruses, F-specific RNA bacteriophages Qβ and MS2, were investigated during the coagulation process by using river water spiked with these bacteriophages. The particle size and electrophoretic mobility of Qβ and MS2 were similar, but the removal performances of infectious Qβ and MS2, as measured by a plaque forming unit (PFU) method, differed markedly during the coagulation process. The removal ratio of the infectious Qβ concentration was approximately 2 log higher than that of the infectious MS2 concentration at all coagulant doses tested. The total Qβ and MS2 bacteriophage concentrations, which were measured by a real-time reverse transcription-polymerase chain reaction (RT-PCR) method and represented the total number of bacteriophages regardless of their infectivity, were similar after the coagulation process, suggesting that the behaviors of Qβ and MS2 as particles were similar during the coagulation process. The difference between total concentration and infectious concentration indicated that some of the bacteriophages were probably inactivated during the coagulation process. This difference was larger for Qβ than MS2, meaning that Qβ was more sensitive to the virucidal activity of the aluminum coagulant. Analysis of the PFU and real-time RT-PCR findings together suggested that the difference in removal performances of Qβ and MS2 during the coagulation process was probably caused by differences not in the extent of bacteriophage entrapment in the aluminum floc particles but in the sensitivity to virucidal activity of the aluminum coagulant.

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1. Introduction

During drinking water treatment, coagulation is an essential process for combining small particles into larger aggregates. Small particles in the drinking water source, such as viruses, that will not settle from suspension by gravity are destabilized and combined into larger aggregates during the coagulation process; this allows the small particles to be effectively removed by subsequent sedimentation and filtration processes. Several studies have reported the usefulness of the coagulation process for the removal of enteric viruses and bacteriophages, which are viruses that infect bacteria (Guy et al., 1977; Havelaar et al., 1995; Nasser et al., 1995).

Some bacteriophages have been evaluated as possible indicators for enteric viruses. For instance, F-specific RNA bacteriophage concentrations are highly correlated with those of enteric viruses in a wide range of water environments and water treatment processes (Havelaar et al., 1993). Because of their morphological similarity to hepatitis A viruses and polioviruses, F-specific RNA bacteriophage MS2 is widely used

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as a surrogate for pathogenic waterborne viruses in Europe and the United States (Jacangelo et al., 1995; Meng and Gerba, 1996; Redman et al., 1997; Shin and Sobsey, 1998, 2003; Sobsey et al., 1998; Meschke and Sobsey, 2003; Thurston-Enriquez et al., 2003; Zhu et al., 2005; Fiksdal and Leiknes, 2006), whereas the F-specific RNA bacteriophage Qβ tends to be used as a surrogate in Japan (Kamiko and Ohgaki, 1989; Urase et al., 1996; Otaki et al., 1998).

Previous studies have compared the behaviors of Qβ and MS2 in the physicochemical treatment process. Their behaviors are similar in the process of adsorption onto solid surfaces (such as cellulose, kaolin, carbon black and river sediment; Sakoda et al., 1997), the sandy aquifer treatment process (Dowd et al., 1998), and the direct microfiltration treatment process (Herath et al., 2000), whereas the sensitivities of Qβ and MS2 to ultraviolet radiation are significantly different (Blatchley et al., 2008). The behaviors of these two bacteriophages in the coagulation process may be different as well, although these differences have not been investigated except for previous studies of our research group (Matsui et al., 2003; Matsushita et al., 2004). If significant differences do exist, then Qβ and MS2 cannot be used as equivalent surrogates to evaluate the effectiveness of treatment processes for the removal of pathogenic waterborne viruses.

Virucidal activity during the water treatment process has been widely investigated. Ultraviolet radiation (Kamiko and Ohgaki, 1989; Meng and Gerba, 1996; Sobsey et al., 1998; Thurston-Enriquez et al., 2003) as well as disinfectants such as free chlorine, chlorine dioxide (Sobsey et al., 1998), chloramines (Shin and Sobsey, 1998) and ozone (Shin and Sobsey, 2003) are well known to inactivate viruses. Our research group has reported the virucidal activity of aluminum coagulant (Matsui et al., 2003; Matsushita et al., 2004). In our previous studies, we used the plaque forming unit (PFU) method in an attempt to determine the mechanisms and kinetics of the virucidal activity of aluminum coagulant. During coagulation of the virus suspension, although the majority of viruses were entrapped in the aluminum floc particles, some of the viruses remained suspended in the liquid phase. Not all the suspended viruses were infectious; some were likely inactivated by the virucidal activity of aluminum coagulant. Thus, virus removal during the coagulation process includes two mechanisms: entrapment in floc particles and inactivation. The PFU method, however, can detect neither the viruses entrapped in the floc particles nor the inactivated viruses judging from its measurement principle. Therefore, even though the virus concentration measured by the PFU method decreases in the liquid phase during the coagulation process, it remains unclear whether the decrease results from the entrapment in floc particles, inactivation, or both.

The reverse transcription-polymerase chain reaction (RT-PCR) method is capable of amplifying small regions of viral nucleic acid. Thus, RT-PCR can detect infectious as well as inactivated viruses, unless the target nucleic acid is lost, but not all viruses entrapped in the floc particles can be detected by this method. Calculation of the concentration of entrapped virus particles simply entails subtracting the concentration of total viruses including infectious and inactivated viruses in the liquid phase, as determined by RT-PCR, from the total virus concentration as measured in the raw water. Likewise, subtracting the concentration of infectious viruses, as determined by the PFU method, from the total virus concentration, as determined by RT-PCR, gives the number of inactivated viruses. Therefore, combining the PFU and RT-PCR methods allows entrapped viruses and inactivated viruses in the coagulation process to be clearly distinguished.

Our objectives were to investigate and compare the behaviors of two bacteriophages, Qβ and MS2, during the coagulation process by using both PFU and real-time RT-PCR methods and to elucidate what caused the differences.

2. Materials and methods

2.1. *Source water and coagulant*

River water was sampled from the Toyohira River (Sapporo, Japan; water quality shown in Table 1) on 12 October 2007. Polyaluminum chloride (PACl) (250A; 10.5% Al_2O_3, relative density 1.2 at 20 °C; Taki Chemical Co., Ltd., Hyogo, Japan) was used for coagulation experiments.

2.2. *Bacteriophages*

The bacteriophages Qβ (NBRC 20012) and MS2 (NBRC 102619) were obtained from the NITE Biological Research Center (NBRC, Chiba, Japan). Qβ is the prototype member of the genus *Alloleviuirus* in the virus family *Leviviridae*, and MS2 is the prototype member of the genus *Levivirus* in the *Leviviridae*. The genomes of these two bacteriophages contain a single molecule of linear positive-sense, single-stranded RNA, which is encapsulated in an icosahedral protein capsid with a diameter of 24–26 nm (The Universal Virus Database of the International Committee on Taxonomy of Viruses). Each bacteriophage was propagated for 22–24 h at 37 °C in *Escherichia coli* (NBRC 13965) obtained from NBRC. The bacteriophage culture solution was centrifuged (2000×*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., Billerica, MA, USA) to prepare the bacteriophage stock solution. The concentration of each bacteriophage stock solution was approximately 10^{12} PFU/mL.

2.3. *Batch 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 was added to the beaker at

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

approximately 10^6 or 10^8 PFU/mL and mixed with an impeller stirrer. PACl was injected into the water as a coagulant at dosages 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 using 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 aluminum floc particles generated. Samples were taken from the beaker before coagulant dosing and after rapid mixing, slow mixing, and settling for quantification of the bacteriophage concentrations.

To quantify the bacteriophage concentration in the liquid phase of the floc mixture, the suspended floc particles were separated from the mixture by centrifugation ($2000 \times g$, 10 min), and the bacteriophage concentration in the supernatant was measured by the PFU and the real-time RT-PCR methods (see Section 2.4).

In addition, to quantify the bacteriophage concentration in the floc particles, the particles were dissolved by raising the pH of the water to 9.5 with NaOH in 12% beef extract (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) solution and the water was vortexed for 5 h at 4 °C by using direct mixer (DM-301, As One Corp., Osaka, Japan) at 2000 rpm. Beef extract was used in an effort to prevent the inactivation of bacteriophages during floc dissolution (Matsui et al., 2003). Samples of raw water and rapid mixing were taken from the beaker directly for the floc dissolution, because these samples were considered to be mixed completely during the sampling. In contrast, the floc particles settled during the coagulation process were resuspended after the settling process by strong mixing with a magnetic stirrer at 1400 rpm for subsequent sampling and floc dissolution (floc dissolution procedure was described above). After floc dissolution, the bacteriophage concentration in the sample was measured by the PFU and real-time RT-PCR methods.

Because substances such as natural organic matter (Abbaszadegan et al., 1993; Kreader, 1996) and beef extract (Abbaszadegan et al., 1993; Sano et al., 2003) are known to inhibit the amplification of the viral genome by PCR, each sample was diluted 10-fold with Milli-Q water (Milli-Q Advantage, Millipore Corp.) before the real-time RT-PCR quantification.

2.4. *Bacteriophage assays*

2.4.1. *PFU method*

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

2.4.2. *Real-time RT-PCR method*

Viral RNA of bacteriophages was quantified by the real-time RT-PCR method, which detects viruses regardless of their infectivity. We defined concentration measured by the real-time RT-PCR method as total bacteriophage concentration. For quantification of bacteriophages in the raw water and liquid phase of the floc mixture, viral RNA was extracted from 140 μL of sample with a QIAamp Viral RNA Mini Kit (Qiagen K.K., Tokyo, Japan) to obtain a final volume of 60 μL. For quantification of bacteriophages in the floc dissolution sample containing beef extract, a 100-μL sample was heated at 90 °C for 10 min and then cooled to 4 °C for 1 min in a thermal cycler (Thermal Cycler Dice Model TP600, Takara Bio Inc., Shiga, Japan) to extract viral RNA by destroying the capsid. The extracted RNA solution was added to a High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems Japan Ltd., Tokyo, Japan) for the RT reaction, which was conducted at 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 s, followed by cooling to 4 °C in the thermal cycler. The cDNA solution was then amplified by a TaqMan Universal PCR Master Mix with UNG (Applied Biosystems Japan Ltd.), 400 nM of each primer (HQ-SEQ grade, Takara Bio Inc.), and 250 nM of TaqMan probe (Applied Biosystems Japan Ltd.). The oligonucleotide sequences of the primers and the probes are shown in Table 2. Amplification was conducted at 50 °C for 2 min, 95 °C for 10 min, and then 50 cycles of 95 °C for 15 s and 60 °C for 1 min in an ABI Prism 7000 Sequence Detection System (Applied Biosystems Japan Ltd.).

The standard curve for the real-time RT-PCR method was based on the relationship between the infectious bacteriophage concentration of a freshly prepared stock solution measured by the PFU method and the number of cycles for amplification in the PCR process, which is based on the assumption that the freshly prepared stock solution did not contain any inactivated bacteriophages.

2.5. *Particle size distribution*

Particle size distribution of bacteriophages was measured in prepared Milli-Q water and filtered river water. To bring the alkalinity to 20 mg-$CaCO_3$/L, 0.4 mM $NaHCO_3$ was added to the Milli-Q water, and the pH of was adjusted to 6.8 with HCl. River water was filtered through a stirred ultrafiltration cell (Model 8400, Millipore Corp.) with 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 particle size distribution, each bacteriophage was suspended at approximately 10^{10} PFU/mL in the prepared Milli-Q water or filtered river water using the bacteriophage stock solution. The particle size distribution of the bacteriophages was measured with a fiber-optic dynamic light-scattering spectrophotometer (FDLS-3000, Otsuka Electronics Co., Ltd., Osaka, Japan) 200 or 400 times for each sample at 25 °C and at a 90° measurement angle.

2.6. *Electrophoretic mobility*

In Milli-Q water and river water prepared as described in Section 2.5, an electrophoretic light-scattering spectrophotometer (ELS-6000, Otsuka Electronics Co., Ltd.) was used to measure electrophoretic mobility. Just before the measurement, each bacteriophage was suspended at approximately 10^{10} PFU/mL in the prepared Milli-Q water or filtered river water using the bacteriophage stock solution. The electrophoretic mobility of the bacteriophages was measured 25 times for each sample at 25 °C and at a 15° measurement angle.

Table 2 – Oligonucleotide sequences of the primers and probes used in real-time RT-PCR quantification of Qβ and MS2.

Viruses		Oligonucleotide sequences	Positions	References
Qβ	Forward primer	5′-TCA AGC CGT GAT AGT CGT TCC TC-3′	49–71	Katayama et al., 2002
	Reverse primer	5′-AAT CGT TGG CAA TGG AAA GTG C-3′	187–208	
	TaqMan probe	5′-CGA GCC GCG AAC ACA AGA ATT GA-3′	147–169	
MS2	Forward primer	5′-GTC GCG GTA ATT GGC GC-3′	632–648	O'Connell et al., 2006
	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	

3. Results and discussion

3.1. *Particle size distribution*

Fig. 1 shows the particle size distributions of Qβ and MS2 in the prepared Milli-Q water and filtered river water at pH 6.8. In both the Milli-Q and river water, the particle sizes were distributed over the range of 20–30 nm. These values correspond with the particle diameters previously reported for Qβ and MS2 (The Universal Virus Database of the International Committee on Taxonomy of Viruses). Thus, we can assume that no virus–virus aggregate was generated and these two bacteriophages were stably monodispersed in the raw water used (without coagulant dosing).

Langlet et al. (2008) reported that Qβ had a tendency to aggregate in solutions with high ionic strength: Qβ aggregated in deionized water with 100 mM $NaNO_3$ at neutral pH (ionic strength, approximately 1×10^{-1}), whereas it did not aggregate in deionized water with 1 mM $NaNO_3$ (ionic strength, approximately 1×10^{-3}). The ionic strength of both our prepared Milli-Q water and the filtered river water was approximately 1×10^{-3}.

3.2. *Effect of coagulant dose on infectious bacteriophage removal*

Fig. 2 shows the effect of coagulant dose on infectious bacteriophage removal, as measured by the PFU method after settling without centrifugal separation. Bacteriophage removal was not observed without PACl dosing, and the removal ratio of infectious bacteriophages ($\log[C_0/C]$) increased with coagulant dose. At a PACl dose of more than 1.08 mg-Al/L, more than 2-log removal of infectious bacteriophage was achieved for both Qβ and MS2. Therefore, the coagulation process is effective for the removal of infectious bacteriophages. Other studies have reported the usefulness of the coagulation process for removing infectious enteric viruses and infectious bacteriophages (Guy et al., 1977; Havelaar et al., 1995; Nasser et al., 1995).

In our study, however, the removal performances of Qβ and MS2 were quite different. The removal ratio of infectious Qβ was approximately 2 log higher than that of infectious MS2 at each of the PACl doses tested. This difference is detailed in the following section.

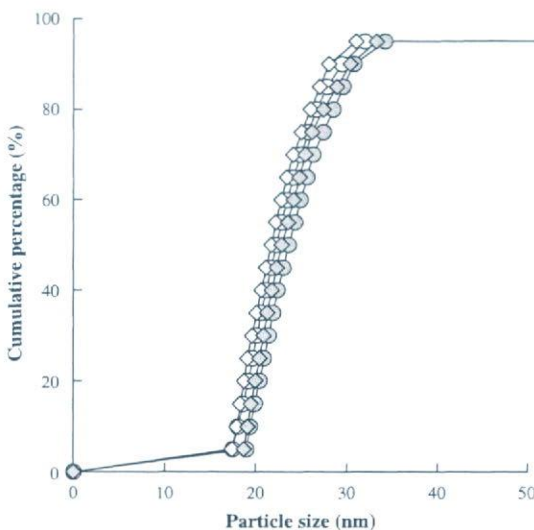

Fig. 1 – Particle size distribution of Qβ (white) and MS2 (gray) in the prepared Milli-Q water (circles) and filtered river water (diamonds), based on the number of particles. Values are the means of 200 or 400 measurements. Bacteriophage concentration in each sample was approximately 10^{10} PFU/mL.

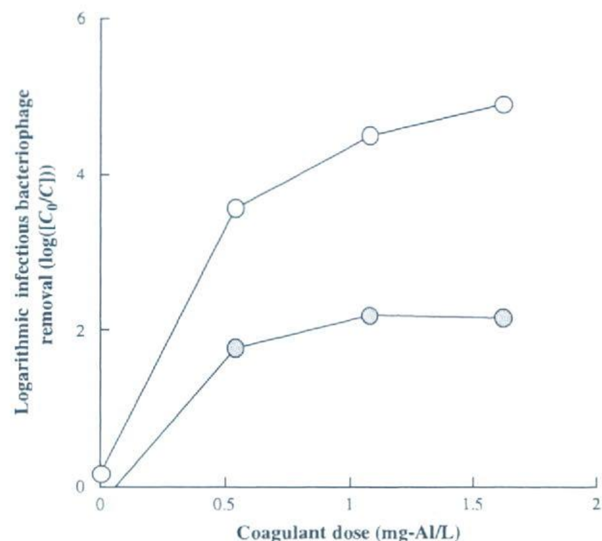

Fig. 2 – Effect of coagulant dose (0.54, 1.08 or 1.62 mg-Al/L) on infectious bacteriophage removal after settling without centrifugal separation. White and gray symbols represent Qβ and MS2, respectively. Initial bacteriophage concentrations in raw water were approximately 10^6 PFU/mL.

3.3. *Changes in the bacteriophage concentrations in the liquid phase of the floc mixture*

To investigate why the removal performances of Qβ and MS2 were different in the coagulation process, the changes in bacteriophage concentrations in the liquid phase of the floc mixture were measured during the coagulation process (Fig. 3). 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 among initial concentrations of 10^6, 10^7 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.

The total bacteriophage concentrations of Qβ and MS2 decreased as coagulation progressed (Fig. 3a). These reductions from the initial concentrations in raw water were probably due to the entrapment of bacteriophages in the aluminum floc particles, because the floc particles entrapping the bacteriophages were excluded from the liquid phase by the centrifugal separation. At each stage of the coagulation process, the reduction in Qβ concentration was similar to that of MS2, with 2-log reductions achieved for both bacteriophages by the end of the process (Fig. 3a). This result suggests that the particle behaviors of Qβ and MS2 were the same during the coagulation process; this possibility is supported by the particles' electrophoretic mobility, an important factor in the coagulation process. The electrophoretic mobilities of Qβ and MS2 were compared using the t-test (two-tail) based on 0.01 level of significance. No significant ($P > 0.01$) difference in the electrophoretic mobility was observed between the two bacteriophages in the filtered river water at pH 6.8, whereas a significant ($P < 0.01$) difference was observed in the prepared Milli-Q water (Fig. 4). Therefore, these two bacteriophages behaved in a similar manner as particles in the river water.

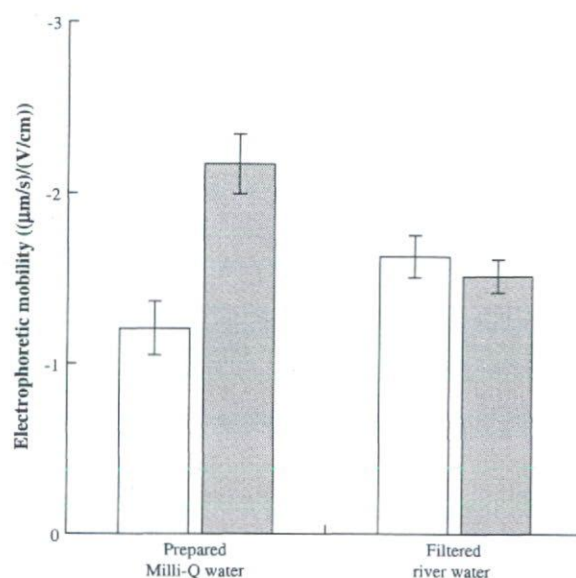

Fig. 4 – Electrophoretic mobility of Qβ (white) and MS2 (gray) in the prepared Milli-Q water and filtered river water. Values are the means and standard deviation of 25 measurements. Bacteriophage concentration of each sample was approximately 10^{10} PFU/mL.

Differences were observed between total bacteriophage concentration (Fig. 3a) and infectious bacteriophage concentration (Fig. 3b). The same phenomenon was

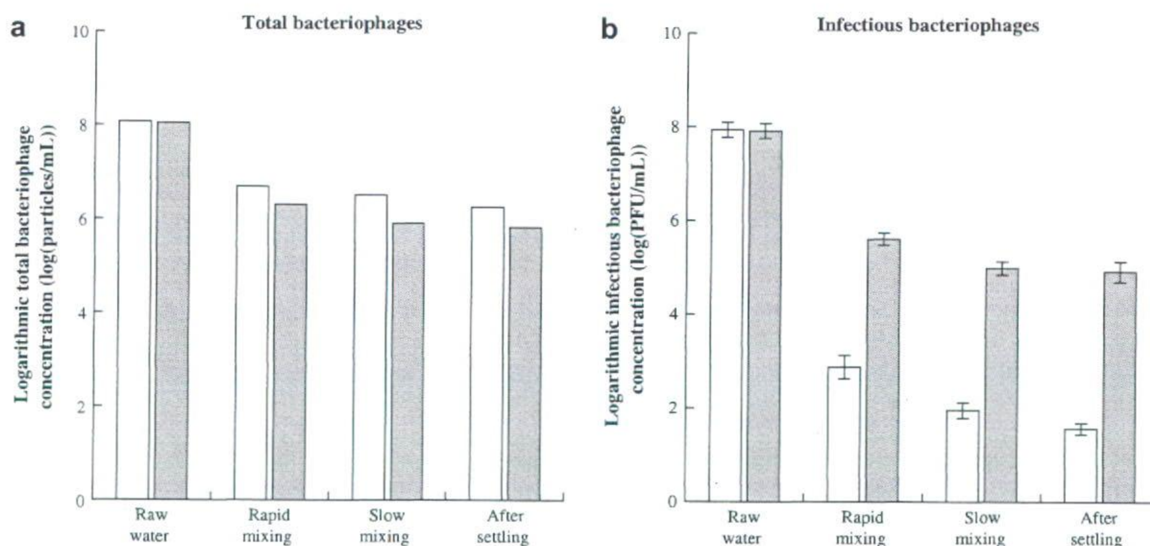

Fig. 3 – Changes in the concentrations of total bacteriophages (a) and infectious bacteriophages (b) in the liquid phase of the floc mixture during the coagulation process. The concentrations of all samples were measured after centrifugal separation. White and gray columns represent Qβ and MS2, respectively. Values of infectious bacteriophage concentrations are means and standard deviation of three replicates for Qβ and five replicates for MS2. Initial bacteriophage concentrations of raw water were approximately 10^8 PFU/mL. PACl dosage was 1.08 mg-Al/L.

observed during the coagulation–microfiltration (MF) process with 1.08 mg-Al/L of PACl (Shirasaki et al., in press): total Qβ concentration was larger than infectious Qβ concentration in the MF permeate. Because the pore size of the MF membrane used in the coagulation–MF process was 0.1 μm, the maximal size of microflocs which passed through the membrane was 0.1 μm. If the microfloc is assumed to be an aggregate of Qβ (50% cumulative percentage of particle size distribution of Qβ was 0.022 μm as shown in Fig. 1) having fractal dimension of 3.0, the 0.1-μm aggregate consists of 94 Qβ particles ($(0.1\ \mu m/0.022\ \mu m)^{3.0} = 94$). In other words, aggregates consisting more than 94 Qβ particles cannot pass through the MF membrane. If the difference between the total and infectious Qβ concentrations in the MF permeate is caused by aggregation alone, the difference is theoretically calculated at 2.0 log ($\log(94) = 2.0$). Tambo and Watanabe (1979) reported that the fractal dimension of aluminum floc particles produced by PACl addition is in the range of 1.5–2.0. Then, the required difference which are theoretically calculated from the reported fractal dimension is in the range of 1.0 log ($\log(0.1\ \mu m/0.022\ \mu m)^{1.5}$)–1.3 log ($\log(0.1\ \mu m/0.022\ \mu m)^{2.0}$). Actually, the differences between total and infectious Qβ concentrations which were observed in the coagulation–MF process were 1.1, 1.7, 2.3 and 2.4 logs at operation time of 1, 2, 3 and 4 h, respectively. These differences are more than the theoretically required differences. This means that the difference in the total and infectious bacteriophage concentrations cannot be explained by aggregation alone. A part of the bacteriophages must have been inactivated during the coagulation process. Therefore, in the present study, we believe that some the bacteriophages were inactivated during the coagulation process. Other researcher has also introduced the combination of the PFU and RT-PCR methods to discuss the virus inactivation during soil column filtration process, and suggest that the difference in the results between these two methods express the virus inactivation (Meschke and Sobsey, 2003).

The total and infectious bacteriophage concentrations differed markedly between Qβ and MS2: a 4-log difference was observed for Qβ, whereas less than a 1-log difference was observed for MS2. Thus, Qβ was more sensitive to virucidal activity of PACl than was MS2. Our research group previously reported on the virucidal activity of PACl (Matsui et al., 2003; Matsushita et al., 2004) and demonstrated that Qβ was the most sensitive to inactivation among four types of bacteriophages tested (Qβ, MS2, P1 and T4). The difference between Qβ and MS2 in their sensitivity to PACl may have resulted in the difference in the reduction of infectious bacteriophage concentrations: after settling, a 6-log reduction was achieved for Qβ, whereas a 3-log reduction was observed for MS2 (Fig. 3b). The virucidal activity of PACl for Qβ probably occurred in the liquid phase during the coagulation process.

The mechanism underlying the virucidal activity of the aluminum coagulant is not understood clearly. Matsui et al. (2003) reported that the infectivity of a bacteriophage did not decrease when the bacteriophage was exposed to a solution containing "preformed" aluminum hydroxide floc prepared by preneutralizing a solution of reagent-grade aluminum chloride with 1 M sodium carbonate at pH 7.5 for 3–19 days. This finding suggests that factors other than the presence of amorphous aluminum hydroxide rendered the bacteriophage inactive. PACl, which contains polymeric aluminum species, has been reported to induce much greater virucidal activity than monomeric aluminum coagulants, including reagent-grade aluminum chloride and aluminum sulfate, and alum used in actual treatment plants (Matsui et al., 2003; Matsushita et al., 2004). Thus, the polymeric aluminum species might play an important role in the virucidal activity. We

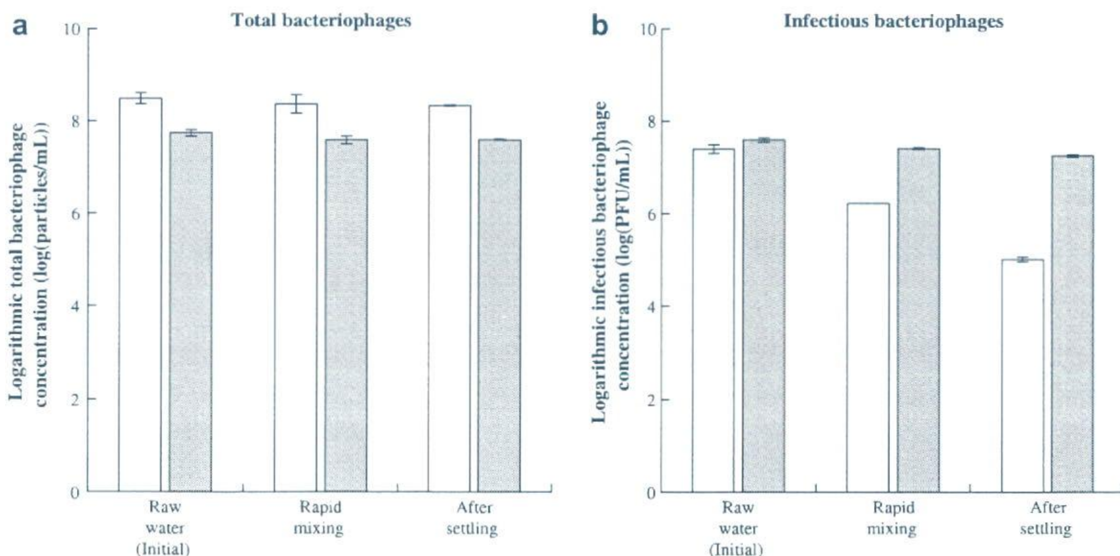

Fig. 5 – Changes in the concentrations of total bacteriophages (a) and infectious bacteriophages (b) of the floc mixture during the coagulation process. The concentrations of all samples were measured after floc dissolution. White and gray columns represent Qβ and MS2, respectively. Values are means and standard deviation of two replicates. Initial bacteriophage concentrations in raw water were approximately 10^8 PFU/mL. PACl dosage was 1.08 mg-Al/L.

imagine that some polymers formed during the hydrolysis of aluminum coagulant might sorb strongly to bacteriophages, either rendering them inactive or preventing infectivity, although further study is needed.

Our results revealed significant differences in the behaviors of Qβ and MS2 during the coagulation process. When the performance of a treatment plant employing the coagulation process is evaluated by using Qβ or MS2 as a surrogate for pathogenic waterborne viruses, the results should be judged carefully while considering the differences in behaviors described in this paper.

3.4. *Changes in the bacteriophage concentrations of the floc mixture after floc dissolution*

Once bacteriophages are entrapped in the aluminum floc particles during the coagulation process, they cannot be enumerated directly by the PFU method or real-time RT-PCR method. To quantify the bacteriophages in the floc particles, these particles were dissolved and the total bacteriophage concentration was measured. This value was then compared with the total bacteriophage concentration in the raw water (initial value) to confirm whether the floc particles formed during the coagulation process were dissolved completely. For both Qβ and MS2, the total bacteriophage concentrations recovered to their initial values at each stage of the coagulation process (Fig. 5a), indicating the complete dissolution of the floc particles.

The infectious MS2 concentrations in samples taken after rapid mixing and settling almost completely recovered to the initial value upon floc dissolution (Fig. 5b), meaning that no significant virus inactivation was observed for MS2 in the floc particles. This finding is in accord with those of Zhu et al. (2005), who suggested that MS2 was not inactivated by aluminum coagulant according to jar tests with 1–5 mg-Al/L: the infectious MS2 concentrations before and after coagulation, settling, and resuspension of the coagulated sludge were not statistically different. In contrast, infectious Qβ concentrations in the sample after rapid mixing and settling were still 1–2 log lower than the initial value after floc dissolution, indicating that the infectious Qβ concentration did not recover to its initial value (Fig. 5b). Therefore, Qβ was inactivated not only in the liquid phase but also in the floc particles during the coagulation process.

When the raw water in a drinking water treatment plant is polluted with a virus, the infectious virus is expected to be concentrated in the treatment sludge produced during the coagulation process; this would potentially increase the risk of infection when exposed to this sludge. In contrast, if the virucidal activity of the aluminum coagulant were consistent with that demonstrated for Qβ, most of the virus in the sludge would be inactivated. Therefore, the risk associated with exposure to the sludge could be decreased by using aluminum coagulant.

4. Conclusions

(1) Although their particle size and electrophoretic mobility were similar, the removal performances of infectious Qβ and MS2 during the coagulation process differed markedly: the removal ratio of infectious Qβ was approximately 2 log higher than that of infectious MS2 at all the PACl doses tested.
(2) Use of a combination of PFU and real-time RT-PCR methods revealed that the difference in behaviors between Qβ and MS2 during the coagulation process was probably not due to the difference in the extent of entrapment in the aluminum floc particles but to the difference in sensitivity to the virucidal activity of PACl. Significant inactivation of Qβ was observed during the coagulation process, whereas little or no inactivation of MS2 was observed.
(3) When the performance of a treatment plant employing the coagulation process is evaluated by using either Qβ or MS2, the results should be carefully judged considering the differences in their behaviors.

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Comparison of removal performance of two surrogates for pathogenic waterborne viruses, bacteriophage Qβ and MS2, in a coagulation–ceramic microfiltration system

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ABSTRACT

The removal performance of two surrogates for pathogenic waterborne viruses, F-specific RNA bacteriophages Qβ and MS2, was evaluated during the coagulation–ceramic microfiltration process. River water spiked with these bacteriophages was used to investigate differences in their behaviors. Infectious and total (infectious + inactivated) bacteriophage concentrations were measured by plaque forming unit and real-time reverse transcription–polymerase chain reaction methods, respectively. Removal of infectious Qβ and MS2 was similar under each coagulation condition. Approximately 6-log reduction was achieved for both bacteriophages at 1.08 mg-Al/L of coagulant dose and 5-min coagulation time. At least 4-log reduction occurred even when coagulant dose and coagulation time were reduced to 0.54 mg-Al/L and 1.8 s, respectively. In contrast, removal of total Qβ and MS2 differed markedly. Removal of total MS2 was approximately 2-log larger than that of total Qβ, possibly owing to selective interaction with the cake layer, although the particle diameters and electrophoretic mobilities of Qβ and MS2 were similar. The total number of bacteriophages retained in the microfiltration compartment after 4-h filtration was similar for Qβ and MS2, but there were approximately 3 log fewer infectious Qβ than infectious MS2, probably owing to the difference in sensitivity to the virucidal activity of aluminum coagulant.

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1. Introduction

Low-pressure membrane (microfiltration and ultrafiltration membranes) technologies provide important alternatives to separation processes such as sedimentation and rapid sand filtration. Microfiltration (MF) is effective for the reduction of turbidity and removal of bacteria, algae, and protozoa. Among MF membranes, ceramic membranes have attracted attention in the field of drinking water treatment, because they can withstand extreme acidity and alkalinity and higher operating pressures than polymeric and other inorganic membranes [1]. These properties of ceramic membranes allow the use of strong acids and bases in chemical cleaning, application of high pressure for hydraulic backwashing, and operation at a high filtration flux. However, MF membranes alone cannot be expected to efficiently remove pathogenic waterborne viruses such as hepatitis A viruses, polioviruses, and noroviruses, because membrane pore sizes are not small enough. Some studies have reported insufficient virus removal by MF membrane alone [2–5], although the U.S. Environmental Protection Agency requires 4-log removal/inactivation of viruses [6].

Using a coagulation process as pretreatment for the MF process may mitigate membrane fouling and improve permeate water quality [7–9]. In addition, a high ratio of virus removal was achieved by the combination of coagulation and MF processes. Zhu et al. [10] reported a >4-log removal of viruses at pH 6.3 with 10 mg-Fe/L of ferric chloride and 0.22-μm pore size of polyvinylidene fluoride MF membrane, whereas MF alone achieved a <0.5-log removal. Fiksdal and Leiknes [11] reported that no virus removal was obtained without a coagulation/flocculation pretreatment process, whereas the combination of coagulation/flocculation and MF (0.2-μm pore size of polyethersulfone) processes with 3–5 mg-Al/L of commercial aluminum-based coagulant provided from 6.7- to >7.5-log removal of viruses. Our research group also reported the effectiveness of a coagulation–ceramic MF system [12,13]: a >6-log removal of viruses was achieved by two types of 0.1-μm pore size ceramic MF systems (positive pressure-driven monolith type and negative pressure-driven immersed type) with 0.5–1.0 mg-Al/L of polyaluminum chloride.

To evaluate the virus removal performance of the membrane filtration process, bacteriophages (i.e., viruses that infect

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bacteria) have been used as possible indicators for enteric viruses. Because of their morphological similarity to hepatitis A viruses and polioviruses, F-specific RNA bacteriophage MS2 is widely used as a surrogate for pathogenic waterborne viruses in Europe and the United States [2,10,11,14–16] and the F-specific RNA bacteriophage Qβ tends to be used as a surrogate in Japan [4,5,12,13,17,18]. Previous studies have compared the behaviors of Qβ and MS2 in the coagulation process and direct MF process. The removal of these bacteriophages in the coagulation process with polyaluminum chloride differed markedly [19,20], whereas their removal was similar in the direct MF process with a 0.1-μm pore size ceramic membrane [21]. Therefore, the removal of Qβ and MS2 in the coagulation–MF process may differ as well, although these differences have not been investigated fully. If marked differences do exist, then Qβ and MS2 cannot be used as equivalent surrogates to evaluate the effectiveness of coagulation–MF processes for the removal of pathogenic waterborne viruses.

The effectiveness of membrane filtration processes, including the coagulation–MF process, for virus removal is generally evaluated based on the results of the plaque forming unit (PFU) method, which can detect only infectious viruses. However, a decrease in the infectious virus concentration is not only due to physical removal (e.g., sieving, adsorption) during the membrane separation process, but also to virucidal activity of aluminum coagulant [19,20] during the coagulation pretreatment. The mechanisms of virucidal activity of aluminum coagulant remain unclear. Inactivated viruses in the MF permeate might recover their infectivity in the water distribution system. However, the PFU method cannot detect the inactivated viruses that leak into MF permeate, which might underestimate the risk of infection. In other words, the removal performance of virus particles during the coagulation–MF process may be overestimated. The PFU method cannot distinguish whether a decrease results from physical removal, virus inactivation during the treatment process, or both. In contrast, the polymerase chain reaction (PCR) method can detect infectious viruses as well as inactivated viruses, unless the target nucleic acid is lost. Accordingly, a combination of the PFU and PCR methods allows physical removal and virus inactivation in the treatment processes to be clearly distinguished. To date, only our research group has investigated the virus removal performance and mechanisms of both infectious and inactivated viruses in the coagulation–MF process [17,18].

Our objectives in the present study were to investigate the behavioral differences of Qβ and MS2 during the coagulation–ceramic MF system using both PFU and real-time reverse transcription–polymerase chain reaction (RT-PCR) methods and to elucidate what caused those differences.

2. Materials and methods

2.1. Source water, coagulant, and MF membranes

River water was sampled on 12 October 2007 from the Toyohira River (Sapporo, Japan; water quality shown in Table 1). Polyaluminum chloride (PACl 250A; 10.5% Al_2O_3, relative density 1.2 at 20 °C; Taki Chemical Co., Ltd., Hyogo, Japan) was used for the coagulation process. The membrane used was a monolithic ceramic MF module (61-channel tubular; nominal pore size 0.1 μm, effective filtration area 0.048 m^2, membrane diameter 0.03 m, membrane length 0.1 m; NGK Insulators, Ltd., Nagoya, Japan), which was installed in a stainless-steel casing.

Table 1
Water quality measures 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

2.2. Bacteriophages

The bacteriophages Qβ (NBRC 20012) and MS2 (NBRC 102619) were obtained from the NITE Biological Research Center (NBRC, Chiba, Japan). Qβ is the prototype member of the genus *Allolevivirus* in the Leviviridae, and MS2 is the prototype member of the genus *Levivirus* in the Leviviridae. The genomes of these two bacteriophages contain a single molecule of linear positive-sense, single-stranded RNA, which is encapsulated in an icosahedral protein capsid with a diameter of 24–26 nm [22]. Each bacteriophage was propagated for 22–24 h at 37 °C in *Escherichia coli* (NBRC 13965) obtained from NBRC. The bacteriophage culture solution was centrifuged (2000 × *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 with a centrifugal filter device (molecular weight cutoff 100,000, regenerated cellulose; Centriplus-100, Millipore Corp., Billerica, MA, USA) to prepare the bacteriophage stock solution.

2.3. Coagulation–ceramic MF experiments

The experimental setup is shown in Fig. 1. The river water was spiked with either Qβ or MS2 in the raw water tank at approximately 10^6 or 10^8 PFU/mL. The raw water tank was constantly mixed with an impeller stirrer during the experiments. The raw water was fed into the system at a constant flow rate (83.3 L/m^2 h) by a peristaltic pump. Hydrochloric acid was added before the first in-line static mixer (hydraulic retention time 1.8 s; 1/4-N40-172-0, Noritake Co., Ltd., Nagoya, Japan) to maintain the MF permeate at pH 6.8. PACl was injected after the first in-line static mixer and before the second in-line static mixer at a constant dose rate (0.54 or 1.08 mg-Al/L). To obtain the two coagulation times, the in-line static mixer (hydraulic retention time 1.8 s) and a combination of the in-line static mixer and a subsequent tygon tube reactor (total hydraulic retention time 5 min) were used as the second in-line static mixer. After the PACl had been mixed in, the water was fed into the ceramic MF module in dead-end mode. Filtration was performed for 4 h without any backwashing. Bacteriophage concentrations in the raw water tank and in the MF permeate were measured every 1 h.

2.4. Extraction of bacteriophage from ceramic MF compartment

After the filtration experiments, the water (floc mixture) in the MF compartment was withdrawn by gravity. To quantify the bacteriophage concentration in the liquid phase of the floc mixture, the suspended floc particles were separated from the mixture by centrifugation (2000 × *g*, 10 min), and then the bacteriophage concentration in the supernatant was measured by the PFU and real-time RT-PCR methods (see Section 2.5): the PFU method measured the concentration of infectious bacteriophages, and the real-time RT-PCR method measured the concentration of total bacteriophages regardless of their infectivity. To quantify the bacteriophage concentrations in the suspended aluminum floc particles, the floc particles were dissolved by raising the pH of the water to 9.5 with aqueous sodium hydroxide in 12% beef extract (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) solution and vortexing the water intensely for 5 h at 4 °C. Beef extract was used in an effort to prevent the inactivation of virus during floc dissolution [19]. The bacteriophage concentrations in the floc mixture were

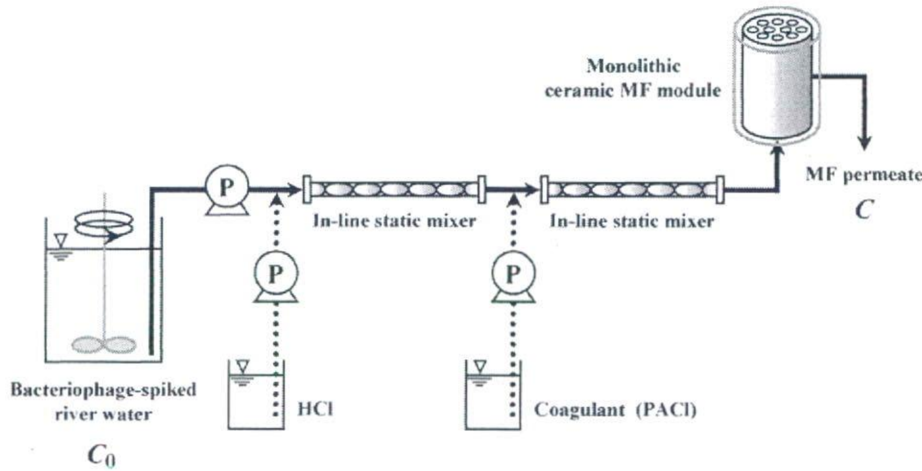

Fig. 1. Schematic diagram of the experimental coagulation–ceramic MF system. C_0 and C are the bacteriophage concentrations (PFU or particles/mL) in the raw water tank and the MF permeate, respectively, at each sampling time.

then measured by the PFU and real-time RT-PCR methods. In addition, to elute floc particles retained on the membrane surface after the floc mixture had been withdrawn by gravity, hydraulic backwashing (pressure 0.5 MPa) with 200 mL of Milli-Q water (Milli-Q Advantage, Millipore Corp.) was conducted using a nitrate gas cylinder. The floc particles in the backwash eluent were dissolved by the method described above. The bacteriophage concentrations in the backwash eluent were measured by the PFU and real-time RT-PCR methods. Finally, the quantities of bacteriophages in the solid phase in the MF compartment were calculated from the bacteriophage concentrations in the floc mixture and the backwash eluent.

Because beef extract is known to inhibit the amplification of the viral genome by PCR [23,24], each floc dissolution sample containing beef extract was diluted 10-fold with Milli-Q water before the real-time RT-PCR quantification.

2.5. Bacteriophage assays

2.5.1. PFU method

The infectious bacteriophages were enumerated according to the double-layer method [25] using the bacterial host *E. coli*. The average of plaque counts of triplicate plates prepared from one sample was considered as the infectious bacteriophage concentration.

2.5.2. Real-time RT-PCR method

Viral RNA of bacteriophages was quantified by the real-time RT-PCR method, which detects viruses regardless of their infectivity. We defined concentration measured by the real-time RT-PCR method as total bacteriophage concentration. For quantification of bacteriophages in the raw water, MF permeate, and liquid phase of the floc mixture, viral RNA was extracted from 200 μL of sample with a QIAamp MinElute Virus Spin Kit (Qiagen K.K., Tokyo, Japan) to obtain a final volume of 20 μL. For quantification of bacteriophages in the floc dissolution sample containing beef extract, a 100-μL sample was heated at 90 °C for 10 min and then cooled to 4 °C for 1 min in a thermal cycler (Thermal Cycler Dice Model TP600, Takara Bio Inc., Shiga, Japan) to extract viral RNA by destroying the capsid. The extracted RNA solution was added to a High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems Japan Ltd., Tokyo, Japan) for the RT reaction, which was conducted at 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 s, followed by cooling to 4 °C in the thermal cycler. The cDNA solution was then amplified by TaqMan Universal PCR Master Mix with UNG (Applied Biosystems Japan Ltd.), 400 nM of each primer (HQ-SEQ grade, Takara Bio Inc.), and 250 nM of TaqMan probe (Applied Biosystems Japan Ltd.). The oligonucleotide sequences of the primers and the probes are shown in Table 2. Amplification was conducted at 50 °C for 2 min, 95 °C for 10 min, and 50 cycles of 95 °C for 15 s and 60 °C for 1 min in an ABI Prism 7000 Sequence Detection System (Applied Biosystems Japan Ltd.).

The standard curve for the real-time RT-PCR method was based on the relationship between the infectious bacteriophage concentration of a freshly prepared stock solution measured by the PFU method and the number of cycles for amplification in the PCR process, which is based on the assumption that the freshly prepared stock solution did not contain any inactivated bacteriophages.

2.6. Electron micrography

Qβ and MS2 were observed with an electron microscope. Ten microliters of each bacteriophage stock solution (see Section 2.2) was placed on a 400-mesh copper grid with collodion membrane (Nissin EM Corp., Tokyo, Japan) and adsorbed to the grid for 1 min. Excess solution on the grid was drained with filter paper, and

Table 2
Oligonucleotide sequences of the primers and the probes used in real-time RT-PCR quantification of Qβ and MS2.

Viruses		Oligonucleotide sequences	Positions	References
Qβ	Forward primer	5′-TCA AGC CGT GAT AGT CGT TCC TC-3′	49–71	[26]
	Reverse primer	5′-AAT CGT TGG CAA TGG AAA GTG C-3′	187–208	
	TaqMan probe	5′-CGA GCC GCG AAC ACA AGA ATT GA-3′	147–169	
MS2	Forward primer	5′-GTC GCG GTA ATT GGC GC-3′	632–648	[27]
	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	

bacteriophages were negatively stained with 10 μL of 2% phosphotungstic acid (pH 7.0) for 45 s. After the excess strain was drained off, each grid was examined with a transmission electron microscope (TEM, JEM-1210, Jeol Ltd., Tokyo, Japan). Particle diameter of each bacteriophage was expressed as the mean and standard deviation of 20 randomly chosen particles on the electron micrograph.

2.7. Electrophoretic mobility

Electrophoretic mobility of bacteriophages was measured in prepared Milli-Q water and filtered river water. To bring the alkalinity to 20 mg-$CaCO_3$/L, 0.4 mM $NaHCO_3$ was added to the Milli-Q water, and the pH was adjusted to 6.8 with HCl. River water was filtered through a stirred ultrafiltration cell (Model 8400, Millipore Corp.) with 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 electrophoretic mobility, each bacteriophage was suspended at approximately 10^{10} PFU/mL in the prepared Milli-Q water or filtered river water using the bacteriophage stock solution. The electrophoretic mobility of the bacteriophages was measured with an electrophoretic light-scattering spectrophotometer (ELS-6000, Otsuka Electronics Co., Ltd., Osaka, Japan) 25 times for each sample at 25 °C and at a 15° measurement angle.

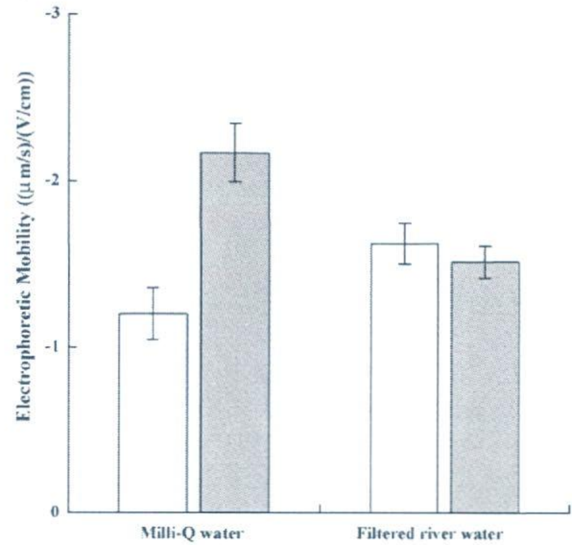

Fig. 3. Electrophoretic mobility of Qβ (white) and MS2 (gray) in the prepared Milli-Q water and filtered river water. Values represent the mean and standard deviation of 25 measurements. Bacteriophage concentration of each sample was approximately 10^{10} PFU/mL.

3. Results and discussion

3.1. Particle diameter and electrophoretic mobility

Fig. 2 shows the electron micrographs of Qβ and MS2. Nearly the same particle diameters were observed: 23.5 ± 0.8 nm for Qβ and 22.5 ± 1.0 nm for MS2. These values correspond with the particle diameters previously reported for Qβ and MS2 [22].

To investigate the electrokinetic properties of bacteriophages, the electrophoretic mobility (i.e., surface charge) was measured in the prepared Milli-Q water and filtered river water (Fig. 3). The surface charge on virus particles is often invoked to discuss the virus removal by physicochemical water treatment processes such as coagulation [20], media filtration [28–30], and membrane filtration [4,14]. The electrophoretic mobility of Qβ and MS2 were compared using the t-test (two tail) based on 0.01 level of significance. In the present study, the electrophoretic mobility of Qβ in the prepared Milli-Q water was significantly ($P < 0.01$) less than that of MS2. Thus, the capsid proteins of these two bacteriophages consist of different types of amino acid, so that the surface charges of the bacteriophages differed according to the difference in the dissociation of functional groups in constitutive amino acids. In the prepared Milli-Q water, the behaviors of these bacteriophages might be different during the coagulation–ceramic MF process. In contrast, no significant ($P > 0.01$) difference in electrophoretic mobility was observed in the filtered river water, possibly because the ionic strength of the river water is large enough to compress the effective thickness of the diffuse layer around the bacteriophage particles, which would allow the attractive van der Waals interaction to dominate [31] and reduce the difference in the electrophoretic mobility accordingly. Alternatively, multivalent ions present in the river water influenced the electrophoretic mobility. In this river water, Qβ and MS2 are expected to behave in a similar manner as would particles during the coagulation–ceramic MF process. Moreover, the surface charges of these two bacteriophages were negative at this pH condition (Fig. 3). This result is supported by previous studies [30,32] in which the isoelectric point (pI) of Qβ and MS2 were reported at around 5.3 and 3.9, respectively. The negative surface charge of Qβ and MS2 indicate that virus–virus aggregates were unlikely to be generated

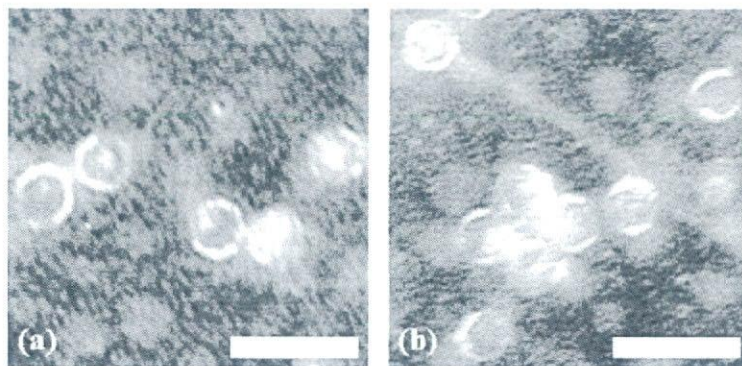

Fig. 2. Negative-strain electron micrographs of (a) Qβ and (b) MS2. The scale bar represents 50 nm.

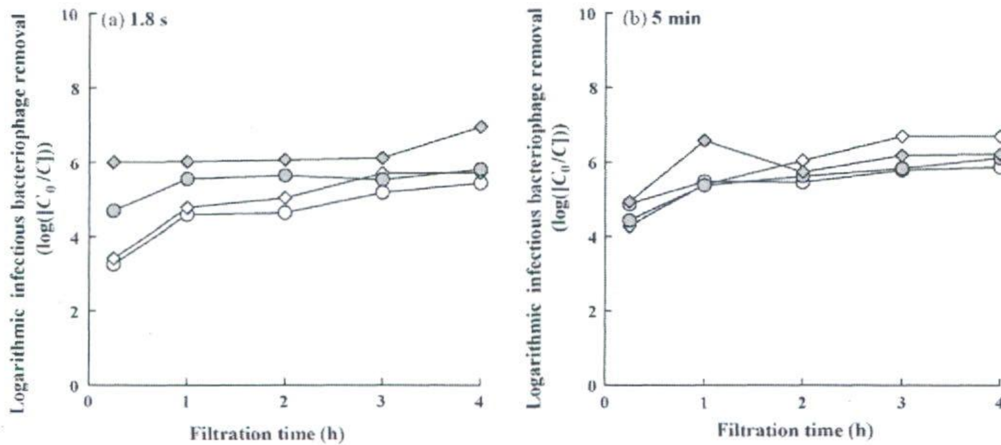

Fig. 4. Effect of coagulant dose and coagulation time on infectious bacteriophage removal. Open and solid symbols represent Qβ and MS2, respectively. Circles and diamonds represent coagulant dosage of 0.54 and 1.08 mg-Al/L, respectively. Coagulation times were 1.8 s (a) and 5 min (b). Initial bacteriophage concentrations of raw water were approximately 10^6 PFU/mL.

and these two bacteriophages were stably monodispersed in the raw water used (without coagulant dosing).

3.2. Effect of coagulant dose and coagulation time on infectious bacteriophage removal

Fig. 4 shows the effect of coagulant dose and coagulation time on the infectious bacteriophage removal obtained by the PFU method in the coagulation–ceramic MF system (initial concentration of each bacteriophage was approximately 10^6 PFU/mL). Because the diameters of both bacteriophages, as confirmed by electron microscopy, are smaller than the nominal pore size of MF membrane (0.1 μm), nonaggregated viruses are expected to pass through the MF membrane. Although other researchers have reported adsorptive interactions between virus and membrane surface, such as electrostatic and hydrophobic interactions, as some of the important factors for virus removal in the MF process [4,14], no removal of Qβ and MS2 was observed during the 4-h filtration without coagulation pretreatment (data not shown). This result suggests that the effect of electrostatic and hydrophobic interactions were negligible in this ceramic MF process. In contrast, addition of the coagulation pretreatment was effective in removing infectious bacteriophages under all coagulation conditions (Fig. 4). The removal ratio ($\log[C_0/C]$) of both infectious bacteriophages gradually increased with filtration time, and a >5-log removal ratio was achieved for each infectious bacteriophage after 4 h of filtration. In a study using Qβ, our research group previously reported the factors that contribute to the time-course increase in the virus removal ratio [18]: growth of a cake layer that accumulated on the membrane surface and accumulation of foulants on the internal structure of the membrane pores probably account for the time-course increase in the virus removal ratio in the coagulation–ceramic MF system.

The effect of coagulant dose (0.54 mg-Al/L vs. 1.08 mg-Al/L) on infectious bacteriophage removal was observed with the 1.8-s coagulation time (Fig. 4a). The removal ratio of each bacteriophage increased with coagulant dose, but the effect was not large, perhaps because the infectious bacteriophage concentrations in the MF permeate were close to its detection limit (10^0 PFU/mL, approximately 6-log removal) with the 1.08 mg-Al/L coagulant dose. In contrast, Matsushita et al. [13] reported that the coagulant dose strongly affected the virus removal by the coagulation–ceramic MF system: the time-averaged virus removal was only 2.8 log at a 0.54 mg-Al/L coagulant dose, whereas 1.08 mg-Al/L achieved a 6.4-log time-averaged virus removal of infectious Qβ. This result does not correspond with the present findings, even though the same coagulation–ceramic MF system was used in both studies. One reason for the discrepancy is the difference in source water. The previous study used Toyokawa River (Aichi, Japan) water (DOC 1.1 mg/L, OD260 0.037 cm^{-1}) as the source [13]. DOC and OD260 of Toyokawa River water were higher than those of Toyohira River water (Table 1). These water qualities possibly affected the virus removal performance in the coagulation process. Accordingly, the removal performances of infectious bacteriophages between the previous and the present study were different at 0.54 mg-Al/L coagulant dose. The coagulation time also affected the infectious bacteriophage removal: extending the coagulation time from 1.8 s to 5 min somewhat increased the removal ratio of infectious bacteriophages. Thus, coagulant dose and coagulation time affected the infectious bacteriophage removal in this coagulation–ceramic MF system.

Fig. 5 summarizes the time-averaged infectious bacteriophage removal in the coagulation–ceramic MF system. According to the U.S. Environmental Protection Agency National Primary Drinking

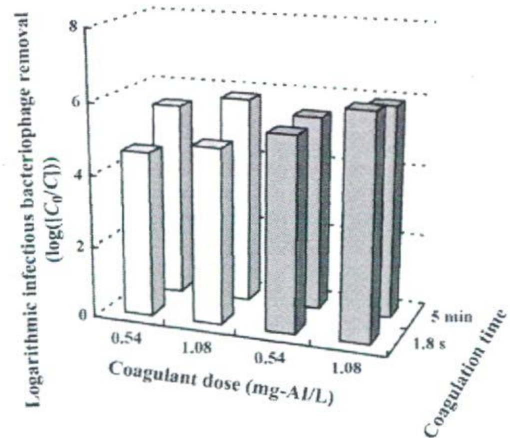

Fig. 5. Filtration time-averaged infectious bacteriophage removal. White and gray columns represent Qβ and MS2, respectively. Initial bacteriophage concentrations of raw water were approximately 10^6 PFU/mL.