２．第27回SIAMでの審議状況

（１）初期評価文書の審議結果

初期評価文書の審議は、スポンサー国または産業界が初期評価文書の原案をCDGに掲載し、CDG上で行う事前討議（コメントの提出、コメントへの返答、コメントに応じたSIAPの修正）およびSIAMでの対面討議で行われる。第27回SIAMでの初期評価文書の審議は、CDGでの事前討議を基に修正したSIAPを用いて行われた。日本は、日本政府が原案を作成したSodium *p*-toluenesulfonate（CAS: 657-84-1）、ICCAが原案作成した1,3-Benzenediol（CAS: 108-46-3）、および日本政府とドイツがEUの評価文書をもとに作成したN-cyclohexylbenzothiazole-2-sulphenamide (CAS: 95-33-0）の初期評価文書を提出した。今回の会議では、35物質（37CAS）の初期評価文書が審議され、すべての初期リスク評価結果が合意された（表1)。中でも、次の物質については通常の審議と異なる点があったため特筆する。

1) 1,3-Benzenediol (CAS: 108-46-3)

1,3-Benzenediol (CAS: 108-46-3）については、HPV点検プログラムと国化学物質安全性計画(IPCS: International Programme on Chemical Safety）の国際簡潔評価文書（CICAD: Concise International Chemical Assessment Document）プログラムによる重複作業を防ぐための試みとして、CICADをSIARとして提出することに合意が得られていたが（松本他 2006)、スポンサーである日本／ICCAが、CICADの評価結果と異なる評価を行ったため、今回のSIAMでは独自に作成された初期評価文書が審議された。今回のSIAMで初期評価内容に合意が得られたが、Dossierに記載されていない情報があったため、SIAM後に詳細データを回覧することになった。

2) 物質カテゴリー：Nickels and nickel compounds (CAS: 3333-67-3, 7440-02-0, 77185-4-9, 7786-81-4, 12122-15-5, 12607-70-4, 13138-45-9)

デンマーク：eu (注：この記号は欧州連合でのリスク評価文書を基にしたことを意味する)が担当した物質カテゴリー（Nickels and nickel compounds）は第24回SIAMで審議され、ヒト健康影響についてのみ合意された（松本他 2007)。今回は環境影響について再審議され合意が得られた。合意された環境影響についての初期評価文書は既存化学物質タスクフォースの承認を得た後に、ヒト健康影響についての初期評価文書に結合される。

3) Antimony(Ⅲ)oxide(Sb_2O_3)(CAS: 1309-64-4)

スウェーデン：euが担当したAntimony(Ⅲ)oxide(Sb_2O_3)（CAS: 1309-64-4)は環境中でSenarmontite (CAS: 12412-52-1) および Valentinite (CAS: 1317-98-2) の構造で存在することがあり、これら３つのCAS番号で示される物質の曝露や毒性影響の差についての情報がないことから、前述の2つのCAS番号が初期評価文書に追記されることになった。

（２）HPV点検プログラムにおける全般的な議題

1) Dossierの化学物質用途の記述について

化学物質の用途はSIDS項目の一つであり、一般的な用途、消費者製品としての使用方法および製造方法を記載することになっている。しかし、近年カテゴリー評価を用いた場合に、化学物質の放出や曝露の可能性を推論しにくいという問題が生じている。そこで、今回のSIAMに先立て、Dossierの用途記載方法を修正するための案が報告された。アメリカ、カナダ、日本が事前にコメントを提出した。ほとんどのコメントは修正案を支持するものであり、最近行われた既存化学物質タスクフォースでも修正案に合意が得られた。第27回SIAMも修正案を支

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持したが、Dossier は入力しやすい形式であるとともに、化学物質の環境放出の可能性について個別に分類できることが必要であるとした。次回の SIAM では、米国とカナダの事例を参考にした HPV 点検プログラムのマニュアル修正文書および、より詳細なマニュアルのガイダンスが紹介される予定である。

2) CSR/SIAR プラグイン機能について

IUCLID5 は OECD の HPV 点検プログラムだけでなく、欧州連合における **REACH** (Registration, Evaluation, Authorisation and Restriction of Chemicals) でも使用されている。今回の SIAM では IUCLID 5 に入力したデータから必要な情報を抜き出して、SIAR または **CSR** (Chemical Safety Report ; REACH で使用) を作成するプラグイン機能の開発について報告された。現時点では CSR を作成するためのプラグイン機能のみが開発されており、SIAR を作成するためのプラグイン機能を作成するには別に資金が必要である。第 27 回 SIAM は、SIAR 作成のプラグイン機能は大変有用であるとしたが、SIAR 用に資金を提供する前に、CSR プラグイン機能の使用経験を集積すべきであるとした。または、CSR プラグイン機能で作成したレポートから不要な部分を削除することによって、SIAR を作成することも可能であるとした。

3) OECD HPV点検プログラムの発展について

US チャレンジプログラムでは、化学物質の有害性の特徴を示す文書 (HC : Hazard Characterizations) を公開しており (EPA 2008)、第 26 回 SIAM は HC を SIAR の代わりに OECD の HPV 点検プログラムに提出することを承認した。HC の文書には生産量、用途および曝露についての情報は含まれていないので、HPV 点検プログラムに提出する際には情報の追加が必要とされる。第 27 回 SIAM では、HC を OECD へ提出する際の手続き方法についての案が報告され、次のアウトラインに合意が得られた。

<u>米国から提出する場合</u>

- HC が公表前の場合、米国は HC 草案を SIAM に提出し SIAM での審議をもとに HC の最終文書を作成する。EPA は修正済み HC をウェブサイトで公開する。OECD は SIAP を公開し HC の文書を参照できるようにする。HC には、どこの部分が OECD での審議の結果を反映したもので、どこの部分が US チャレンジプログラム特有の評価かを示す。もしくは、米国が同じ有害性評価の結論を含む２セットの文書を US チャレンジプログラム用と、OECD のプログラム用に別々に作成する。
- HC が公表されている場合、SIAM での審議をもとに米国が文書を修正し、修正文書を EPA のウェブサイトで公開するか、または通常の HPV 点検プログラムの文書と同様に OECD 事務局に提出し、出版の手続きを取る。

<u>米国以外の国から提出する場合</u>

HC を HPV 点検プログラムに提出したい国は事前に EPA に連絡し、もし有害性について HC と異なる結論に至る場合は、SIAM 前に協議により問題を解決するよう努力する。スポンサー国は HC を本プログラムに提出するために不足している情報の追加を行う。もし、使用方法についての情報がスポンサー国内にない場合は、米国の製造者から情報が得られるかもしれない。SIAM で合意が得られた場合、スポンサー国は修正文書を OECD 事務局に提出する。

<u>産業界から提出する場合</u>

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HC を OECD の HPV 点検プログラムに提出したいスポンサー（会社および会社のコンソーシアム）は EPA に事前に連絡し、もし有害性について HC と異なる結論に至る場合は、SIAM 前に協議により問題を解決するよう努力する。SIAM で合意が得られた場合、スポンサーは修正文書を OECD 事務局に提出する。

米国は HC を作成するにあたっては、産業界から提出される Dossier に加え、文献検索も行う予定であるとした。BIAC は HC を HPV 点検プログラムに提出するためのスポンサーを見つける努力をするとともに、今後も有害性情報の提供を続けるよう努めるとした。

4) OECD HPV 点検プログラムにおける選択的評価の使用について

OECD の HPV 点検プログラムの未評価物質の審議を効率よく終えるために、特定のエンドポイントのみを評価する手法（選択的評価：Targeted assessment）の導入の可能性について審議された。選択的評価は、環境影響またはヒト健康影響について入手可能な全ての情報から、有害性評価に最も関連の強い一つもしくは複数のエンドポイントに焦点を絞って評価する手法である。カナダはカナダ環境保護法 1999（**CEPA 1999**: Canadian Environmental Protection Act 1999）で行われているリスク評価を例に評価手法を紹介した。また、欧州化学物質庁（**ECHA**：European Chemicals Agency）は EU が準備している REACH の添付書 XV Dossier を紹介し、特定の有害性、例えば PBT（残留性・蓄積性・有害性のある物質）または CMR（発がん性・変異原性・生殖毒性のある物質）などに対して、どのように選択的評価が行われるかを紹介した。

OECD 事務局は HPV 点検プログラム内でどのように選択的評価が使用されていくかのアウトラインを提示した。

- OECD の既存点検プログラムにおける選択的評価とは、限定的な数の有害なエンドポイントのみの（すなわち SIDS 項目を満たさない）有害性の評価と定義される。
- 選択的評価は、より決定的な手法がまとまるまでは、non-HPV に限定して適用すべきであり、OECD 加盟国にとって有用なものであるためには、リスク評価、分類と表示およびリスク削減などの目的に関連するエンドポイントが評価されるべきである。
- 有害性の初期評価について結論が導けるあらゆるエンドポイントが評価対象となる。SIDS 項目のエンドポイントから結論を導くためには、HPV 点検プログラムで示されている最小限の情報を満たしている必要がある。
- SIDS 項目でないエンドポイントを用いた選択的評価も可能であり、生体濃縮などの SIDS 項目でないエンドポイントだけで評価することも予想され得る。
- 結論を導き出した根拠となるエンドポイントの情報はすべて記載すべきであり、そのほかのエンドポイントについては、情報を収集していないことを明記する。
- 物質カテゴリーの場合も選択的評価も同様に行うことができるが、カテゴリーの正当性を示す根拠（例えば物理化学的性質）となるエンドポイントの結果が必要とされる。
- 選択的評価を行った場合、文書の読者がフルの初期評価文書と異なることを理解できるよう記されている必要がある。また、結論を導き出したエンドポイントをリストに示す必要がある。こういった免責事項は SIAP の上部および SIAR のカバーページに示される。
- 評価文書は SIAP, SIAR, Dossier と同じ形式を使用し、必要な情報のみを入力する。SIAR としての目的を満たすのであれば、別のタイプのレポートが使われることも可能である。
- 選択的評価は、初期評価文書と同様の手順を用い SIAM で審議され得るが、異なる議題として審議さる。選択的評価における評価文書は、OECD から公開されるとともに、UNEP からも公表され得る。

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- 選択的評価の文書作成におけるスポンサーシップについても、OECD のデータベースにより公開され、プログラムへの貢献としてカウントされる。しかし、統計的にはフルの初期評価文書の提出と、選択的評価の評価文書の提出は分けて処理される。

SIAMはOECDが示したアウトラインにほぼ合意したが、次のような勧告がなされた。

- 選択的評価を行うことを正当とする理由を明記する必要がある。
- 入手可能な全ての有害性情報を限定的な範囲で示すべきである。

OECD の HPV 点検プログラムでは、日本の化審法のスクリーニング試験として要求されていない生殖発生毒性試験結果が必要とされる。今後、選択的評価による評価文書が受け入れられることになると、化審法のみで評価された物質を選択的評価の評価文書として OECD に提出し得ると考えられた。OECD 事務局は選択的評価手法についての文書を修正し、回覧することになった。最終草案は 2009 年 4 月の既存化学物質タスクフォースまでに準備される。さらに、OECD 事務局はカナダ、ECHA および英国とともに選択的評価の文書例を、第 28 回 SIAM に試験的に提出できないか検討することになった。

5）優先順位設定ツールを用いた評価方法について

OECD の HPV 点検プログラムの未評価物質の審議を効率よく終えるために、評価すべき物質の優先順位をつけるためのツール（優先順位設定ツール：Priority setting tools）を用いた物質選定の利用を検討した。カナダは国内物質リストに掲載されている化学物質（23,000 物質）のカテゴリー化と優先順位の設定方法について紹介した。カナダでは、難分解・高蓄性の性質および急性・慢性の水生毒性が環境影響の優先順位を定める基準とされ、ヒト健康影響では、曝露の可能性および物質特有の毒性（Inherent toxicity）が基準とされている。また、米国は化学物質アセスメント・管理計画（**ChAMP**: Chemical Assessment and Management Program）の中での HPV および年間 25,000 ポンド以上生産している中生産量化学物質（**MPV**：Moderate production volume chemical）に対する優先順位付け方法を報告した。また、REACH の添付書 IV には有害性が低いと考えられる化学物質のリストが掲載されている。

OECD 事務局は HPV 点検プログラム内での優先順位付け方法や優先順位が低いとされる物質（保留物質：set aside chemicals）をどのように評価するかなどのアウトラインを提示した。

- 保留物質を設定するための基準は、現在までのHPV点検プログラムで使用されているLPやFWを定める基準や、国や地域で使用されている基準を使用することが可能と考えられる。
- 保留物質の評価には、物理化学的性質や環境運命や経路などの情報は不要であると考えられる。
- 保留物質についてSIDS項目の情報がない場合、(Q)SARアプリケーションツールボックスがデータギャップを補完するためのツールとして有用である。
- SIDS項目ではないが、優先順位を定める基準に関係するエンドポイント（例えば生体濃縮性など）については記載が必要である。
- 文書ついては定型書式を設けず、国や地域で使用されている書式を用いる。決定された優先順位については、その設定根拠についてOECD事務局が定型句を用いて表示する。しかし、国や地域のプログラムで精査されない文書用に、基本的なガイダンスは作成され得る。
- 有害性が低いと考えられた保留物質であっても、全てのエンドポイントについて再評価する候補物質となり得ることを、優先順位設定根拠と共に記載する。また、国や地域のプロ

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グラムとHPV点検プログラムでの免責事項の基準についての相違点などを記載する必要があるかもしれない。

- HPV点検プログラム内での手順については、次の二つが考えられる。①保留物質または保留物質カテゴリーについての文書は、SIAMで審議されるが異なる議題として審議され、合意された文書はJoint Meetingで承認を得た後、背景文書と共にOECDより出版される。②保留物質または保留物質カテゴリーについての文書を精査する代わりに、国や地域の評価手法を把握し、もしその基準を満たしている場合はそのまま承認する。ただし、OECDによる優先順位決定根拠の記載が必要であり、その決定を公表することが可能である。背景文書については、OECDから公開されるか、または国や地域の公表を参照できるようにする。
- 有害性が低いとされる保留物質の文書は、OECD加盟国、会社、または会社のコンソーシアムがスポンサーとして提出することができる。保留物質の文書作成におけるスポンサーシップについてもOECDのデータベースにより公開され、プログラムへの貢献としてカウントされる。しかし、統計的にはフルの初期評価文書の提出と、保留物質文書の提出は分けて処理される。
- 保留物質について新しい情報が入手され、その有害性に懸念が感じられた場合は、フルの初期評価文書を作成するためのスポンサーが必要となる。または、選択的評価を用いるという方法も可能である。

SIAMはOECDが示したアウトラインにほぼ合意したが、次のような勧告がなされた。

- 保留物質の設定基準は厳格なものであり、その基準は限定的な数まで減らすことが可能である。
- 優先順位の決定を環境影響とヒト健康影響で分けて定められるかの可能性を調査すべきである。
- OECDの活動が国による優先順位設定を承認するだけに限定されるべきではない。

OECD事務局は選択的評価手法についての文書を修正し、回覧することになった。最終草案は2009年4月の既存化学物質タスクフォースまでに準備される。さらに、OECD事務局はカナダ、米国、ECおよび英国とともに保留物質の文書例を第28回SIAMに試験的に提出できないか検討することになった。

6）SIAM前・SIAM後のCDG上での審議について

第18回SIAMで米国・日本／ICCAが提出し合意された物質カテゴリー：Short Chain Alkyl Methacrylate（CAS：97-63-2, 97-86-9, 97-88-1, 688-84-6）については、環境影響について新しい情報が得られたので、修正SIAPがCDG上に提出され合意が得られた。第18回SIAMでは、Ethyl methacrylate （CAS: 97-63-2）、iso-Butyl methacrylate（CAS: 97-86-9）およびn-Butyl methacrylate（CAS: 97-88-1）はLP、2-Ethylhexyl methacrylate （CAS: 688-84-6）はFWと合意されたが、今回これらの化学物質の生体濃縮性の可能性が低いことが新たに分かったので、本カテゴリーの環境影響はLPと結論された。修正SIAPは第27回SIAMで合意されたSIAPと共に既存化学物質タスクフォースに提出される。

おわりに

OECDのHPV点検プログラムでは、2005年から2010年の間に1,000物質の評価を終えることを目標としており、2005年から2008年6月までには、386物質が審議された。しかし残

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りの624物質中188物質については、スポンサーが決まっておらず、プログラムの効率化が要求されている。今回のSIAMでは、未評価物質の審議を効率よく終えるために、選択的評価や優先順位設定ツールの使用などの新しい評価手法導入の可能性について審議された。また、米国のHCをHPV点検プログラムに提出するためのアウトラインが紹介され、HPV点検プログラムへの貢献が期待された。

参照資料：

1. EPA (2008) HPV Chemical Hazard Characterizations. http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report
2. OECD (2008a) Draft Summary Record for SIAM 27. EXCH_SIAM_M(2008)2_draft
3. OECD (2008b) OECD integrated HPV database. http://cs3-hq.oecd.org/scripts/hpv/
4. OECD (2008c) Screening Information Datasets (SIDS) for High Production Volume Chemicals in IUCLID format. http://www.oecd.org/document/55/0,3343,en_2649_34379_31743223_1_1_1_1,00.html
5. OECD (2008d) Series on Testing and Assessment / Adopted Guidance and Review Documents. No 88: Report of a Workshop on Integrated Approaches to Testing and Assessment (IATA) http://www.oecd.org/document/30/0,3343,fr_2649_34377_1916638_1_1_1_1,00.html
6. UNEP (2008) Chemicals Screening information dataset (SIDS) for high volume chemicals. http://www.chem.unep.ch/irptc/sids/OECDSIDS/sidspub.html
7. 江馬　眞 (2006)：OECD の高生産量化学物質安全性点検プログラムとその実施手順. 化学生物総合管理, 2-1, 83-103
8. 高橋美加, 松本真理子, 川原和三, 菅野誠一郎, 菅谷芳雄, 広瀬明彦, 鎌田栄一, 江馬　眞 (2006a)：OECD化学物質対策の動向（第8報）. 化学生物総合管理, 2-1, 147-162
9. 高橋美加, 松本真理子, 川原和三, 菅野誠一郎, 菅谷芳雄, 広瀬明彦, 鎌田栄一, 江馬　眞 (2006b)：OECD化学物質対策の動向（第9報）. 化学生物総合管理, 2-1, 163-175
10. 高橋美加, 松本真理子, 川原和三, 菅野誠一郎, 菅谷芳雄, 広瀬明彦, 鎌田栄一, 江馬　眞 (2006c)：OECD 化学物質対策の動向（第11報）. 国立医薬品食品衛生研究所報告, 124, 62-68
11. 高橋美加, 松本真理子, 川原和三, 菅野誠一郎, 菅谷芳雄, 広瀬明彦, 鎌田栄一, 江馬　眞 (2007a)：OECD化学物質対策の動向（第10報）. 化学生物総合管理, 2-2, 286-301
12. 高橋美加, 松本真理子, 川原和三, 菅野誠一郎, 菅谷芳雄, 広瀬明彦, 鎌田栄一, 江馬　眞 (2007b)：OECD化学物質対策の動向（第12報）. 化学生物総合管理, 3-1, 43-55
13. 高橋美加, 松本真理子, 川原和三, 菅野誠一郎, 菅谷芳雄, 広瀬明彦, 鎌田栄一, 江馬　眞 (2007c)：OECD化学物質対策の動向（第12報）. 国立医薬品食品衛生研究所報告,125,101-106
14. 松本真理子、高橋美加、平田睦子、広瀬明彦、鎌田栄一、長谷川隆一、江馬　眞 (2006)：OECD 高生産量化学物質点検プログラム：第18回初期評価会議までの概要. 化学生物総合管理, 2-1, 104-134
15. 松本真理子、川原和三、菅谷芳雄、江馬　眞 (2006)：OECD 高生産量化学物質点検プログラム：第21回初期評価会議概要. 化学生物総合管理, 2-1, 135-146
16. 松本真理子、山本展裕、宮地繁樹、菅谷芳雄、江馬　眞 (2007)：OECD 高生産量化学物質点検プログラム：第24回初期評価会議概要. 化学生物総合管理, 3-2, 180-189

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表1　第27回SIAMで審議された化学物質と合意結果

CAS/ 物質カテゴリー	化学物質名	スポンサー	勧告	
			ヒト健康	環境
80-07-9	Sulfone, bis(p-chlorophenyl)	SE/ICCA	FW	FW
108-46-3	1,3-Benzenediol	JP/ICCA	LP	LP
物質カテゴリー	Nickels and nickel compounds	DK:eu		FW
3333-67-3	Nickel carbonate			
7440-02-0	Nickel			
7718-54-9	Nickel chloride			
7786-81-4	Nickel sulfate			
12122-15-5	2:3 basic carbonate			
12607-70-4	1:2 basic carbonate			
13138-45-9	Nickel nitrate			
109-60-4	Propyl acetate	US/ICCA	N/A	N/A
657-84-1	Sodium p-toluenesulfonate	JP	LP	LP
1309-64-4 12412-52-1 1317-98-2	Antimony (III) oxide (Sb2O3)	SE:eu	FW	LP
10361-37-2	Barium chloride	KO	LP	LP
物質カテゴリー	Acid Chloride Category	US/ICCA	N/A	N/A
760-67-8	Hexanoyl chloride, 2-ethyl-			
764-85-2	Nonanoyl chloride			
3282-30-2	Propanoyl chloride, 2,2-dimethyl-			
40292-82-8	Neodecanoyl chloride			
物質カテゴリー	Linear alkylbenzene alkylate bottoms	US/ICCA	N/A	N/A
68515-32-2	Benzene, mono-C12-14-alkyl derivs., fractionation bottoms			
68515-34-4	Benzene, mono-C12-14-alkyl derivs., fractionation bottoms, light ends			
68855-24-3	Benzene, C14-30-alkyl derivs.			
84961-70-6	Benzene, mono-C10-13-alkyl derivs., distn. Residues			
85117-41-5	Benzene, mono-C10-14-alkyl derivs., fractionation bottoms			

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CAS/ 物質カテゴリー	化学物質名	スポンサー	勧告	
			ヒト健康	環境
94094-93-6	Benzene, mono-C10-13-alkyl derivs., fractionation bottoms, heavy ends			
129813-62-3	Benzene, mono-C10-13-alkyl derivs., fractionation bottoms, light ends			
151911-58-9	Benzene, mono-C12-13-branched alkyl derivs., fractionation bottoms			
95-33-0	N-Cyclohexyl-2-benzothiazolsulfenamide	JP+DE:eu	LP	FW
物質カテゴリー	Ethyl silicates	US/ICCA	N/A	N/A
78-10-4	Silicic acid, (H4SiO4), tetraethyl ester			
11099-06-2	Silicic acid, ethyl ester			
68412-37-3	Silicic acid (H4SiO4), tetraethyl ester, hydrolyzed			
物質カテゴリー	Hydroperoxides	US/ICCA	N/A	N/A
80-15-9	Hydroperoxide, 1-methyl-1-phenylethyl-			
3425-61-4	Hydroperoxide, 1,1-dimethylpropyl-			
物質カテゴリー	Methyl mercaptons Category	US/ICCA	N/A	N/A
74-93-1	Methanethiol			
5188-07-8	Methanethiol, sodium salt			
10039-54-0	Bis(hydroxylammonium) sulphate	DE:eu	LP	FW
25167-70-8	Pentene, 2,4,4-trimethyl-	DE:eu	LP	FW

FW = The substance is a candidate for further work.（追加の調査研究作業が必要）
LP = The substance is currently of low priority for further work.（現状では追加作業の必要なし）
N/A=Not applicable (勧告が定められなかった)
ICCA は国際化学工業協会協議会による原案提出を示す。
eu は欧州連合でのリスク評価文書を基にしたことを意味する。
略号は、DE:ドイツ、DK: デンマーク、JP：日本、KO：韓国、SE: スウェーデン、US：米国である。

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Evaluation of norovirus removal performance in a coagulation–ceramic microfiltration process by using recombinant norovirus virus-like particles

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ABSTRACT

Norovirus (NV) is a prototype strain of a group of human caliciviruses responsible for epidemic outbreaks of acute gastroenteritis worldwide. Because of the lack of a cell culture system or an animal model for this virus, studies on drinking water treatment such as separation and disinfection processes are still hampered. In the present study, we investigated NV removal performance as particles during a coagulation–ceramic microfiltration (MF) process by using recombinant NV virus-like particles (rNV-VLPs), which are morphologically and antigenically similar to native NV. We also experimentally investigated the behaviors of two widely accepted surrogates for pathogenic waterborne viruses, bacteriophages Qβ and MS2, for comparison with the behavior of rNV-VLPs. More than 4-log removal was observed for rNV-VLPs with a 1.08 mg-Al/L dose of polyaluminium chloride in the coagulation–ceramic MF process. This high removal ratio of rNV-VLPs satisfies the U.S. Environmental Protection Agency requirement of 4-log removal or inactivation. In addition, the removal ratios of Qβ and MS2 were approximately 2-log and 1-log, smaller than the ratio of rNV-VLPs. Accordingly, both bacteriophages have the potential to become appropriate surrogates for native NV in the coagulation–ceramic MF process, and, of the two, Qβ is the more conservative surrogate.

Key words | coagulation–ceramic microfiltration, ELISA, norovirus, real-time RT-PCR, virus-like particles

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INTRODUCTION

Ceramic microfiltration (MF) 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 or other inorganic membranes. These properties of ceramic membranes allow the use of strong acids and bases for chemical cleaning, application of high pressure for hydraulic backwashing, and operation at a high filtration flux. However, ceramic MF membranes alone cannot remove pathogenic waterborne viruses efficiently, because the membrane pore sizes are not small enough to remove viruses. To compensate for this disadvantage, a coagulation process has sometimes been used as a pretreatment before ceramic MF. In fact, coagulation–ceramic MF systems have already been applied to the treatment of drinking water in Japan, and our research group has showed these systems to be useful for virus removal by using the bacteriophages Qβ and MS2 as surrogates for pathogenic waterborne viruses (Matsui *et al.* 2003; Matsushita *et al.* 2005; Shirasaki *et al.* 2009*a*,*b*).

Among the pathogenic waterborne viruses, norovirus (NV; formerly known as small round structural virus and Norwalk-like virus) is one of most important human pathogens, causing epidemic and acute gastroenteritis worldwide. Although it has been 30 years since NV was first identified, studies of this virus, including experiments in the field of drinking water treatment, are still hampered by

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the lack of a cell culture system or an animal model (Zheng *et al.* 2006). Accordingly, the removal performance of NV in the drinking water treatment process has not been investigated fully. In recent years, expression of the NV genome in a baculovirus expression system has resulted in the production of recombinant NV virus-like particles (rNV-VLPs) (Jiang *et al.* 1992) that are morphologically and antigenically similar to native NV (White *et al.* 1996). In the present study, we used rNV-VLPs to investigate the removal performance of NV in a coagulation–ceramic MF process. We also experimentally investigated the behaviors of the bacteriophages Qβ and MS2 for comparison with the behavior of rNV-VLPs, and evaluated their suitability as surrogates for NV. This study represents the first attempt to apply rNV-VLPs to the evaluation of the removal performance of NV in a drinking water treatment process.

MATERIALS AND METHODS

Source water, coagulant, and MF membranes

Water was sampled from the Toyohira River (Sapporo, Japan; water quality shown in Table 1) on 12 June 2008. Two commercial aluminium coagulants, polyaluminium chloride (PACl 250A; 10.5% Al_2O_3, relative density 1.2 at 20°C; Taki Chemical Co., Ltd., Hyogo, Japan) and alum (8.1% Al_2O_3, relative density 1.3 at 20°C; Taki Chemical Co., Ltd.), were used for the coagulation process. 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.

Preparation of rNV-VLPs

Subgenomic cDNA fragments of NV (AB042808, GI/4, Chiba407/1987/JP) genome were artificially synthesized. The fragments contained the entire second and third open reading frames of the NV genome. The cDNA was subcloned into a baculovirus transfer vector, and then the transfer vector was transfected into silkworm cells. The expressed rNV-VLPs were separated from the cell lysate by centrifugation and dialysis.

Table 1 | Water quality of the Toyohira River

pH	7.2
DOC (mg/L)	0.76
OD260 (cm)	0.019
Turbidity (NTU)	0.63
Alkalinity (mg-$CaCO_3$/L)	17.2

Quantification of rNV-VLPs

rNV-VLPs were detected by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (NV-AD (II), Denka Seiken Co., Ltd., Tokyo, Japan). The assay was performed according to the manufacturer's instructions. Optical densities at 450 nm and 630 nm in a 96-well microplate were measured with a microplate reader (MTP-300, Corona Electric Co., Ltd., Ibaraki, Japan). The rNV-VLP concentration detection limit with the ELISA kit was approximately 10^8 VLPs/mL.

Preparation of bacteriophages

F-specific RNA bacteriophages Qβ (NBRC 20012) and MS2 (NBRC 102619) were obtained from the NITE Biological Research Center (NBRC, Chiba, Japan). The bacteriophages Qβ and MS2 are widely used as surrogates for pathogenic waterborne viruses because of their morphological similarities to hepatitis A viruses and polioviruses, which are important viruses to remove during drinking water treatment. The genomes of these two bacteriophages each 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. 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 (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; Amicon Ultra-15, Millipore Corp., Billerica, MA, USA) to prepare the bacteriophage stock solution.

Quantification of bacteriophages

Viral RNA of the bacteriophages was quantified by real-time RT-PCR. 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. 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 (Thermal Cycler Dice Model TP600, Takara Bio Inc., Shiga, Japan). 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 40 cycles of 95°C for 15 s and 60°C for 1 min in an Applied Biosystems 7300 Real-Time PCR 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 plaque forming unit (PFU) method (Adams 1959) and the number of cycles of PCR amplification.

Coagulation–ceramic MF process

Batch coagulation experiments were conducted with 400 mL of river water in glass beakers at 20°C. rNV-VLPs, Qβ, and MS2 were simultaneously added to the beakers at approximately 10^{11} VLPs/mL and 10^{8} PFU/mL, respectively, and mixed with an impeller stirrer. PACl or alum was injected into the water as the coagulant at a dose of 0.54 or 1.08 mg-Al/L. The pH of the water was immediately adjusted to, and maintained at, 6.8 using HCl or NaOH. The water was stirred rapidly for 2 min ($G = 200\,\mathrm{s}^{-1}$) and then slowly for 28 min ($G = 20\,\mathrm{s}^{-1}$). The water was then left at rest for 20 min to allow the generated aluminium floc particles to settle. The supernatant was fed through a ceramic MF membrane by a peristaltic pump at 83 L/(m^2·h). The raw water (C_0; before coagulant dosing) and the MF permeate (C; after 15, 30, 60, 90, and 120 min of filtration) was collected for quantification of the rNV-VLPs, Qβ, and MS2.

Electron microscopy

rNV-VLPs were observed with an electron microscope. Ten microlitres of rNV-VLP stock solution was put on a 400-mesh copper grid with a collodion membrane (Nissin EM Corp., Tokyo, Japan) and adsorbed to the grid for 1 min. Excess solution on the grid was drained from the side of the grid with filter paper, and rNV-VLPs were negatively stained with 10 μL of 2% phosphotungstic acid (pH 5.5) for 45 s. After the excess stain was drained off, the grid was examined with a transmission electron microscope (TEM, H-7650, Hitachi High-Technologies Corp., Tokyo, Japan). Particle diameter of rNV-VLPs was expressed as the mean and standard deviation of any 10 particles on the electron micrograph.

RESULTS AND DISCUSSION

Characteristics of the rNV-VLPs produced

Figure 1 shows an electron micrograph of rNV-VLPs. The presence of particles was confirmed on the electron

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	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	

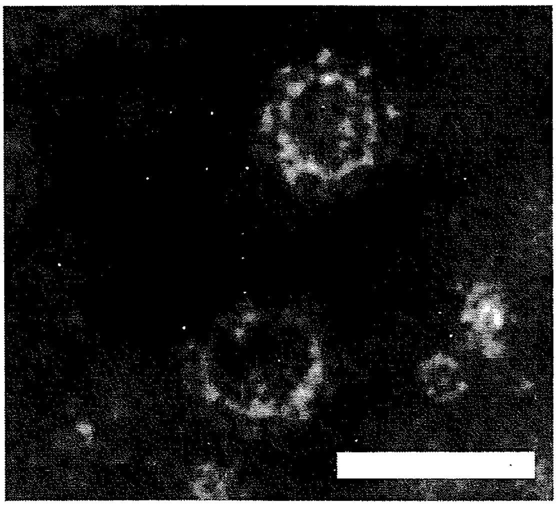

Figure 1 | Negatively stained electron micrograph of rNV-VLPs. Scale bar, 50 nm.

micrograph, and rNV capsid proteins spontaneously self-assembled into VLPs during expression. The rNV-VLP particle diameter was 35.7 ± 3.2 nm, measured by randomly choosing 10 particles on the electron micrograph. This value roughly corresponds to the particle diameter (approximately 38 nm) previously reported for native NV (Someya *et al.* 2000). This result suggests that rNV-VLPs were successfully produced by the baculovirus-silkworm expression system, and were morphologically similar to native NV.

Removal performance in the coagulation–ceramic MF process with PACl

Figure 2 shows the change in the removal ratio ($\log[C_0/C]$) of rNV-VLPs, Qβ, and MS2 with filtration time in the coagulation–ceramic MF process with PACl. Because the diameters of rNV-VLPs (35.7 ± 3.2 nm), Qβ, and MS2 (24–26 nm) are smaller than the nominal pore size of the ceramic MF membrane (0.1 μm), no removal (<0.2-log) of rNV-VLPs or either bacteriophage was observed without coagulation pretreatment (data not shown). In contrast, the coagulation–ceramic MF process effectively removed rNV-VLPs, Qβ, and MS2 at PACl doses of 0.54 (Figure 2a) and 1.08 mg-Al/L (Figure 2b). In addition, an effect of the coagulant dose (0.54 mg-Al/L vs. 1.08 mg-Al/L) on the removal of rNV-VLPs and both bacteriophages was observed: the filtration time-averaged removals of rNV-VLPs, Qβ, and MS2 were only 1.6-log, 0.5-log, and 0.9-log, respectively, at the coagulant dose of 0.54 mg-Al/L, whereas with the dose of 1.08 mg-Al/L, the removals were > 2.9-log, 2.4-log, and 3.3-log for rNV-VLPs, Qβ, and MS2, respectively. This dose effect was most likely due to an increase in floc size with the increased coagulant dose. Increasing the coagulant dose from 0.54 to 1.08 mg-Al/L increased the size of the aluminium floc particles with adsorbed/entrapped rNV-VLPs or bacteriophages, and subsequently increased the amount of aluminium floc particles that settled out from the suspension during the

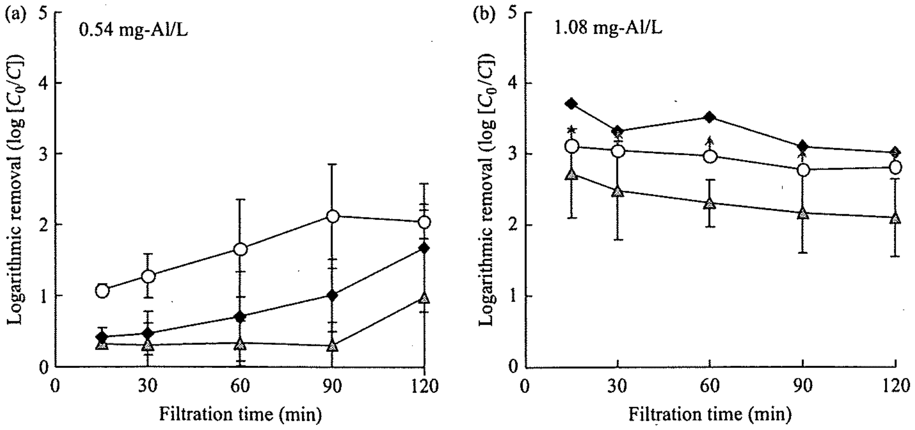

Figure 2 | rNV-VLPs, Qβ and MS2 removal by a coagulation–ceramic MF process with PACl coagulant at doses of (a) 0.54 mg-Al/L and (b) 1.08 mg-Al/L. Circles, triangles, and diamonds represent rNV-VLPs, Qβ and MS2, respectively. The arrows indicate values greater than those that could be estimated accurately by ELISA.

settling process. Consequently, the amount of aluminium floc particles that passed through a ceramic MF membrane was decreased, leading to a difference in the removal ratios of the rNV-VLPs and the two bacteriophages between PACl doses of 0.54 and 1.08 mg-Al/L.

The removal ratios of rNV-VLPs, Qβ, and MS2 gradually increased with filtration time at the PACl dose of 0.54 mg-Al/L. Ultimately, 2.0-log, 1.0-log, and 1.7-log removals were obtained for rNV-VLPs, Qβ, and MS2, respectively, at the end of the 2-h filtration. As described above, because the size and amount of aluminium floc particles that settled out from suspension were probably smaller with a PACl dose of 0.54 mg-Al/L than with one of 1.08 mg-Al/L, many of the aluminium floc particles were fed into the ceramic MF membrane and accumulated on the membrane surface as a cake layer during the MF process. This cake layer would act as a barrier to rNV-VLPs and both bacteriophages, causing the removal ratios of rNV-VLPs, Qβ, and MS2 to gradually increase with the growth of the cake layer at the PACl dose of 0.54 mg-Al/L.

In contrast, the removal ratios of rNV-VLPs, Qβ, and MS2 gradually decreased with filtration time at the PACl dose of 1.08 mg-Al/L. Our research group has demonstrated that the number of virus particles in the MF compartment increases with filtration time in the coagulation–ceramic MF system (Shirasaki *et al.* 2007). Although the experimental procedures of the present study and our previous study (Shirasaki *et al.* 2007) differed in terms of the coagulation process (batch coagulation vs. in-line coagulation), rNV-VLPs and both bacteriophages also probably accumulated in the MF compartment with filtration time in the present study. Accordingly, the concentrations of rNV-VLPs, Qβ, and MS2 in the MF permeate increased with the accumulation of rNV-VLPs and both bacteriophages in the MF compartment. In addition, because many of the aluminium floc particles had settled out from suspension during the settling process prior to the MF process with the 1.08 mg-Al/L PACl dose, the effect of the cake layer was probably smaller than with the 0.54 mg-Al/L PACl dose. The combination of these two phenomena caused the leakage of the rNV-VLPs and the two bacteriophages into the MF permeate, decreasing their removal ratios with filtration time.

The removal performance for rNV-VLPs, Qβ, and MS2 with the 1.08 mg-Al/L PACl dose at the end of the filtration (2 h) was still higher than that with the 0.54 mg-Al/L PACl dose, although the removal ratios decreased with filtration time as described above. Therefore, a PACl dose of 1.08 mg-Al/L was more effective for the removal of rNV-VLPs and both bacteriophages than the dose of 0.54 mg-Al/L in the coagulation–ceramic MF process.

Removal performance in the coagulation–ceramic MF process with alum

Figure 3 shows the change in the removal ratio of rNV-VLPs, Qβ, and MS2 with filtration time in the coagulation–ceramic MF process with alum. The coagulation–ceramic MF process effectively removed rNV-VLPs, Qβ, and MS2 at alum doses of 0.54 (Figure 3a) and 1.08 mg-Al/L (Figure 3b). In addition, an effect of the coagulant dose (0.54 mg-Al/L vs. 1.08 mg-Al/L) on the removal of rNV-VLPs and of both bacteriophages was also observed with alum: the filtration time-averaged removals of rNV-VLPs, Qβ, and MS2 were only 1.9-log, 0.9-log, and 2.3-log, respectively, at the 0.54 mg-Al/L coagulant dose, whereas the 1.08 mg-Al/L dose achieved time-averaged removals of > 3.1-log, 1.3-log, and 3.1-log for rNV-VLPs, Qβ, and MS2, respectively.

The removal ratios of rNV-VLPs, Qβ, and MS2 gradually increased with filtration time at the 0.54 mg-Al/L alum dose, probably owing to the aluminium floc particles accumulating on the membrane surface as a cake layer during the MF process. Ultimately, removals of 2.3-log, 1.0-log, and 2.7-log were obtained for rNV-VLPs, Qβ, and MS2, respectively, at the end of the 2-h filtration. In contrast, the removal ratios of rNV-VLPs, Qβ, and MS2 gradually decreased with filtration time at the 1.08 mg-Al/L alum dose, for the same reason as in the PACl experiment, described above.

The removal performance for rNV-VLPs, Qβ, and MS2 with the 1.08 mg-Al/L alum dose at the end of the filtration (2 h) was also higher than that with the 0.54 mg-Al/L alum dose, as in the PACl experiments. Accordingly, the alum dose of 1.08 mg-Al/L was more effective for removal of rNV-VLPs and both bacteriophages than the 0.54 mg-Al/L alum dose in the coagulation–ceramic MF process.

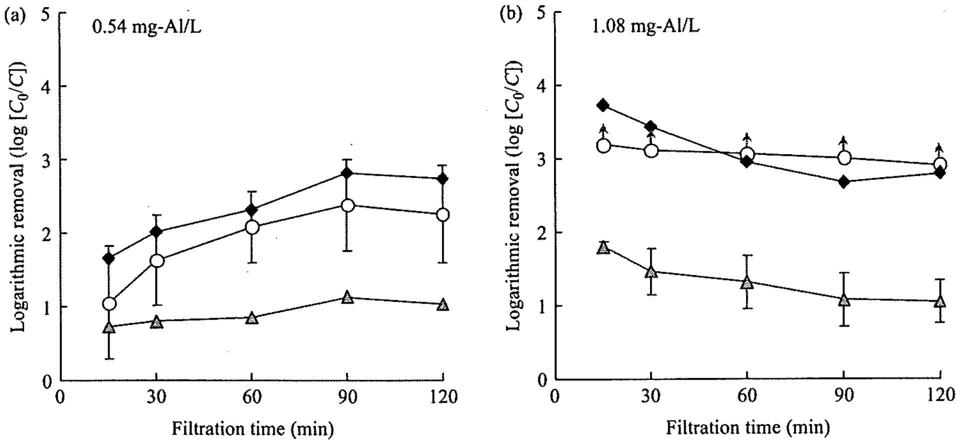

Figure 3 | rNV-VLPs, Qβ and MS2 removal by a coagulation–ceramic MF process with alum coagulant at doses of (a) 0.54 mg-Al/L and (b) 1.08 mg-Al/L. Circles, triangles, and diamonds represent rNV-VLPs, Qβ and MS2, respectively. The arrows indicate values greater than those that could be estimated accurately by ELISA.

Comparison of removal performances between PACl and alum

Because the concentration of rNV-VLPs in the MF permeate with the 1.08 mg-Al/L coagulant dose (Figures 2b and 3b) was less than the detection limit of the ELISA kit (approximately 10^8 VLPs/mL), further concentration using a centrifugal filter device (molecular weight cutoff 30,000, regenerated cellulose; Amicon Ultra-15, Millipore Corp.) was performed in the present study to evaluate 4-log removal, as regulated by the U.S. Environmental Protection Agency (USEPA) National Primary Drinking Water Standards (U.S. Environmental Protection Agency 2001). An approximately 10-fold concentration was obtained by the concentration method for rNV-VLPs in the MF permeate.

Figure 4 shows the effect of coagulant type (PACl vs. alum) on the removals of rNV-VLPs, Qβ, and MS2 in the coagulation–ceramic MF process with a 1.08 mg-Al/L coagulant dose. The experiment with PACl achieved >4-log removal of rNV-VLPs regardless of the filtration time, whereas with alum 3.3-log to >3.9-log removals were observed. This means that the experiment with PACl more effectively removed rNV-VLPs than that with alum, and that it satisfied the USEPA requirement of 4-log removal/inactivation. The difference in removal performance between PACl and alum was possibly due to differences in the characteristics of the aluminium floc particles generated during the coagulation process. Gregory & Dupont (2001) reported that aluminium floc particles formed with PACl are larger, stronger, and more readily separated by the settling process than those formed with alum. In the present study, because the rNV-VLPs were adsorbed to or entrapped in the aluminium floc particles, and settled out from suspension during the settling process or were separated during the MF process, the difference in the characteristics of the aluminium floc particles between PACl and alum may account for the difference in the removal performance for rNV-VLPs of the coagulation–ceramic MF process. Further investigation is needed.

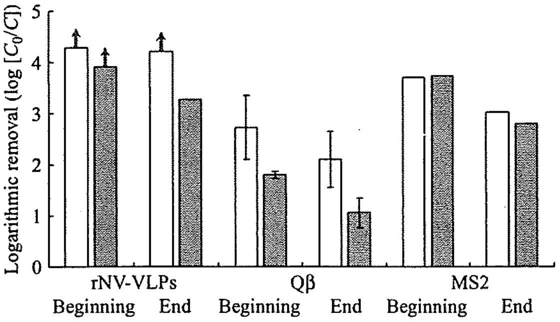

Figure 4 | Effect of coagulant type on the removal of rNV-VLPs Qβ and MS2 at the beginning and end of filtration in a coagulation–ceramic MF process. White and gray columns represent PACl and alum, respectively. The coagulant dose was 1.08 mg-Al/L. The arrows indicate values greater than those that could be estimated accurately by ELISA.

The removal ratios of Qβ and MS2 were smaller than those of rNV-VLPs in the coagulation–ceramic MF process with both aluminium coagulants: the ratio of Qβ was approximately 2-log smaller than that of rNV-VLPs, and the MS2 ratio was approximately 1-log smaller. Accordingly, both bacteriophages have the potential to become appropriate surrogates for native NV in the coagulation–ceramic MF process, with Qβ being the more conservative surrogate of the two. We imagine that the characteristics of rNV-VLPs, Qβ, and MS2 such as surface charge and hydrophobicity affect the removal performance in the coagulation–MF process. Further investigation of differences in surface properties between rNV-VLPs and the two bacteriophages is needed.

Our research group has shown that PACl exhibits virucidal activity during the coagulation process (Shirasaki *et al.* 2009*a*,*b*): an approximately 4-log difference between the total (infectious + inactivated) concentration measured by real-time RT-PCR and the infectious concentration measured by the PFU method was observed during the coagulation process, indicating that some of the bacteriophages were probably inactivated by PACl. Because rNV-VLPs lack RNA, which is necessary for infection and replication in host cells, we cannot discuss the fate of infectivity of NV in the treatment process. If PACl exhibits virucidal activity for native NV as well as for bacteriophages, >4-log removal (including inactivation) of native NV might be easily achieved during the coagulation–ceramic MF process.

CONCLUSIONS

- The removal performance of NV as particles was evaluated by using rNV-VLPs in a coagulation–ceramic MF process.
- Both coagulation type and coagulant dose affected the removal performance of rNV-VLPs: the experiment with a 1.08 mg-Al/L PACl dose achieved high ratios of rNV-VLP removal, >4-log, which satisfies the USEPA requirement of 4-log removal/inactivation.
- The removal ratios of rNV-VLPs were higher than those of Qβ and MS2 in the experiments with the 1.08 mg-Al/L coagulant dose: the Qβ ratio was approximately 2-log smaller than that of rNV-VLPs, and the MS2 ratio was approximately 1-log smaller. This means that both bacteriophages have the potential to become appropriate surrogates for native NV in the coagulation–ceramic MF process, with Qβ being the more conservative of the two.

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REFERENCES

Adams, M. H. 1959 *Bacteriophages*. Interscience Publishers, New York, NY, USA, pp. 450–454.

Gregory, J. & Dupont, V. 2001 Properties of flocs produced by water treatment coagulants. *Water Sci. Technol.* **44**(10), 231–236.

Jiang, X., Wang, M., Graham, D. Y. & Estes, M. K. 1992 Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. *J. Virol.* **66**(11), 6527–6532.

Katayama, H., Shimazaki, A. & Ohgaki, S. 2002 Development of virus concentration method using negatively charged membrane by alkaline elution after acid rinse. *J. Jpn Soc. Water Environ.* **25**(8), 469–475 (in Japanese).

Matsui, Y., Matsushita, T., Inoue, T., Yamamoto, M., Hayashi, Y., Yonekawa, H. & Tsutsumi, Y. 2003 Virus removal by ceramic membrane microfiltration with coagulation pretreatment. *Water Sci. Technol. Water Supply* 3(5), 93–99.

Matsushita, T., Matsui, Y., Shirasaki, N. & Kato, Y. 2005 Effect of membrane pore size, coagulation time, and coagulant dose on virus removal by a coagulation–ceramic microfiltration hybrid system. *Desalination* **178**(1–3), 21–26.

O'Connell, K. P., Bucher, J. R., Anderson, P. E., Cao, C. J., Khan, A. S., Gostomski, M. V. & Valdes, J. J. 2006 Real time fluorogenic reverse transcription-PCR assays for detection of bacteriophage MS2. *Appl. Environ. Microbiol.* **72**(1), 478–483.

Shirasaki, N., Matsushita, T., Matsui, Y., Ohno, K. & Kobuke, M. 2007 Virus removal in a hybrid coagulation-microfiltration system-Investigating mechanisms of virus removal by a combination of PCR and PFU methods. *Water Sci. Technol. Water Supply* 7(5–6), 1–8.

Shirasaki, N., Matsushita, T., Matsui, Y., Kobuke, M. & Ohno, K. 2009*a* Comparison of removal performance of two surrogates for pathogenic waterborne viruses, bacteriophages Qβ and MS2, in a coagulation–ceramic microfiltration system. *J. Membr. Sci.* **326**(2), 564–571.

Shirasaki, N., Matsushita, T., Matsui, Y., Urasaki, T. & Ohno, K. 2009*b* Comparison of behaviors of two surrogates for pathogenic waterborne viruses, bacteriophages Qβ and MS2, during the aluminum coagulation process. *Water Res.* **43**(3), 605–612.

Someya, Y., Takeda, N. & Miyamura, T. 2000 Complete nucleotide sequence of the chiba virus genome and functional expression of the 3C-like protease in *Escherichia coli*. *Virology* **278**(2), 490–500.

U. S. Environmental Protection Agency 2001 *National Primary Drinking Water Standards*, Office of Water, U.S. Environmental Protection Agency, Washington, DC, EPA816-F-01-007.

White, L. J., Ball, J. M., Hardy, M. E., Tanaka, T. N., Kitamoto, N. & Estes, M. K. 1996 Attachment and entry of recombinant Norwalk virus capsids to cultured human and animal cell lines. *J. Virol.* **70**(10), 6589–6597.

Zheng, D. P., Ando, T., Fankhauser, R. L., Beard, R. S., Glass, R. I. & Monroe, S. S. 2006 Norovirus classification and proposed strain nomenclature. *Virology* **346**(2), 312–323.

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NF membrane fouling by aluminum and iron coagulant residuals after coagulation–MF pretreatment

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ABSTRACT

The effects of coagulant residuals on fouling of a nanofiltration (NF) membrane were investigated. Experiments were carried out with a laboratory-scale microfiltration (MF)–NF setup and a pilot MF–NF plant. In the laboratory-scale experiments, NF feed water was pretreated with poly-aluminum chloride (PACl) or alum followed by MF. NF membrane permeability declined when the feed water contained residual aluminum at 18 μg/L or more, but not when it was lower than 9 μg/L. When pretreated with ferric chloride, no substantial decline of NF membrane permeability was observed; residual iron did not affect the permeability. When SiO_2 was added to the water before the pretreatment with PACl, the NF membrane permeability declined at about double the speed. Thermodynamic calculations and elemental analysis of foulants recovered from the membranes indicated that the majority of inorganic foulants were compounds composed of aluminum, silicate, and possibly potassium. In the pilot plant, NF feed was pretreated by PACl. Transmembrane pressure for NF doubled over 4.5 months of operation. Although the aluminum concentration in the NF feed was not high (30 μg/L), analysis of membrane foulants revealed excessive accumulation of aluminum and silicate, also suggesting that aluminum residuals caused the membrane fouling by alumino-silicates or aluminum hydroxide.

1. Introduction

Nanofiltration (NF) is a promising advanced drinking water treatment process that offers an efficient alternative to conventional advanced treatment (ozone-activated carbon) and has the potential to produce potable water of better quality. NF is expected to perform better than conventional advanced treatment in removing natural organic matter, precursors of disinfection by-products [1,2] and trace hazardous chemicals such as pesticides [3] from water; however, NF is still more expensive, and its cost needs to be reduced if it is to be widely accepted.

Membrane fouling leads to a continuous decline in membrane permeability, and fouling mitigation considerably reduces the cost of designing and operating membrane filtration systems. In the case of NF of surface waters, the accumulation of particulate matter severely decreases the permeability of the NF membranes; such particulates must be removed by pretreatment processes such as coagulation, followed by clarification and then multi-media filtration or microfiltration (MF).

Although these pretreatments can alleviate the effect of organic foulants as well as that of particulate, the pretreatment increases coagulant residuals to NF feed and they may precipitate on the membrane surface and reduce membrane permeability. Kim et al. [4] used three types of NF feed: untreated raw water (RAW water), pretreated by in-line coagulation followed by MF (MF water) and pretreated by coagulation, sedimentation and sand filtration (CS water). They found that the order of the ratio of inorganic foulants to the total amounts of foulants was CS water>MF water>RAW water. Gabelich et al. tested reverse osmosis (RO) membrane using feed pretreated with conventional or direct filtration treatment plants. They used either alum or ferric chloride, and also used cationic polymer and chloramines for pretreatment. The tests using alum with RO elements revealed rapid deterioration in specific flux, on the other hand, the specific flux using ferric chloride did not decrease over time [5]. They also suggested that three types of aluminum-based foulants: aluminum silicates, aluminum hydroxides, and aluminum phosphates [6]. Accordingly, both pretreatment methods and types of coagulants may play a crucial role in the control of NF/RO fouling. Application of conventional coagulation, clarification and multi-media filtration can

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take advantage of preexisting facilities; however, the processes offset the benefit of small area required for NF membrane process. Pre-coagulated MF would be more advantageous process for pretreatment for NF.

Most of the surface water treatment plants in Japan that have coagulation process currently use aluminum coagulants [7]. During our pilot plant experiment [8], which is also discussed in this study, we found that residual aluminum coagulants in the NF feed might cause the membrane fouling. Therefore, the objective of this study was to investigate the effects of coagulant residuals on NF membrane fouling when NF is applied as an advanced water treatment process of surface or ground water. In laboratory-scale experiments, we used two types of aluminum coagulant: poly-aluminum chloride (PACl) and alum and pretreated by in-line coagulation and ceramic MF. As an alternative coagulant, iron coagulant (ferric chloride) was also used. To clarify the effect of residual coagulants, groundwater that contained low organic matter was used as raw experimental water. To elucidate the effects of SiO_2 on the NF membrane fouling, we added SiO_2 to the raw water in some of the experiments. The results of the pilot scale experiment, in which PACl was used as a coagulant agent, were also presented and discussed.

2. Experimental

2.1. Laboratory-scale experiment

In the laboratory-scale experiment, Hokkaido University groundwater was used as raw experimental water. The average quality of this water was: DOC 0.5 mg/L, EC 450 µS/cm, pH 7.2, Na 29 mg/L, K 9.6 mg/L, Ca 50 mg/L, Mg 15 mg/L, Cl 25 mg/L, SO_4 40 mg/L. Types of coagulants used were PACl (10% Al_2O_3, basicity 52%, Japanese Industrial Standard (JIS) grade), alum (Aluminum Sulfate 14–18 Water, reagent grade, Wako Pure Chemicals, Osaka, Japan) or ferric chloride (Iron (III) Chloride Hexahydrate, reagent grade, Wake Pure Chemicals). The raw water was firstly treated with activated carbon cartridge filter to quench residual chlorine and then pretreated by in-line coagulation followed by MF. The MF filtrate (pH 7.0–7.1) was then pumped at a rate of 1.5 L/h to a flat sheet membrane test cell (filtration area 60 cm^2, C10-T, Nitto Denko Matex Corp., Tokyo, Japan; Fig. 1) that housed the NF membrane (UTC-60, nominal NaCl rejection 55%, Toray Industries, Inc.). NF was performed at a filtration flux of 2.5 cm/h and a water recovery rate of 10%. The system flow is shown in Fig. 2. We conducted nine experimental runs and their conditions are summarized in Table 1. In all experimental runs, the coagulant dose was set at 0.04 mM (1.1 mg-Al/L, 2.2 mg-Fe/L). MF was performed with a laboratory-use ceramic membrane (nominal pore size 0.1 µm, membrane area 0.4 m^2, NGK Insulators, Nagoya, Japan) at very low filtration flux (0.83 cm/h), without periodic hydraulic backwashing; the MF membrane was replaced with a chemically cleaned membrane when the inlet pressure reached 0.05 MPa. After the NF experiments, spent NF membranes were cleaned with 2% citric acid, and the aluminum and iron concentrations in the citric acid drain were analyzed.

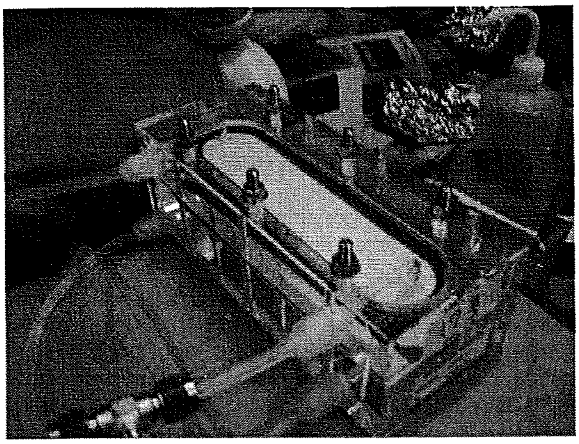

Fig. 1. Flat sheet membrane test cell.

After five experimental runs with the laboratory-scale experimental setup, an automatic hydraulic backwash system was introduced to the MF step; the MF was performed at a normal filtration flux (6.25 cm/h), and the MF membrane was hydraulically cleaned by backwash at a pressure of 500 kPa for 10 s every 2 h [9]. MF permeate (pH 6.8–7.0) was introduced to the NF membrane test cell at a filtration flux of 2.08 cm/h, which is slightly lower than the fluxes used for the previous experiments. Runs 6–9 were conducted with this system. Run 6 used PACl as a coagulant, and Run 7 used ferric chloride.

In the next two experimental runs (Runs 8 and 9), SiO_2 was added to the chlorine-quenched groundwater before coagulation with PACl and MF pretreatment. By comparing these results with the results of Run 6, in which the mean silicate concentration was 35 mg-SiO_2/L, we investigated the effect of silicate on the NF membrane fouling. Diluted sodium silicate was added so that the silicate concentration in the NF feed would be approximately 80 mg-SiO_2/L. Sodium silicate is a basic reagent, and thus the pH was adjusted to approximately 7 with hydrochloric acid. After Runs 6, 8, and 9, the spent NF membranes were cleaned sequentially with HCl, NaOH, and citric acid; and then the aluminum, silicate, calcium, and potassium concentrations in the cleaning water were analyzed.

2.2. Pilot MF–NF plant experiment

The pilot plant received water at a rate of 24 m^3/h from the outlet of a sedimentation basin of the Ishikawa Water Treatment Plant, Okinawa, Japan, after PACl (basicity 50%, JIS grade) coagulation. In the pilot plant, MF (polyvinylidene fluoride membrane, nominal pore size 0.1 µm; Toray Industries, Inc., Tokyo, Japan) filtrates (pH 6.5–7.3) were transferred to the NF modules (nominal NaCl rejection 55%; polyamide SU-610, Toray Industries, Inc.), which were operated at constant flux (2.5 cm/h) and water recovery rate (95%) by adjustment of the feed pressure. Average quality of MF filtered water was: TOC 0.9 mg/L, EC 185 µS/cm, Na 19 mg/L, Ca 11 mg/L, Cl 27 mg/L, SO_4 14 mg/L, and residual Al was 0.03 mg/L. The 15 NF modules were placed in a multistage array (8, 4, 2, and 1 modules in series), and the water recovery rate of each element was about 13%. Details of the process configuration and operation are given elsewhere [8]. After 4.5 months of system operation, foulants on the NF membrane surface were collected by gentle scraping of the membrane deposits. The foulants were then dried, weighed, combusted for 30 min in a muffle furnace at 550 °C, and then weighed again to obtain the mass of fixed solid. The recovered foulant was analyzed for Al, Ca, Fe, S, and Si.

2.3. Analytical methods

Aluminum and iron concentrations were measured by an inductively coupled plasma-mass spectrometer (ICP-MS; HP-4500; Agilent Technologies, Inc., Palo Alto, CA, USA). Ion concentrations (Na^+, K^+, Mg^+, Ca^+, Cl^-, NO_3^-, SO_4^{2-}) were measured by ion chromatograph (DX-120, Nippon Dionex K.K., Osaka, Japan). SiO_2 was measure by molybdenum yellow colorimetric method. TOC and DOC were measured by TOC-5000A (Shimadzu Corporation, Kyoto, Japan) or Sievers 900 Laboratory TOC analyzer (GE Analytical Instruments, Boulder, CO, USA).

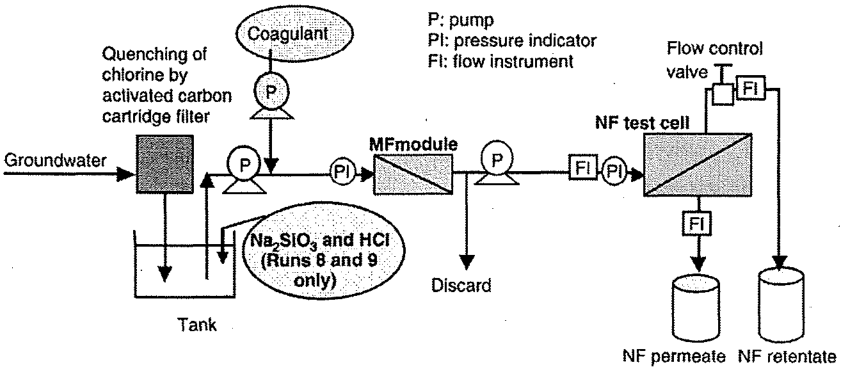

Fig. 2. Experimental setup for laboratory-scale MF–NF experiments.

3. Results and discussion

3.1. Laboratory-scale experiment pretreated with aluminum coagulants (Runs 1–3)

Changes in NF membrane permeability over time in the laboratory-scale experiment with different aluminum coagulants (i.e., PACl and alum) are compared in Fig. 3. Because variation in the initial filtration flux of the pieces of NF membrane sheet used in the NF cross-flow cell was small, nanofilter permeability was evaluated in terms of the dimensionless standardized filtration flux, which is the standardized flux at 1 MPa and 25 °C divided by the standardized flux for pure water, as described by the following equations [10]:

$$J = \frac{Q}{A} \cdot \frac{\alpha}{\Delta P} \tag{1}$$

where J is standardized filtration flux [m/(h MPa)], Q is filtration rate (m^3/h), A is membrane surface area (m^2), α is a temperature compensation factor to 25 °C, and ΔP is transmembrane pressure (TMP) (MPa); and

$$J^* = \frac{J}{J_W} \tag{2}$$

where J^* is dimensionless standardized flux, and J_W is standardized filtration flux for pure water [m/(h MPa)].

After 25 days of operation of the laboratory-scale experiment with PACl coagulant (Run 1) in which MF permeate (NF feed) had a mean aluminum concentration of 20 μg/L, the filtration flux decreased by about 15% (Fig. 3). In an experiment with alum coagulant (Run 2) in which NF feed contained aluminum at a mean concentration of 18 μg/L, the filtration flux again decreased by about 15%. Lower mean aluminum concentration (8.7 μg/L) in NF feed was observed in another experiment with PACl coagulant (Run 3); we did not change any specific condition of coagulation and MF, and we could not elucidate the reason why we could achieve this lower residual aluminum concentration. In this Run 3, the NF membrane permeability did not change substantially. The percentage rejection of aluminum by NF was more than 85%; most of the aluminum remaining after MF obviously could not permeate the NF membrane and thus had the potential to be deposited on the NF membrane, reducing membrane permeability. However, the aluminum concentrations in the NF retentates were only slightly higher than the concentrations in the corresponding NF feed; more than 98% of the aluminum fed to the NF was discharged with the NF retentate. Therefore, the high rejection ratio of aluminum did not produce a retentate that was highly concentrated in comparison with the feed and did not necessarily result in a high deposition rate on the NF membrane.

Table 1
Laboratory-scale experimental conditions and mean concentrations of residual coagulant in NF feed water.

Run	Type of coagulant	Automatic backwash system in MF and filtration flux (cm/h)	SiO$_2$ was added to be ~80 mg/L	Mean concentration of residual coagulant in NF feed water	NF filtration flux (cm/h)
1	PACl	No (0.83)		20 μg-Al/L	2.5
2	Alum	No		18 μg-Al/L	2.5
3	PACl	No		8.7 μg-Al/L	2.5
4	FeCl$_3$	No		10 μg-Fe/L	2.5
5	FeCl$_3$	No		18 μg-Fe/L	2.5
6	PACl	Yes (6.25)		40 μg-Al/L	2.08
7	FeCl$_3$	Yes		8.6 μg-Fe/L	2.08
8	PACl	Yes	Yes	103 μg-Al/L	2.08
9	PACl	Yes	Yes	85 μg-Al/L	2.08

3.2. Laboratory-scale experiment with iron coagulant pretreatment (Runs 4 and 5)

Unlike Runs 1 and 2, Runs 4 and 5 did not show a large change in nanofilter permeability (Fig. 4). Mean iron concentrations in the NF feed after the ferric chloride coagulation and MF pretreatments in Runs 4 and 5 were 10 and 18 μg/L, respectively, and the aluminum concentration in the NF feeds was less than 2 μg/L. The percentage rejection of iron by NF was 60–90%, which was not as high as that of aluminum. These results suggest that the concentration of residual aluminum after coagulation influenced NF fouling more strongly than did the residual iron concentration.

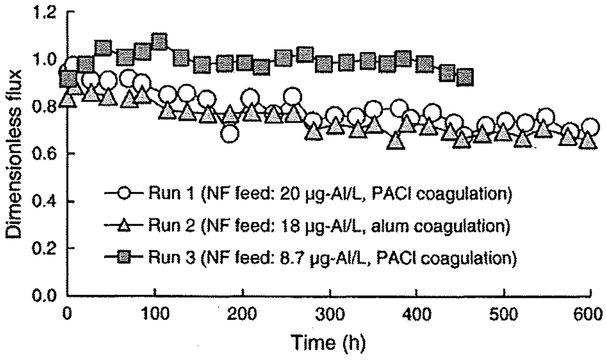

Fig. 3. Time dependence of dimensionless standardized flux (standardized flux/standardized flux for pure water) for NF after coagulation and MF in the laboratory-scale experiment (Runs 1–3).

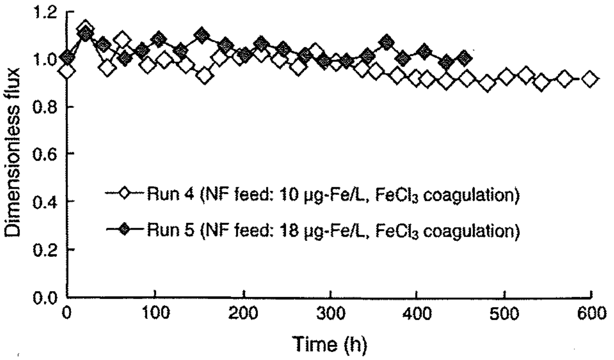

Fig. 4. Time dependence of dimensionless standardized flux (standardized flux/standardized flux for pure water) for NF after coagulation and MF in the laboratory-scale experiment (Runs 4 and 5).

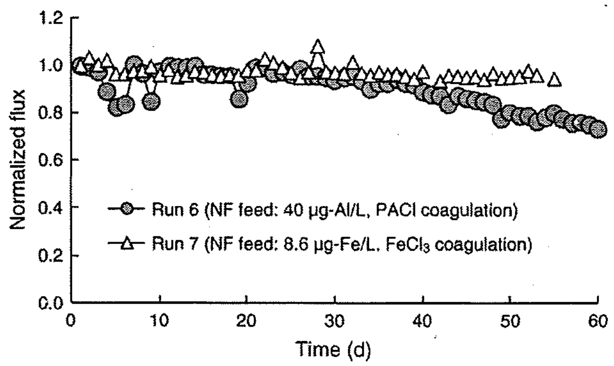

Fig. 5. Time dependence of normalized flux (standardized flux/first day standardized flux) for NF after coagulation and MF in the laboratory-scale experiment (Runs 6 and 7).

Table 2 summarizes the masses of aluminum and iron eluted from the spent NF membranes by citric acid. The membranes used in Runs 1 and 2, which showed larger permeability declines, contained more aluminum (3.6 and 4.0 mg/m^2-membrane surface) than the others. The amount of iron eluted from the spent membranes was similar to the amount of aluminum (Table 2), but unlike the mass of aluminum, the mass of iron was not obviously correlated with membrane permeability decline (Figs. 3 and 4).

3.3. Laboratory-scale experiment with backwashing in the MF step

3.3.1. Comparison of aluminum and iron coagulation (Runs 6 and 7)

An automatic backwash was introduced to the MF step after Run 5, and this alteration permitted MF at a normal filtration flux. Under this altered condition, experimental runs with PACl coagulant (Run 6) and ferric chloride (Run 7) were performed. Nanofilter permeability declined during Run 6 (Fig. 5). In this run, the NF feed contained residual aluminum of 40 µg/L in average, and the membrane permeability declined by 25% after 60 days (1440 h) of operation. The permeability declined at lower rates in this run than in Runs 1 and 2; this result was probably due to the fact that the filtration flux (2.08 cm/h) was lower than in Runs 1 and 2 (2.5 cm/h). In the experiment with ferric chloride (Run 7), no substantial decline in NF membrane permeability was observed; this result was the same as those for Runs 4 and 5.

3.3.2. Effect of SiO_2 addition (Runs 6, 8 and 9)

When SiO_2 was added to the water before the pretreatment by coagulation with PACl and MF (Runs 8 and 9), the NF membrane permeability declined at about double the speed observed for Run 6 (Fig. 6). The pH in Run 8 was not strictly controlled (the pH of the NF feed water fluctuated between 6.6 and 7.5). Aluminum is more soluble at alkaline pH than at neutral pH [11], and therefore the mean aluminum concentration in the NF feed was as high as 103 µg/L. In Run 9, the pH was controlled more strictly (6.7–7.0 over the course of the run); nevertheless, the mean aluminum concentration was also rather high (85 µg/L) in this run. This higher residual aluminum may have been due to the effect of excess silicate. Lartiges et al. [12] reported that flocculation of colloidal silica with polymerized aluminum begins with the formation of negatively charged aluminosilicate sites. Duan and Gregory [13,14] investigated the interaction of aluminum coagulants with silica and found that a small amount of dissolved silica can improve coagulation by affecting the charge-neutralizing behavior of hydrolyzed aluminum species, but silica coagulation is inhibited as the amount of silica is increased, as a result of the increasing negative charge of the particles.

Table 2
NF membrane foulants in the laboratory-scale MF–NF experiment in Runs 1–5.

	Coagulant	Foulant	
		Al (mg/m^2)	Fe (mg/m^2)
Run 1	PACl	3.6	2.5
Run 2	Alum	4.0	1.7
Run 3	PACl	1.8	2.1
Run 4	$FeCl_3$	0.2	3.3
Run 5	$FeCl_3$	1.2	1.8

To explore further the effect of a large amount of silicate on the faster decline of NF membrane permeability, we cleaned the spent membrane sequentially with HCl, NaOH, and citric acid after the experimental runs. Results after chemical cleaning with HCl are shown in Table 3; aluminum and silicate were recovered in molar ratios of 1:0.49 (Run 6), 1:1.52 (Run 8), and 1:1.78 (Run 9). These results suggest that aluminum and silicate were major inorganic foulants of the nanofilter.

We calculated the aluminum solubility diagram for the NF feed solution used in Runs 6, 8, and 9 (Fig. 7) with Geochemist's Workbench (ver. 6, RockWare, Inc., Golden, CO, USA). The major difference in the feed water quality of these runs was silicate concentration. The aluminum concentration in the NF feed water exceeded the upper solubility limit in the case of gibbsite ($Al(OH)_3$), pyrophyllite ($AlSi_2O_5OH$), and kaolinite ($Al_2Si_2O_5(OH)_4$), that is, an aluminum compound and two compounds containing both aluminum and silicate. Furthermore, changing the silicate concentration from 40 to 80 mg-SiO_2/L led to a large decrease in the solubility of pyrophyllite and kaolinite (from dotted line to solid line), although the solubility of gibbsite did not change. This result implies that more aluminum silicate compounds may have deposited on the NF membrane as the amount of silicate in the feed water increased. Therefore,

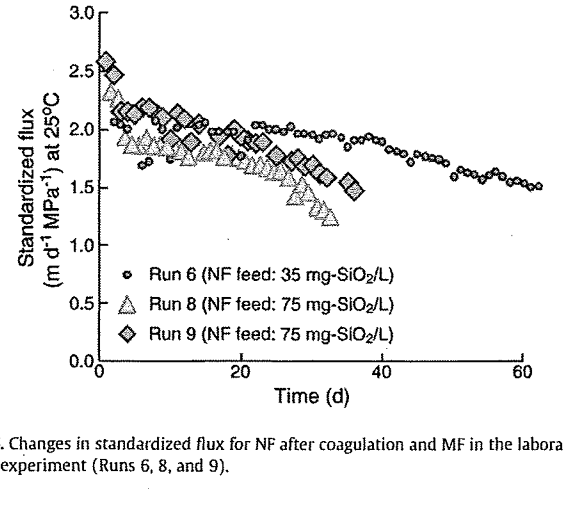

Fig. 6. Changes in standardized flux for NF after coagulation and MF in the laboratory-scale experiment (Runs 6, 8, and 9).