



Removal of phages and nanoparticles by ceramic membranes

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Title

Removal of phages and nanoparticles by ceramic membranes

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Summary

The study examines the removal of natural nanoparticles including phages by ceramic membranes without application of flocculants. Micro- and ultrafiltration membranes were considered, which were made from different inorganic materials including Al_2O_3 , SiC and TiO_2 .

Phages and nanoparticles were removed in general in correspondence with the membrane pore diameter. Size exclusion was found to be a major but not the only mechanism which influences the efficiency of phages removal by filtration with ceramic membranes. Under conditions of the study ceramic membranes showed a 0.5 to 0.9 log higher log-removal for negatively charged MS2-phages compared to the uncharged ΦX174 -phages, despite of the same diameter for both phages. This effect may be due to charge interactions, but requires additional examinations to support this hypothesis.

Nanoparticle analysis measured by Laser Induced Breakdown Detection (LIBD) was found to provide similar conclusions compared to phages analysis. Therefore LIBD-technique could be used as a highly sensitive tool for online control of membranes as support of grab sampling analysis of phages by cultivation methods.

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1 INTRODUCTION

Applications of micro- and ultrafiltration membranes in drinking water treatment showed a considerable increase in recent years. The membranes are used to remove particulate matter such as inorganic particles as well as microorganisms including bacteria and viruses. Examinations of mechanisms for virus removal during membrane filtration is of practical importance especially if no flocculants are applied, because of their small size of about 20-30 nm.

Micro- and ultrafiltration membranes, which are currently in operation in European waterworks are made from organic materials. Recent developments showed that membranes made from inorganic materials could be promising in membrane technology in the future, due to their unique characteristics including a hydrophilic surface and a high resistance against mechanical, chemical or thermal stress (Lerch et al. 2005, Heijman and Bakker 2007, NGK 2008, METAWATER 2009). Studies for removal of viruses by ceramic microfiltration membranes made from Al_2O_3 were conducted in combination with flocculants (Matsui et al. 2003, Matsushita et al. 2005).

This study examined the removal of natural occurring nanoparticles including phages by ceramic membranes without application of flocculants. Micro- and ultrafiltration membranes were considered, which were made from different materials including Al_2O_3 , SiC and TiO_2 .

2 MATERIALS AND METHODS

2.1 Membranes and experimental pilot setup

Membranes made from several ceramic materials were considered in this study. As can be seen in Tab. 2.1 a silicon carbide/silicon oxide microfiltration membrane as well as pure and titanium dioxide coated aluminium oxide ultrafiltration membranes were included. Membranes were obtained from four different companies.

Tab. 2.1: Characteristics of ceramic membrane test elements employed in this study

| pore size nm | material | | area test module m ² | producer |
|-----------------|--|--|------------------------------------|----------|
| | membrane | carrier | | |
| 200 | SiC/SiO ₂ | | 0.43 | D |
| 50 | α -Al ₂ O ₃ | | 0.20 | B |
| 50 | TiO ₂ | α -Al ₂ O ₃ | 0.13 | A |
| 10 | TiO ₂ , ZrO ₂ | α -Al ₂ O ₃ | 0.20 | C |

Ceramic membranes were applied in pilot scale tests in a waterworks. Pretreated dam water, taken from the full scale plant was used as feed for the pilot plant. Pretreatment included a rapid sand prefiltration, ozonation, flocculation and dual media sand filtration. A more detailed description may be found in previous reports [MUELLER et al., 2007]. For the experiments concerning the removal of phages, MS2- or Φ X174-phages were spiked into the feed water in concentrations between 10⁵ to 10⁷ pfp/mL. Samples collected in feed, filtrate and backwash water were analyzed for their phages concentrations.

2.2 Nanoparticle Analysis

Nanoparticles were determined by using a nanoparticle analyzer base on Laser Induced Breakdown Detection (NPA/LIBD). LIBD is a highly sensitive method to detect nanoparticles in the size range between 10 to 1,000 nm [Bundschuh et al., 2005]. NPA contained a solid state Nd:YAG-laser, providing a detection limit of about 10⁸ to 10⁹ particles/L for a particle size of 20 nm [WAGNER, 2005]. NPA/LIBD measurements were conducted in grab samples from feed and filtrate. Every analysis bases on triple sampling and detection. NPA/LIBD analysis was provided by Research Center Karlsruhe, Germany.

2.3 Fluorescence Analysis

Fluorescence analysis was conducted using a combined UV/VIS adsorption and fluorescence detector according to a method suggested by Schmidt et al. (2005). Fluorescence analysis provides a “finger print” of fluorescent sub-

stances from dissolved organic matter in water samples, including humic substances and proteins. Fluorescence analysis was used in this study to examine the composition of organic membrane foulants.

2.4 Phages Detection by Cultivation Methods

For the experiments concerning the removal of phages by ceramic membranes, 2 different types of phages were used: *MS2*-bacteriophages and *ΦX174*-phages which are relatively similar in diameter but differing in electrical charge. They are described in 3.1.

The analytical techniques for the enumeration of phages in water consist in mixing the water sample with a semi-solid nutrient medium and adding a bacterial culture sensitive to that phage. The complete mixture is then plated on a solid nutrient medium. After incubation, the phages are enumerated as plaques in the bacterial culture (number of plaque forming particles (pfp)).

MS2-bacteriophages were analyzed according the method for enumeration of F-specific RNA-bacteriophages (DIN EN ISO 10 705-1) and the stock solutions for the experiments were prepared according to Annex C of this standard method. The concentration in the stock solutions was about 10^{12} pfp/mL.

The *ΦX174*-phages belong to the group of somatic coliphages and therefore were analyzed with the method for enumeration of somatic coliphages (DIN EN ISO 10705-2). The stock solution for the experiments was prepared according to Annex C of this standard method. The concentration in the stock solution was about 10^9 pfp/mL.

The stock solutions of the respective phages were added at first to 10 L of water with drinking water quality (without chlorine residuals) and mixed. After that, these 10 L were mixed into 150 L water in a 200 L tank. The feed concentration was approximately 10^7 /mL *MS2*-phages and 10^5 /mL *ΦX174*-phages.

10-20 L of the spiked water was filtered by the membranes and phages concentration was measured in the feed and the filtrate, before back-washing. The backwash water was also analysed to allow for mass balances. For reproducibility this procedure was repeated three times for each tested membrane.

The mass balance for the phages was determined by measuring the phages concentrations (c) in the feed, filtrate und backwash water as well as the respective volumes (V) and calculating the absolute phages numbers N from these ($N = c \cdot V$). This was done by collecting the whole backwash water in special containers during the backwash process. Weight was determined from the filled containers. Samples from the backwash water were taken for phages concentration analysis after each run. Every experimental setup involved three runs under comparable conditions. The recovery (R) was determined on

the sum of absolute phages numbers in filtrate and backwash water divided by the absolute phages numbers in the feed according to:

$$R = \frac{N_{phages,filtrate} + N_{phages,backwash}}{N_{phages,feed}} * 100$$

2.5 Detection of MS2-phages by PCR

In addition to the cultural detection of MS2-phages a molecular detection by PCR was used in two runs, to be sure that no inactivation takes place during the filter studies.

The concentration of samples, the RNA-extraction and nucleic acid analysis as well as the qualitative PCR for detection of MS2 bacteriophages was done as described by Zawadsky et al. 2007. Additionally, for quantification, a real time PCR was done. For this approach the standard curve method was applied. Samples were analyzed in triplicate.

2 μ L of cDNA was mixed with 23 μ L of a reaction mix containing 12.5 μ L 2x SensiMix (Bioline), 0.5 μ L SYBR Green (Bioline), 10 pmol/ μ L forward primer (5'-CTGGGCAATAGTCAAA) and 10 pmol/ μ L reverse primer (5'-CGTGGATCTGACATAC). The PCR was conducted at 95°C for 15 min followed by 45 cycles at 94°C for 15 s, 50°C for 30 s and 72°C for 30 s, and 55°C-99°C Melt.

The measurements were done after concentration of 10 mL, 100 mL and 1000 mL to allow also semi-quantitative direct PCR. The analyses were also done in feed, filtrate and backwash water.

3 RESULTS AND DISCUSSION

3.1 Characterization of phages and membranes concerning size and charge

Pore size of membranes including their distribution may be the most important factor also influencing the removal efficiency of particles by ceramic membranes. However, other factors such as membranes and particles charge may affect the filtration result. To examine the effect of charge on phages removal phages and membranes, both different in charge, were employed. Ceramic membranes produced from several inorganic materials were considered in this study. Examined materials are listed in Table 3.1 together with their isoelectric point (IEP). Data for IEP were obtained from the literature. The IEP describes the pH-value at which the surface of the membrane has no net electrical charge. The silicon carbide/silicon oxide material has a considerable lower IEP compared to the aluminium oxide. TiO_2 and ZrO_2 are used in some products as membrane layer material on Al_2O_3 carriers. IEP of ZrO_2 membrane layers are dependent on production process. WEGMANN et al. (2008) showed that IEP of ZrO_2 coatings were about 9 and 5.5 for a calcination temperature of 250 °C and 400 °C respectively. ZrO_2 -coatings produced at a calcination temperature of 250 °C are not durable. Therefore, it is unlikely that the products with ZrO_2 -membranes tested in this study are characterized by an IEP in the range of 9.

Spherical phages with about the same mean dimension but a different IEP were identified: bacteriophage *MS2* and Φ *X174*-phage. Under these initial conditions steric factors may play a secondary role in relation to charge effects if applied in an examination on filtration effects. Moreover, both phages are known to be acceptable in cultivation and handling. In Table 3.2 the diameters and IEP of *MS2*- and Φ *X174*-phages are shown. The mean diameters are 24 or 27 nm, respectively, the *MS2*-phages having a lower IEP compared to Φ *X174*-phages.

Tab. 3.1 Isoelectric point (IEP) for ceramic membrane materials used in this study

| Membrane material | IEP | Reference |
|--|------------|--------------------------|
| SiC/SiO ₂ | 2.7 | Piwonski, 2005 |
| | 2.2 | Steigerwald et al., 1997 |
| α -Al ₂ O ₃ | 8.3 | Piwonski, 2005 |
| | 9.0 | Steigerwald et al., 1997 |
| TiO ₂ | 5.1 .. 6.7 | Zhang et al., 2003 |
| | 6.2 | Steigerwald et al., 1997 |
| ZrO ₂ | 6.7 | Brunelle, 1978 |
| | 6.7 | Steigerwald et al., 1997 |
| | 5.5 (.. 9) | Wegener et al., 2008 |

Tab. 3.2 Isoelectric point (IEP) and size for phages used in this study (Dowd et al., 1998)

| Phages | size in nm | IEP |
|-------------|------------|-----|
| MS2 | 24 | 3.9 |
| Φ X174 | 27 | 6.6 |

Conductivity of feed water used in this study was about 3.8 mS/m. The corresponding ionic strength (I) was estimated with about 0.7 mmol/L. Debye length (κ^{-1}) was determined using an approximative relation for water (25 °C) [Wikipedia, 2009]

$$\kappa^{-1}(nm) = \frac{10}{\sqrt{I(mM/L)}}$$

with 12 nm. This indicates that charge interactions should be relevant for the feed water tested in this study.

Feed water used in this study with pH-values between 6.8 and 7.1 implies surface charges for membranes and phages according to Table 3.3. Information on charge for the investigated membranes were given for the membrane layer as well as for the carrier material. In natural waters as specified above membranes made from silicon carbide or silicon oxide respectively are characterized by a negative surface charge whereas aluminium oxide membranes are charged positively. Membranes with an Al₂O₃-carrier material and a TiO₂- or a TiO₂-ZrO₂-membrane layer are assumed to have a slightly negative charge on the feed side but a positive charge on the carrier side. The runs were conducted with virgin, cleaned or pre-fouled membranes.

Tab. 3.3: Surface charge of membranes and organisms in natural water with pH 6.8-7.1 under conditions of this study

| Membranes | | Phages | | Remark |
|--|---------------------|--------|-------------|---------------------|
| material | charge [#] | Charge | type | |
| SiC | -/- | - | MS2 | <i>not examined</i> |
| SiC | -/- | 0 | Φ X174 | |
| Al ₂ O ₃ | +/+ | - | MS2 | |
| Al ₂ O ₃ | +/+ | 0 | Φ X174 | |
| TiO ₂ /Al ₂ O ₃ | -/+ ## | - | MS2 | |
| TiO ₂ /Al ₂ O ₃ | -/+ ## | 0 | Φ X174 | |
| TiO ₂ -ZrO ₂ /Al ₂ O ₃ | -/+ ## | - | MS2 | |
| TiO ₂ -ZrO ₂ /Al ₂ O ₃ | -/+ ## | 0 | Φ X174 | |
| polymeric | -* | - | MS2 | |
| polymeric | -* | 0 | Φ X174 | |

[#] membrane/carrier

^{##} charge expected from the material, see discussion in chapter 3.3.2

* IEP of polyethersulfone membranes: 2.2 - 2.4 (Ricq et al., 1997)

The raw water conditions considered in this study imply a negative surface charge of *MS2*-phages whereas *ΦX174*-phages are practically not charged or may show a slightly negative charge.

3.2 Results

3.2.1 *Recovery of phages*

Different ceramic membrane materials (SiC/SiO₂, TiO₂/Al₂O₃, TiO₂/ZrO₂/Al₂O₃ and Al₂O₃) with pore diameters of 200 nm, 50 nm and 10 nm were tested for phage removal efficiency. The 200 nm SiC/SiO₂ and the 50 nm TiO₂/Al₂O₃ membranes were also tested after pre-fouling with pre-filtered dam water. A polymeric ultrafiltration membrane was additionally investigated as comparison.

As already mentioned, all runs were conducted without dosing any flocculant or flocculation aid.

The concentrations, volumes and recoveries applied for each run and sub-run are summarized in Table 3.4. Run 8 was conducted without recovery test. A laboratory equipment was used in this run, which did not allow to backwash the membrane.

The recovery in general was very variable. Especially for virgin membranes (Al₂O₃, filter run 6, and TiO₂/ZrO₂/Al₂O₃, filter run 10) the recovery was changing over 1-2 orders of magnitude over the 3 consecutive filter runs (6: 5%-30%, 10: 0,9%-38%). For membranes after CIP or fouled membranes the recovery was mostly much better and more constant over the 3 consecutive runs. About 50% to 100% were reached in the runs 1, 2, 4, 5, 7, 9, 11.

From 3 runs with *ΦX174* (5, 7 and 12) the runs 5 (50nm, TiO₂/Al₂O₃) and 7 (50nm, Al₂O₃) showed better recovery than the same membrane for *MS2*, whereas the run 12 (10nm, TiO₂/ZrO₂/Al₂O₃) was showing considerably lower recovery than the respective run with *MS2*.

Table 3.4: Recovery in the different filter runs

| No. | Phage | Membrane material condition | Pore size nm | C ₀ pfp/mL | Volume mL | C _{filtrate} pfp/mL | Volume mL | C _{backwash} pfp/mL | Volume mL | Recovery |
|-------------|-----------|--|--------------|-----------------------|-----------|------------------------------|-----------|------------------------------|-----------|----------|
| 1 | I MS2 | SiC/SiO ₂ after CIP | 200 | 1.50E+05 | 18,550 | 2.80E+04 | 18,550 | - | 12,650 | - |
| | II MS2 | | | 1.50E+05 | 19,300 | 7.80E+04 | 19,300 | 9.20E+02 | 14,800 | 0.52 |
| | III MS2 | | | 1.50E+05 | 19,550 | 8.30E+04 | 19,550 | 2.40E+03 | 14,450 | 0.57 |
| 0.54 | | | | | | | | | | |
| 2 | I MS2 | SiC/SiO ₂ fouled | 200 | 9.33E+06 | 18,550 | 1.54E+07 | 18,550 | 7.55E+06 | 8,350 | 2.02 |
| | II MS2 | | | 9.33E+06 | 19,150 | 7.60E+06 | 19,150 | 2.15E+06 | 7,850 | 0.91 |
| | III MS2 | | | 9.33E+06 | 19,750 | 1.20E+07 | 19,850 | 1.55E+06 | 9,250 | 1.37 |
| 1.43 | | | | | | | | | | |
| 3 | I MS2 | TiO ₂ /Al ₂ O ₃ after CIP | 50 | 2.43E+07 | 20,100 | 2.15E+05 | 20,100 | 2.23E+06 | 2,250 | 0.02 |
| | II MS2 | | | 2.43E+07 | 20,000 | 7.20E+05 | 20,000 | 2.30E+07 | 3,250 | 0.18 |
| | III MS2 | | | 2.43E+07 | 20,150 | 1.17E+06 | 20,150 | 5.85E+07 | 3,200 | 0.43 |
| 0.21 | | | | | | | | | | |
| 4 | I MS2 | TiO ₂ /Al ₂ O ₃ fouled | 50 | 2.04E+07 | 18,300 | 4.13E+04 | 18,300 | 2.70E+07 | 5,700 | 0.41 |
| | II MS2 | | | 2.04E+07 | 12,050 | 3.06E+05 | 12,050 | 2.17E+07 | 5,050 | 0.46 |
| | III MS2 | | | 2.04E+07 | 13,850 | 1.85E+05 | 13,850 | 1.80E+07 | 5,800 | 0.38 |
| 0.42 | | | | | | | | | | |
| 5 | I ΦX174 | TiO ₂ /Al ₂ O ₃ after CIP | 50 | 6.25E+05 | 20,150 | 5.40E+04 | 20,150 | 1.10E+06 | 5,500 | 0.57 |
| | II ΦX174 | | | 6.25E+05 | 18,900 | 6.35E+04 | 18,900 | 2.16E+06 | 4,550 | 0.93 |
| | III ΦX174 | | | 6.25E+05 | 20,450 | 4.60E+04 | 20,450 | 1.49E+06 | 4,500 | 0.60 |
| 0.70 | | | | | | | | | | |
| 6 | I MS2 | Al ₂ O ₃ virgin | 50 | 2.41E+07 | 19,700 | 2.34E+05 | 19,700 | 5.45E+06 | 3,700 | 0.05 |
| | II MS2 | | | 2.41E+07 | 19,550 | 3.27E+05 | 19,550 | 2.18E+07 | 4,100 | 0.20 |
| | III MS2 | | | 2.41E+07 | 19,850 | 5.42E+05 | 19,850 | 3.00E+07 | 4,300 | 0.29 |
| 0.18 | | | | | | | | | | |
| 7 | I ΦX174 | Al ₂ O ₃ after CIP | 50 | 4.97E+05 | 19,250 | 5.40E+04 | 19,250 | 7.90E+06 | 2,800 | 2.42 |
| | II ΦX174 | | | 4.97E+05 | 20,500 | 6.35E+04 | 20,500 | 2.15E+06 | 2,950 | 0.75 |
| | III ΦX174 | | | 4.97E+05 | 20,500 | 4.60E+04 | 20,500 | 3.65E+06 | 2,850 | 1.11 |
| 1.43 | | | | | | | | | | |
| 8 | I MS2 | PESM flushed | 20 | 2.04E+07 | | | | | | |
| | II MS2 | | | 7.85E+06 | | 9.75E+04 | | | | |
| | III MS2 | | | 9.30E+06 | | 4.44E+05 | | | | |
| 9 | I MS2 | PESM flushed | 20 | 2.36E+07 | 250 | 4.98E+04 | 250 | 3.67E+08 | 10 | 0.62 |
| | II MS2 | | | 2.36E+07 | 250 | 8.35E+04 | 250 | 5.13E+08 | 10 | 0.87 |
| 0.75 | | | | | | | | | | |
| 10 | I MS2 | TiO ₂ /ZrO ₂ /Al ₂ O ₃ virgin | 10 | 2.81E+07 | 19,950 | 1.69E+04 | 19,950 | 1.41E+06 | 3,200 | 0.01 |
| | II MS2 | | | 2.81E+07 | 19,550 | 2.20E+04 | 19,550 | 1.97E+06 | 3,600 | 0.01 |
| | III MS2 | | | 2.81E+07 | 20,000 | 3.07E+05 | 20,000 | 5.85E+07 | 3,500 | 0.38 |
| 0.13 | | | | | | | | | | |
| 11 | I MS2 | TiO ₂ /ZrO ₂ /Al ₂ O ₃ after CIP | 10 | 1.70E+05 | 20,900 | 1.10E+01 | 20,900 | 6.05E+05 | 4,200 | 0.72 |
| | II MS2 | | | 1.70E+05 | 20,450 | 1.57E+01 | 20,450 | 6.75E+05 | 2,850 | 0.55 |
| | III MS2 | | | 1.70E+05 | 19,900 | 2.96E+01 | 19,900 | 9.60E+05 | 3,750 | 1.06 |
| 0.78 | | | | | | | | | | |
| 12 | I ΦX174 | TiO ₂ /ZrO ₂ /Al ₂ O ₃ after CIP | 10 | 3.88E+05 | 19,750 | 1.34E+03 | 19,750 | 9.28E+03 | 2,500 | 0.01 |
| | II ΦX174 | | | 3.88E+05 | 20,100 | 1.05E+03 | 20,100 | 4.95E+04 | 2,300 | 0.02 |
| | III ΦX174 | | | 3.88E+05 | 19,950 | 1.83E+03 | 19,950 | 7.38E+05 | 2,650 | 0.26 |
| 0.09 | | | | | | | | | | |

3.2.2 Removal of phages

In Table 3.5 log-removals for all filter runs are shown. As expected, for the 200 nm ceramic membranes (filter runs 1 and 2), no phages removal could be detected. For 50 nm ceramic membranes, a removal between 1 and 2 log was found (filter runs 3-7). For the 10 nm membranes (filter runs 10-12), a higher removal between 2.5 and 4 log was found.

Tab. 3.5: Removal efficiency for MS2- and Φ X174-phages phages in different filter runs under different conditions

| No. | Phage | Membrane material condition | Pore size nm | C_0 pfp/mL | C_{filtrate} pfp/mL | Removal log |
|-----|-----------------|--|--------------|--------------|------------------------------|--------------|
| 1 | I MS2 | SiC/SiO ₂ | 200 | 1.50E+05 | 2.80E+04 | - |
| | II MS2 | after CIP | | 1.50E+05 | 7.80E+04 | 0.28 |
| | III MS2 | | | 1.50E+05 | 8.30E+04 | 0.26 |
| | | | | | | 0.27 |
| 2 | I MS2 | SiC/SiO ₂ | 200 | 9.33E+06 | 1.54E+07 | -0.22 |
| | II MS2 | fouled | | 9.33E+06 | 7.60E+06 | 0.09 |
| | III MS2 | | | 9.33E+06 | 1.20E+07 | -0.11 |
| | | | | | | -0.08 |
| 3 | I MS2 | TiO ₂ /Al ₂ O ₃ | 50 | 2.43E+07 | 2.15E+05 | 2.05 |
| | II MS2 | after CIP | | 2.43E+07 | 7.20E+05 | 1.53 |
| | III MS2 | | | 2.43E+07 | 1.17E+06 | 1.32 |
| | | | | | | 1.63 |
| 4 | I MS2 | TiO ₂ /Al ₂ O ₃ | 50 | 2.04E+07 | 4.13E+04 | 2.69 |
| | II MS2 | fouled | | 2.04E+07 | 3.06E+05 | 1.82 |
| | III MS2 | | | 2.04E+07 | 1.85E+05 | 2.04 |
| | | | | | | 2.19 |
| 5 | I Φ X174 | TiO ₂ /Al ₂ O ₃ | 50 | 6.25E+05 | 5.40E+04 | 1.06 |
| | II Φ X174 | after CIP | | 6.25E+05 | 6.35E+04 | 0.99 |
| | III Φ X174 | | | 6.25E+05 | 4.60E+04 | 1.13 |
| | | | | | | 1.06 |
| 6 | I MS2 | Al ₂ O ₃ | 50 | 2.41E+07 | 2.34E+05 | 2.01 |
| | II MS2 | virgin | | 2.41E+07 | 3.27E+05 | 1.87 |
| | III MS2 | | | 2.41E+07 | 5.42E+05 | 1.65 |
| | | | | | | 1.84 |
| 7 | I Φ X174 | Al ₂ O ₃ | 50 | 4.97E+05 | 5.40E+04 | 0.96 |
| | II Φ X174 | after CIP | | 4.97E+05 | 6.35E+04 | 0.89 |
| | III Φ X174 | | | 4.97E+05 | 4.60E+04 | 1.03 |
| | | | | | | 0.96 |
| 8 | I MS2 | PESM | 20 | 2.04E+07 | | |
| | II MS2 | flushed | | 7.85E+06 | 9.75E+04 | 1.91 |
| | III MS2 | | | 9.30E+06 | 4.44E+05 | 1.32 |
| | | | | | | 1.61 |
| 9 | I MS2 | PESM | 20 | 2.36E+07 | 4.98E+04 | 2.68 |
| | II MS2 | flushed | | 2.36E+07 | 8.35E+04 | 2.45 |
| | | | | | | 2.56 |
| 10 | I MS2 | TiO ₂ /ZrO ₂ /Al ₂ O ₃ | 10 | 2.81E+07 | 1.69E+04 | 3.22 |
| | II MS2 | virgin | | 2.81E+07 | 2.20E+04 | 3.11 |
| | III MS2 | | | 2.81E+07 | 3.07E+05 | 1.96 |
| | | | | | | 2.76 |
| 11 | I MS2 | TiO ₂ /ZrO ₂ /Al ₂ O ₃ | 10 | 1.70E+05 | 1.10E+01 | 4.19 |
| | II MS2 | after CIP | | 1.70E+05 | 1.57E+01 | 4.03 |
| | III MS2 | | | 1.70E+05 | 2.96E+01 | 3.76 |
| | | | | | | 3.99 |
| 12 | I Φ X174 | TiO ₂ /ZrO ₂ /Al ₂ O ₃ | 10 | 3.88E+05 | 1.34E+03 | 2.46 |
| | II Φ X174 | after CIP | | 3.88E+05 | 1.05E+03 | 2.57 |
| | III Φ X174 | | | 3.88E+05 | 1.83E+03 | 2.33 |
| | | | | | | 2.45 |

3.2.3 Parallel analyses of culture and PCR

In the filter runs 04/III and 10/III the MS2-phages have additionally been quantified by real-time-PCR. The results are shown in parallel to the cultural results in Table 3.6. The values were mostly in the same order of magnitude. It can be concluded that the phages were not inactivated by membrane filtration process, and also no extreme coagulation of the phages was occurring.

Tab. 3.6: Comparison of PCR- and cultural detection of MS2-phages

| No. | Material | Size nm | | C_0 pfp/mL | C_{filtrate} pfp/mL | C_{backwash} pfp/mL | Removal log |
|-----|---|---------|---------|--------------|------------------------------|------------------------------|-------------|
| 4 | III TiO ₂ /Al ₂ O ₃ fouled | 50 | culture | 2.04E+07 | 1.85E+05 | 1.80E+07 | 2.042 |
| | | | PCR | 2.20E+07 | 1.60E+06 | 1.80E+07 | 1.138 |
| 10 | III TiO ₂ /ZrO ₂ /Al ₂ O ₃ virgin | 10 | culture | 2.81E+07 | 3.07E+05 | 5.85E+07 | 1.962 |
| | | | PCR | 5.40E+08 | 5.20E+06 | 1.00E+08 | 2.016 |

3.3 Discussion

3.3.1 Influence of pore size on phages removal efficiency

According to results summarized in Fig. 3.1 removal of both MS2- and Φ X174-phages depends on membrane pore size in general. Ceramic membranes with pore sizes of 10 nm provided a 2.5 to 4.0 phages removal under the conditions tested. For pore sizes of 50 nm the log-removal dropped to 0.96 to 1.8. Log-removal for a polymeric membrane whose pore size is 20 nm falls in between the log removal of the ceramic membranes. The silicon carbide/silicon dioxide (SiC/SiO₂) microfiltration membrane with a pore size of 200 nm did not remove phages as can be expected if size exclusion is the main removal mechanism. SiC/SiO₂-membranes were only available as microfiltration membranes within this study.

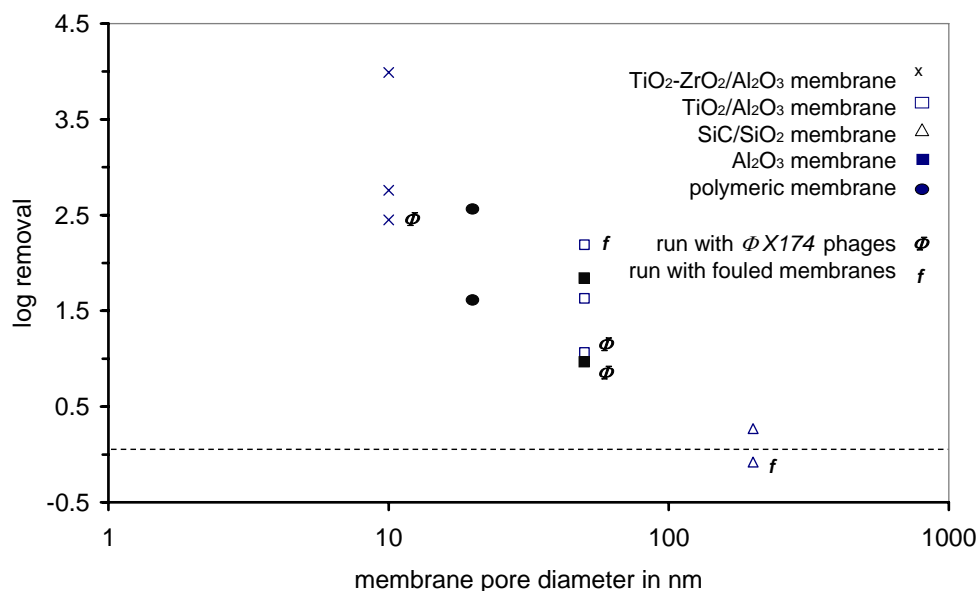


Fig. 3.1: Log-removal of MS2- and Φ X174-phages for different pore sizes and membrane materials

It is obvious from these results that for a given pore size the log-removal for phages varies up to 2 log, indicating that other factors influence the removal of phages which are worthy for a more detailed discussion.

3.3.2 *Influence of charge on phages removal efficiency*

Dimension of spherical shaped $\Phi X174$ -phages tends to be comparable with $MS2$ -phages. Therefore, size exclusion should be similar for both phages and not be responsible for differences in removal efficiency.

Due to their isoelectric point $MS2$ -phages have a stronger negative charge compared to $\Phi X174$ -phages. Positive surfaces should initiate higher $MS2$ -phages counts at the membrane surface due to charge attraction. This may be concluded from examinations from WEGMANN et al. (2008), showing for adsorbing ceramic materials that phages retention was improved if phages and filter surface had opposite charges.

Results of the study showed that removal was 0.5 to 0.9 log higher for $MS2$ -phages compared to $\Phi X174$ -phages as can be seen from Fig. 3.2. This effect may be explained by charge attraction. $MS2$ -phages adsorb better on positive surfaces compared to $\Phi X174$ -phages. Under these circumstances, higher counts for $MS2$ -phages are expected within the boundary layer between membrane surface and bulk liquid. Due to adsorption lower $MS2$ -phages counts are found in the filtrate.

Removal efficiency between the 50 nm pure aluminium oxide membrane and the 50 nm coated aluminium oxide membrane showed roughly comparable removal efficiency for both phages. This result was unexpected according the previous proposed mechanism and could be explained with a predominance of the charge of the carrier material against the charge of the membrane layer. This would mean that even the TiO_2/Al_2O_3 -membrane is characterized by a positive charge in contrast to the theoretical expected negative surface charge according to Table 3.3. To verify this hypothesis detailed measurements of membrane surface charge are required.

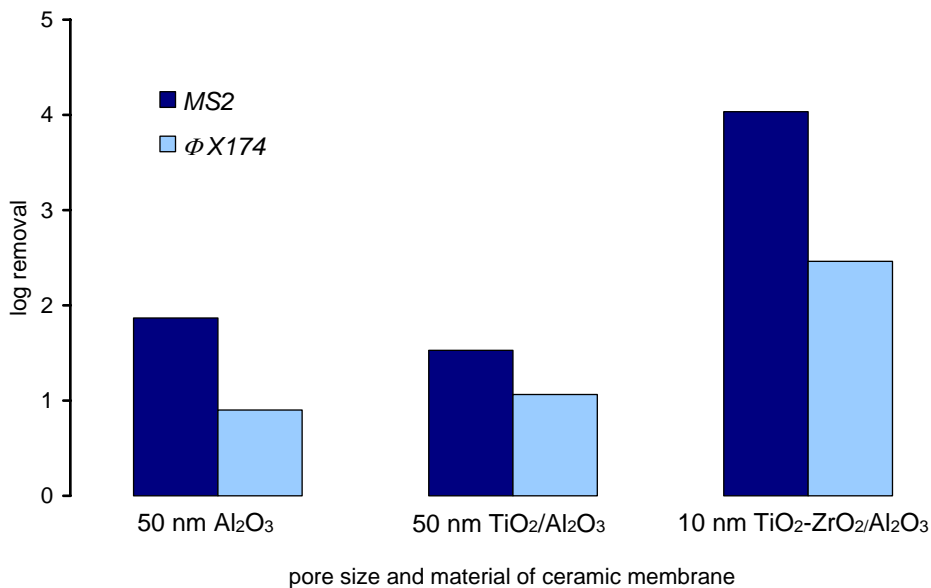


Fig. 3.2: Removal of MS2-phages compared to Φ X174-phages for different pore sizes and materials of ceramic membranes

3.3.3 Influence of pre-fouling on phages removal efficiency

During water treatment membrane fouling with dissolved and undissolved organic and inorganic substances such as particles (particulate fouling), polysaccharides (organic fouling) and metabolites (biofouling) is an unwanted but natural process. For two runs membranes were pre-fouled with the non spiked feed water under operating conditions typical for water treatment up to a resistance where a chemical cleaning (CIP) would be reasonable. Background for these experiments was to simulate conditions in the waterworks, which may influence the phages removal due to organic fouling.

The virgin SiC/SiO₂-microfiltration membrane with a pore diameter of 200 nm showed no removal of MS2-phages due to the membrane pore diameter and the negative charge of both membrane and MS2-phages resulting charge repulsion on the membrane surface. No elimination of phages was found for 100 nm Al₂O₃-microfiltration membranes in other studies without dosage of flocculant (Matsushita et al., 2005), too. This is in agreement with the expectations because the pore diameter is much larger compared to the phages diameter, despite the Al₂O₃-membranes are characterized by a positive surface charge.

Pre-fouling the SiC/SiO₂-microfiltration membrane had no effect on MS2-phages removal efficiency. The slight release of MS2-phages which can be seen in Fig. 3.3 is within the analytical error. For the 50 nm ultrafiltration membrane MS2-removal was somewhat higher after pre-fouling the membrane. This result could be due to size exclusion of fouled pores. On the other hand pre-fouling with organic matter may cause a stronger negative membrane surface charge, which was found for nanofiltration membranes by Ver-

liefde (2008). Therefore pre-fouled membranes could initiate elevated charge repulsion with the negatively charged *MS2*-phages. Pre-fouling may be also responsible for changes in surface structure leading to different physico-chemical interactions (e.g. charge, hydrophilicity), which complicates the identification of a main mechanism for phages removal by pre-fouled membranes.

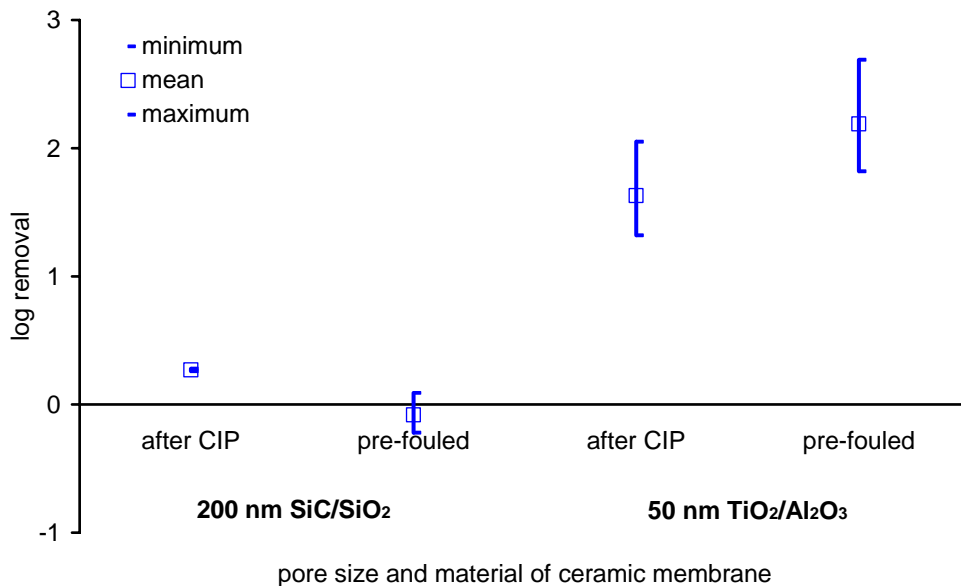


Fig. 3.3: Removal of *MS2*-phages with virgin and pre-fouled ceramic membranes

3.3.4 Log-removal determined by phages detection and nanoparticle analysis

As described above pretreated dam water was spiked with phages to determine log-removal by ceramic membranes. During certain runs (run 1, 2, 3, 6, 9, 10) nanoparticles were determined in feeds and filtrates in parallel with microbiological phages analysis. Nanoparticles were detected using a Nanoparticle Analyzer based on Laser Induced Breakdown Detection (NPA/LIBD). Nanoparticle analyse by LIBD provides as results a mean, number weighted nanoparticle count and a mean nanoparticle diameter. Assuming spherical nanoparticles a nanoparticle volume was computed using nanoparticle count and diameter.

A comparison between log removal determined by phages cultivation and by nanoparticle analysis is shown in Fig. 3.4. Log-removal was computed for nanoparticle analysis by nanoparticle count and volume. Similar log-removal rates were found for both methods, microbiological and physical analysis. Therefore LIBD-technique could be used as a highly sensitive tool for online control of membranes to support grab sampling analysis of phages by cultivation methods.

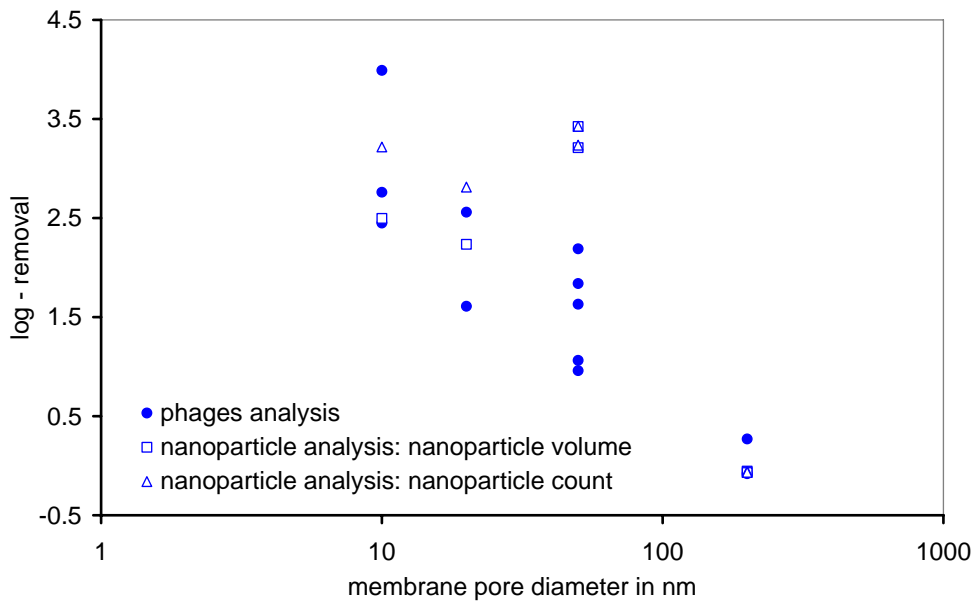


Fig. 3.4: Log removal determined by phages analysis (cultivation) and nanoparticle analysis (breakdown detection)

Nanoparticle diameter in feed was determined between 37 and 99 nm, depending on the feed water composition (Fig. 3.5). In filtrates for ceramic ultrafiltration membranes mean nanoparticle diameter was between 8 and 31 nm, which is in relatively good accordance with the membrane pore size. Nanoparticle diameters for the ceramic microfiltration membrane with a pore diameter of 200 nm were found to be comparable in feed and filtrate. This correlates with the previous described phages tests, where no phages removal was detected for microfiltration membranes.

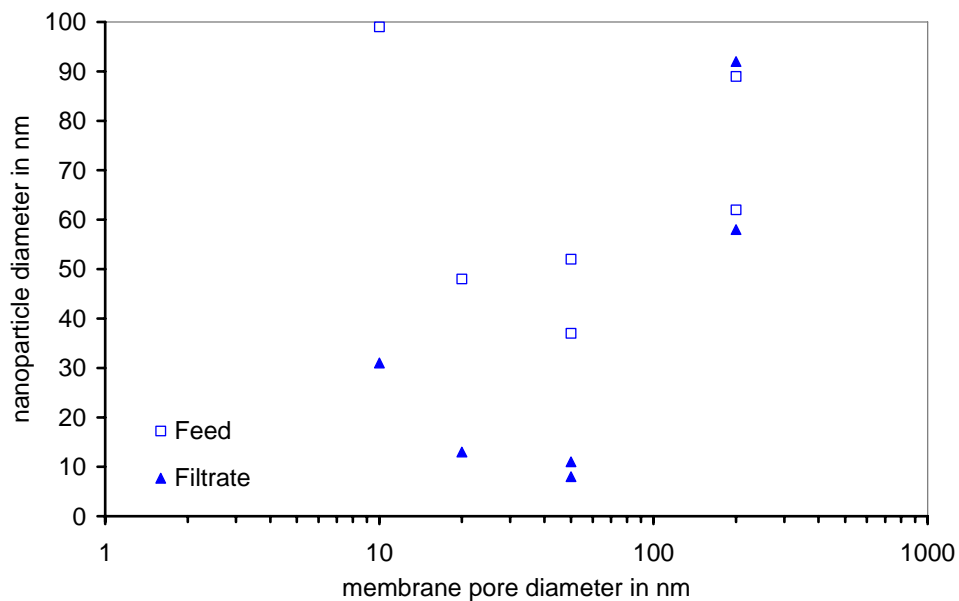


Fig. 3.5: Mean nanoparticle diameter in feed and filtrate, determined by nanoparticle analysis (breakdown detection)

As expected, diameters of the nanoparticles in the feed were always greater than in the filtrate, which corresponds with size exclusion.

Comparison between results of cultivation methods for phages and detection of nanosized particles showed a relatively good agreement. This indicates, that LIBD nanoparticle analysis might be a suitable method for membrane integrity control.

3.3.5 *Comparison of organic fouling of ceramic and polymeric membranes*

A ceramic and a polymeric membrane were loaded with natural organic matter by operation of the pilot plant with prefiltered dam water. The ceramic membrane was made from aluminium oxide and coated with titanium oxide. The pore size was given by the manufacturer with 50 nm. The polymeric membrane material was polyethersulfone with a pore size of 20 nm. Both membranes were cleaned at pH 11. Alkaline CIP-waste was examined for composition of organic substances by LCOCD-analysis. Results of LCOCD-analysis were described earlier and showed that the polysaccharide fraction in CIP-waste was higher for the polymeric membrane compared to the ceramic membrane (Mueller et al., 2008). Polysaccharides are assumed to be a major foulant. This indicates that ceramic membranes may show a lower potential for organic fouling compared to the polymeric membranes for the water tested in this study.

To verify these results fluorescence analysis was employed to characterize the composition of organic substances in CIP-waste. Fluorescence analysis may be another analytical tool to get an impression on occurrence of potential membrane foulants.

Figures 3.6 to 3.8 show the fluorescence matrices of the feed water and the alkaline CIP-wastes for the ceramic and polymeric membrane. It is obvious that the fluorescence of organic substances in both CIP-waste samples is quite different. According to a comparison with matrices of trace compounds it can be concluded, that CIP-waste of polymeric membranes show a more intensive fluorescence for the protein-like organic compounds. CIP-waste of ceramic membranes showed a higher fluorescence of humic substances. These results support the findings of LCOCD-analysis [MUELLER et al., 2008] that fouling mechanisms between ceramic and polymeric membranes should be different.

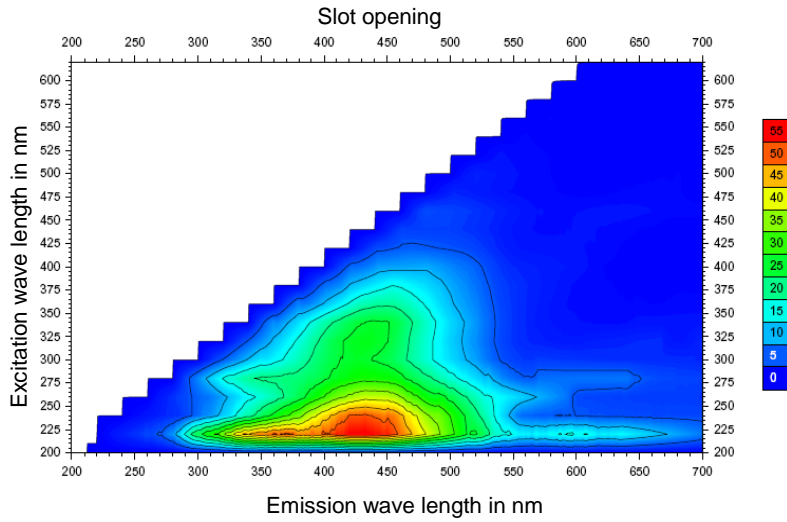


Fig. 3.6: Fluorescence matrix of feed

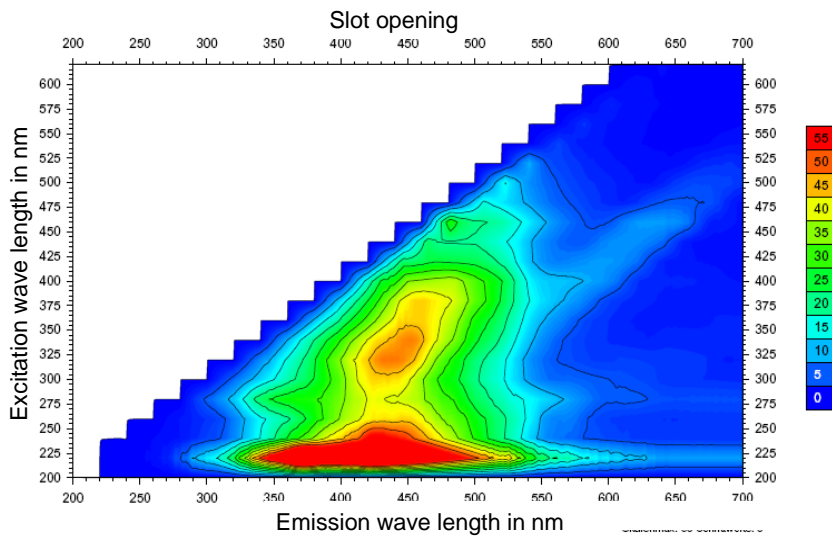


Fig. 3.7: Fluorescence matrix of alkaline CIP-waste of the ceramic membrane

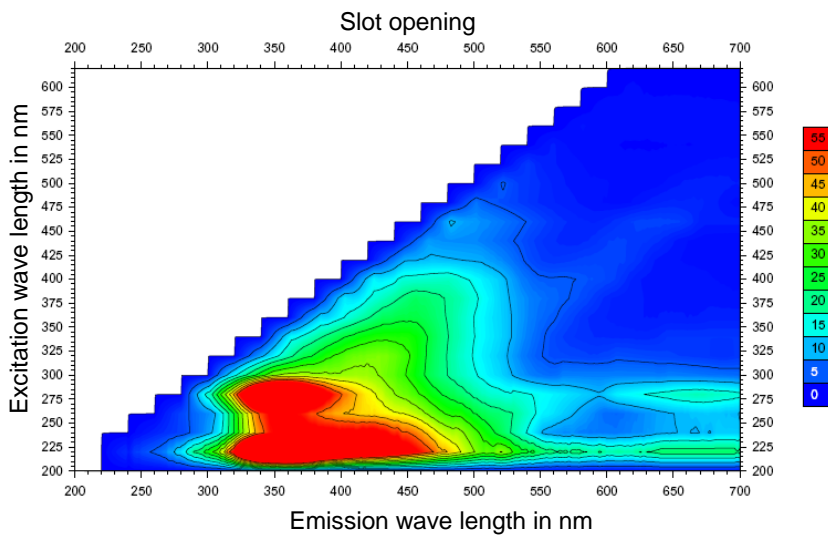


Fig. 3.8: Fluorescence matrix of alkaline CIP-waste of the polymeric membrane

4 SUMMARY AND CONCLUSIONS

In this study the influence of steric and charge mechanisms on removal of particles in the size range of viruses (nanoparticles) were studied during filtration with ceramic membranes without usage of flocculants.

Size exclusion was found to be a major but not the only mechanism which influences the efficiency of phages removal by filtration with ceramic membranes. Under conditions of the study ceramic membranes showed a 0.5 to 0.9 log higher log-removal for negatively charged *MS2*-phages compared to the uncharged *ΦX174*-phages, despite of the same diameter for both phages. Comprehensive examinations of the actual membrane surface charge, e.g. by Zeta-potential, to confirm the membrane charge expected from the membrane material would support the identification of the basic mechanisms, which are responsible for the observed effects.

Pre-fouling was found to improve the removal of *MS2*-phages slightly for the 50 nm ultrafiltration membrane. This result could be due to size exclusion of fouled pores. However, no effect of pre-fouling was measured for the 200 nm SiC/SiO₂-membrane.

Comparison of log-removals determined by cultivation methods for phages and detection of nanosized particles by LIBD-technique showed a relatively good agreement. Therefore, LIBD nanoparticle analysis might be a suitable method for membrane integrity control even for particles in the size range of viruses.

Organic matter from CIP waste of membranes was characterized by fluorescence analysis in addition to LCOCD-analysis. Both analytical methods are independent on each other. They showed a different composition of organic material in CIP-waste for ceramic compared to polymeric membranes. These results support the findings that fouling mechanisms between ceramic and polymeric membranes are different.

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