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Virus removal by ceramic pot filter disks: Effect of biofilm growth and surface cleaning

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ABSTRACT

Ceramic pot filters are household water treatment and safe storage (HWTS) systems designed to improve the microbial quality of drinking water. They yield high log reduction values (LRVs) for bacterial and protozoan pathogens but provide very little removal of viruses. This study investigated virus removal of ceramic filter discs (CFDs), using feed water with 3 different nutrient levels under extended continuous operation and limited cleaning frequency. The results show that filter use without cleaning resulted in biofilm growth and MS2 LRV values increased with increasing feed water nutrient content. Cleaning the filter surface by scrubbing led to a partial or total loss in improved LRVs, indicating the importance of this biological top layer to the removal of MS2. Overall, the removal capacity of a matured biofilm remained constant, regardless of its age. MS2 LRVs ranged between 0.9 ± 0.2 LRV for low nutrient (LN), 1.6 ± 0.2 LRV for medium nutrient (MN) and 2.4 ± 0.5 LRV for high nutrient (HN) biofilms. Interestingly, a change in feed conditions for the HN filters resulted in an unprecedented high LRV of > 4 LRV, which supports further investigation of the mechanistic role of biofilms in virus removal.

1. Introduction

Ceramic pot filters (CPF) are a cost-effective household water treatment and safe storage (HWTS) technology for users without access to microbiologically safe drinking water, such as in rural areas without central treatment works. In a meta-regression study evaluating health improvements of HWTS systems, Hunter et al. (2009) concluded that the CPF provide a greater protective effect than other HWTS systems. The use of CPF reduced waterborne disease risk by a factor of 3. Compared to other HWTS systems, CPF proved to be more sustainable for long term usage and more resilient since it is less dependent on energy, availability of chemicals or weather (Hunter, 2009; WHO, 2011).

CPF manufacturing and use are on the rise in developing countries, yet international production standards and quality control checks do not exist (Rayner et al., 2013a). Manufacturing quality control is limited to visual inspection for cracks and flow rate checks (Lantagne, 2001). No performance checks on the removal of microbes is conducted (Bielefeldt et al., 2010; Rayner et al., 2013a; van Halem et al., 2009a). In lab and field studies, CPFs have been reported to provide high levels of removal for bacteria and protozoa; however, virus removal is very limited (Brown and Sobsey, 2006a; Clopeck, 2009; Van der Laan et al., 2014; van Halem et al., 2017, 2009a). Virus removal has been assessed through challenge studies in the laboratory using bacteriophages (generally F+ coliphage MS2) as viral indicators since they resemble the removal of human enteric viruses (WHO, 2011). Removal of MS2 in CPF found to be less than 1 LRV (Log Reduction Value) under different conditions (water quality, water source, contact time, etc.) (Bielefeldt et al., 2010; Brown and Sobsey, 2010, 2009; Guerrero-Latorre et al., 2015; Salsali et al., 2011; Tsao et al., 2015; Van der Laan et al., 2014; van Halem et al., 2017, 2009a).

To qualify as protective in WHO's verification scheme for HWTS systems, CPFs would need to achieve at least 3 LRV for virusses, demonstrated by MS2 and phiX174 (WHO, 2011). Reported MS2 reductions reached almost 3 LRV after 13 weeks of continuously filtering canal water through CPFs (van Halem, 2006), which was hypothesized to be due to the growth of biofilms on the filter during operation. Similar hypotheses were posed by Rayner et al. (2013b), who claimed that reducing the cleaning frequency would lead to biofilm growth and improved virus removal.

Biofilms have demonstrated the ability to capture viruses (Wingender and Flemming, 2011), Storey and Ashbolt (2003) recovered 10^8 pfu/cm^2 of B40-8 and MS2 phages and 10^7 pfu/cm^2 phiX174 phages from biofilm coupons collected from artificially

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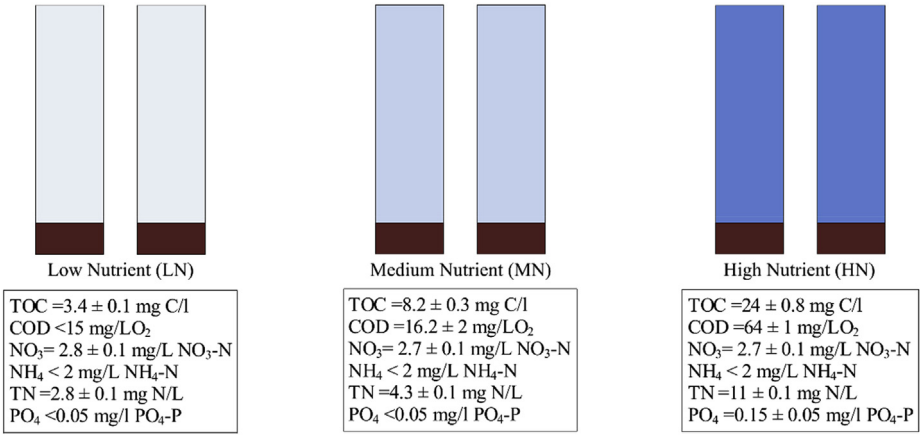

Fig. 1. Experimental set-up and feed water characterisation *(For reference, each set of duplicate CFDs were named after their source feed water).*

challenged drinking water piplelines. Biofilm growth in slow sand filters (SSF), membrane bioreactors (MBR) and constructed wetlands enhances the removal of micro-organisms, including viruses. Ueda (2000) measured an increase of 3.7 LRV in suspended indigenous phage removal following biofilm growth in an MBR unit. Similarly, Purnell et al. (2015) reported a 2.3 LRV increase for MS2 and B-14 spiked in wastewater treated by biofilm-coated MBR. In slow sand filters, the surface biofilm (schmutzdecke) MS2 LRV ranged between 0.08 and 3, depending on the schmutzdecke age and water temperature (Schijven et al., 2013). Moreover, the LRV of MS2 increased by 1.5–2.5 following biofilm growth in ultrafiltration units (ElHadidy et al., 2014; Lu et al., 2013). The differences in LRV between viruses under the same conditions is mainly related to morphology of the viruses, as indicated by the meta-analysis of Amarasiri et al. (2017). The study found MS2 phages to be more difficult to remove than somatic coliphages, F-specific phages and T4 phages, making it a conservative representative for human virus removal.

Biofilm formation starts by the adhesion of bacteria to solid surfaces, which immobilises them. The bacteria may grow (Flemming et al., 2002), dependent on the availability of the compounds necessary for assimilation and dissimilation such as organic matter in water (LeChevallier et al., 1991; Pedersen, 1990; van der Kooij et al., 1982). Many CPF users rely on surface water as their water source (Brown and Sobsey, 2006a; Clopeck, 2009), which may contain significant levels of organic carbon, depending on the level of contamination or eutrophication (Chapman (Ed), 1996; Volk et al., 2002). This implies that a biofilm will form on CPF during operation, and the biofilm formation rate will depend on the level of nutrients in the water source. The objective of the study was to examine the hypothesis that extended filtration periods and reduced cleaning frequency would lead to enhanced virus removal due to biofilm growth. Three source waters containing different nutrient levels were filtered through CFDs, and biofilm growth was monitored by tracking the flow rate of the CFD and measuring bacterial cell counts and ATP on the CFD surface. Virus removal was determined by challenging CFDs at the end of different operational periods, before and after cleaning, with MS2 phages and monitoring virus breakthrough. Finally, the biofilm was evaluated for the presence of MS2 to confirm the role of the biofilm as a virus attachment site and to identify the fate of viruses in the CFD system.

2. Materials and methods

2.1. Ceramic filter disc manufacturing

For this study, CFDs were used to simulate full ceramic pots (Rayner et al., 2013b). CFDs were manufactured at the FilterPure (now Wine to Water) factory in the Dominican Republic by following the manufacturing protocol precisely. Local clay, sawdust and demineralised water were mixed in a weight ratio of 65%: 13%: 22%, respectively. Sawdust was sieved through a 0.5 mm sieve prior to mixing. The components were mixed for 5 min in the mechanical mixer, followed by 5 min of hand mixing. The mixture was moulded and pressed under 2200 psi pressure into CFDs. Moulded CFDs were left to dry for 3–5 days then fired in a kiln for at 860 °C with a ramp rate of 2.4 °C/min and dwell time 1 h. The disks were left in the kiln to cool overnight, no silver or other additives were applied after firing.

2.2. CFDs filtration setup

To facilitate their testing, each CFD (Ø 125 mm & H = 19 ± 2 mm) was fixed using silicone into the bottom of a double socket connector (125 mm Ø PVC). On top of the connector, a 350 mm PVC column (125 mm inner Ø) was placed, providing a maximum water head of 4.5 L above the CFDs. The system was operated as a fed-batch system, filled daily with source water, while resting over an open top polypropylene receptacle to collect filtered water. LN, MN and HN source waters were applied, each in duplicate columns. Nutrient Broth (NB; CM0001, Thermos Scientific) was diluted in unchlorinated tap water. LN was tap water without NB, MN was tap water with 1:500 diluted NB and HN was a 1:125 dilution of NB. PO_4, NO_3 and NH_4 were measured using Hach kits LCK 348, LCK 339, LCK 303. Total (TOC) and dissolved (DOC) organic carbon, and total nitrogen (TN) were measured using a TOC-V CPH Shimadzu analyzer. Dissolved oxygen (DO) was measured using a WTW 3420 portable multimeter. Selected dilution rates aimed to simulate organic carbon concentrations in "clean" (< 10 mg/l) and contaminated (> 10 mg/l) fresh water. The setup was operated inside a Thermo Fisher Heratherm OMH400 incubator at 27 °C throughout the experiment to represent a warm, tropical environment (Fig. 1).

2.3. MS2 challenge test

On the challenge test day, MS2 stock (GAP Environmental, Canada) was diluted in unchlorinated tap water (challenge water) to a concentration of 10^6 to 10^7 pfu/ml. Thus, the term "challenge water" for all experiments refers to LN water spiked with MS2 bacteriophages. The columns were each filled with 4 L and put back in the incubator at 27 °C. Filtrate volume of at least 1 total pore volume (PV) was discarded to ensure that water in the pores was replaced by MS2 challenge water. The pore volume was calculated based on the dry versus saturated weight of the CFDs and corresponded to a PV of 128 ± 2 cm^3. Filtrate samples were collected in a receptacle that had been cleaned and disinfected by subsequent rinses with 0.05% sodium hypochlorite solution, demineralised water, 100 mg/l sodium thiosulfate solution and demineraliszed water (3×). Receptacles were air-dried before use.

Samples were refrigerated and analysed the same day using the double agar layer (DAL) method, according to ISO 10705–1. Nalidixic acid was added to the top agar to limit growth of background flora. Serial sample dilutions were plated in duplicate, and plaque-forming units (PFUs) were counted after incubation for 18 h at 37 °C. PFU results were converted into $\log_{10}$ reduction values (LRVs), where LRV = $\log_{10}$ (measured concentration of MS2 in the influent/measured concentration of MS2 in the filtrate).

2.4. Biofilm analysis

Biofilm growth was monitored by swabbing the CFD surface. An area of 5.31 cm^2 was swabbed using COPAN sterile Dry Swabs with a plastic applicator and rayon tip. The swab was submerged in filter-sterilised mineral water and stored at 4 ± 2 °C until analysis. Bacterial cell counts were measured with a C6 flow cytometer (BD Accuri C6, United States), with distinction between total (TCC) and intact (ICC) cell counts. ATP, TCC and ICC analyses were conducted at Het Waterlaboratorium (Haarlem, the Netherlands) following the protocol described by G Liu et al. (2013).

2.5. Experimental overview

2.5.1. Operational period before cleaning

2.5.1.1. Initial operation (5 weeks). Duplicate LN, MN and HN CFDs were run in fed-batch mode for 5 weeks. Daily flow rates were recorded by measuring filtrate volume over time in a graduated cylinder. MS2 LRVs were measured using the challenge test water at the end of weeks 4 and 5. Water in the columns was replaced by MS2-free source water (LN, MN and HN) directly after completing each challenge test. In addition, the surface of the HN CFDs was swabbed using rayon swabs to quantify TCC, ICC and ATP of the surface grown biofilm.

2.5.1.2. Long-term operation (21 weeks). The LN and MN CFDs were operated in fed-batch mode for an additional 4 months without cleaning. HN CFDs were excluded from this experiment since their flow rates had become too low. Combining the biofilm formation periods of the current and the previous test, the biofilm age reached 21 weeks (5.25 months), which is the longest continuous testing period for ceramic filters reported to our knowledge. MS2 LRVs were determined by challenge tests on weeks 10, 16 and 21. Flow rates were measured daily, and the surface of the CFDs was sampled for biofilm analysis in weeks 10 and 21. The disk surface was swabbed after each challenge test was completed, then columns were refilled with MS2 free source water (LN and MN) to sustain biofilm growth and development.

2.5.2. Effect of cleaning the surface

Cleaning the filter surface and the receptacle is a regular maintenance step recommended and practised by users of CPF. Filter flow rates drop with continued use, especially when surface water is used (Brown and Sobsey, 2007; Lantagne et al., 2010). Thus it is inevitable that the CPF's internal surface needs to be cleaned periodically to restore the flow. CPF factories advise using a stiff laundry brush to scrape the surface once every 4 weeks (Brown and Sobsey, 2007). Nevertheless, Cambodian users were reported to clean the filter every week (Brown and Sobsey, 2007). Cleaning the filters has been studied, although the focus was on water flow restoration (van Halem et al., 2017, 2009a). In this study, the focus is to understand the effect of removing the surface biological top layer (biofilm) on the MS2 LRV. To this end, biofilm amended CFDs were challenged with MS2 before and after cleaning the surface. The cleaning protocol was applied by brushing the surface twice using a stiff hand brush followed by a rinse with demineralised water. This protocol was repeated at each cleaning event.

After running for 21 weeks in fed-batch mode, the LN and MN CFDs surfaces were cleaned. Before and after this cleaning, biofilm swabs were obtained, flow rates were recorded and MS2 challenge tests were conducted. The cleaning event was repeated twice, following CFDs operational periods (i.e. periods of biofilm regrowth) of 4 (week 25) and 1 (week 26) weeks. Also during these regrowth periods, MS2 challenge tests were conducted and flow rates were monitored before and after the cleaning, but biofilm swabs were obtained only before cleaning. After MS2 challenge tests for any cleaning event, CFDs were returned to fed-batch mode with MS2 free source water (LN or MN) to allow for biofilm regrowth and recovery.

HN CFDs were cleaned earlier than LN and MN CFDs given the loss of flow rate after the first experiment lasting 5 weeks. HN CFDs were subsequently fed with LN water for a month instead of regular HN feed to avoid permanent clogging of the CFDs. After this, the CFDs were reloaded with HN water for 4 and 1 weeks, each followed by cleaning events. MS2 challenge tests were run before and after each cleaning event, flow rates were monitored and biofilm swabs were taken to measure TCC and ATP.

2.5.3. The fate of phages in the system

After the experiments with and without cleaning, the fate of MS2 in CFDs was studied in more detail. This experiment was conducted on a 1-week-old re-grown biofilm for all CFDs. The experiment consisted of two stages: (1) MS2 challenge water feed and (2) regular feed without MS2. In the first stage, we examined the stability of the MS2 phage concentration in the feed water residing on the top of CFDs (supernatant) and monitored the MS2 concentration in the filtrate (C_f) over time/pore volume. The second stage examined the MS2 survival/recovery in the biofilm and detachment of MS2 phages from the biofilm/filter into the filtrate. Stage 2 started at the end of Stage 1 and ended when no phages were detected in biofilm or water samples.

At the onset of Stage 1, challenge water was sampled to establish the initial challenge concentration, refered to as C_0 (pfu/ml). Subsequently, duplicate samples were taken from the challenge water every hour after stirring with a sterile serological pipette. The samples were analysed for MS2 to determine the change of pfu concentration over time (C_t). Linear regression of the log concentration change (log C_t/C_0) was used to estimate the inactivation rate (λ) of MS2 in feed water at 27 °C as described by Schijven et al. (2013).

Simultaneously, hourly samples from the filtrate were collected, mixed, volume recorded and analysed for MS2 (C_f). After sampling, a new disinfected receptacle was placed to collect the next hour's filtrate for each time point. Sampling was repeated until > 2 pore volumes (PV) of filtrate were collected, after which the challenge feed water was replaced with regular feed water without MS2 at t_1. Monitoring of the filtrate continued beyond t_1, i.e., Stage 2 of the experiment, in order to assess detachment (C_d) and breakthrough of MS2 from the biofilm/filter.

At the time of feed water replacement (t_1), 3.64 cm^2 of each CFD was swabbed to collect a biofilm sample. Swabs were transferred to a sterile glass tube containing filter-sterilised mineral water then sonicated 3–4 times for 2 min using a Bransonic 521 water bath. Sonicated water was collected in sterile tubes placed on ice, mixed, the volume was recorded and analysed for MS2 phages. Results were converted from PFU/ml into PFU/cm^2, where PFU/cm^2 = (PFU/ml sonicated liquid volume)/surface area swabbed (cm^2). The number of phages in the biofilm of the total CFD surface (N_b) was calculated from the PFU/cm^2 × CFD Surface Area (cm^2).

A MS2 balance was calculated using the total number of phages loaded onto the CFD (N_i) with correction for the inactivation over time (λ), the total number of phages in the filtrate in stage 1 (N_f), the total number of detached phages (N_d, in the Stage 2 filtrate) and the total number of phages recovered from the biofilm (N_b).

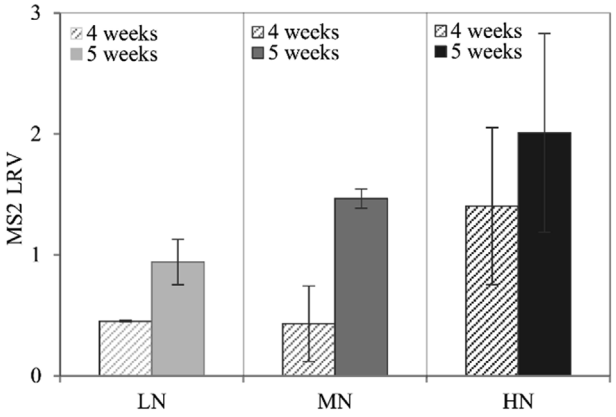

Fig. 2. MS2 bacteriophages removal after 4 and 5 weeks of continuous loading (n = 2). Error bars represent standard deviations (SD).

3. Results and discussion

3.1. Operational period before cleaning

Fig. 2 depicts the average MS2 LRV after the initial operational period of 4 and 5 weeks of CFD filtration of LN, MN and HN source water.

After 4 weeks of initial operation, the average MS2 LRV remained below 0.5 for both LN and MN CFDs, whereas the HN CFDs achieved an average LRV of 1.4. A week later, the MS2 LRV for LN and MN CFDs had increased to 0.9 and 1.5, respectively. The HN CFDs also showed an increased LRV (to 2), although due to the variation between duplicates, this increase is not as apparent.

Continuous loading of the CFDs had an impact on the flow rate as illustrated in Fig. 3. In the first four days, LN, MN and HN CFDs lost 68%, 75% and 83% of their initial flow rate, respectively. The flow rate of HN CFDs continued to drop for 1 week before stabilising. Meanwhile, LN and MN CFD flow rates slowly dropped further during the 5 weeks. Flow rate resistance was higher with higher nutrient feed (LN < MN < HN), suggesting increased biofilm growth with increased nutrient concentration in the feed. Due to the flow rate drop in HN CFDs, these filters were conserved after their cleaning in LN water from week 5 onwards and excluded from further MS2 challenge tests.

For LN and MN CFDs, Fig. 4 depicts the average MS2 LRV and the corresponding flow rates for operational periods from 5 to 21 weeks. This additional operational period did not result in a considerable further increase of MS2 LRVs. CFDs with MN biofilms had a consistently higher MS2 LRV as well as lower flow rate than those with LN biofilms.

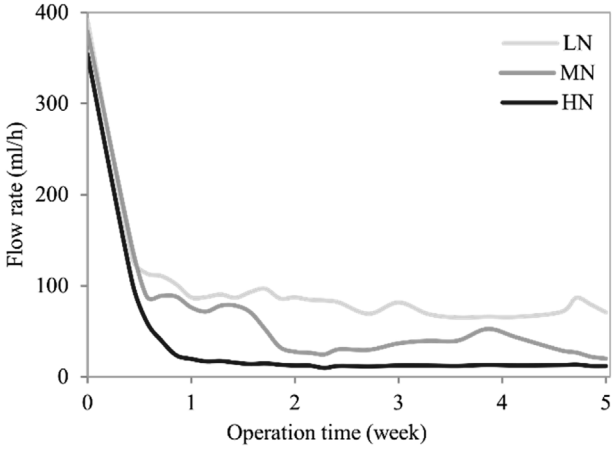

Fig. 3. Flow rate of CFDs during the initial 5 weeks *(Plotted values represent averages of duplicate CFDs)*.

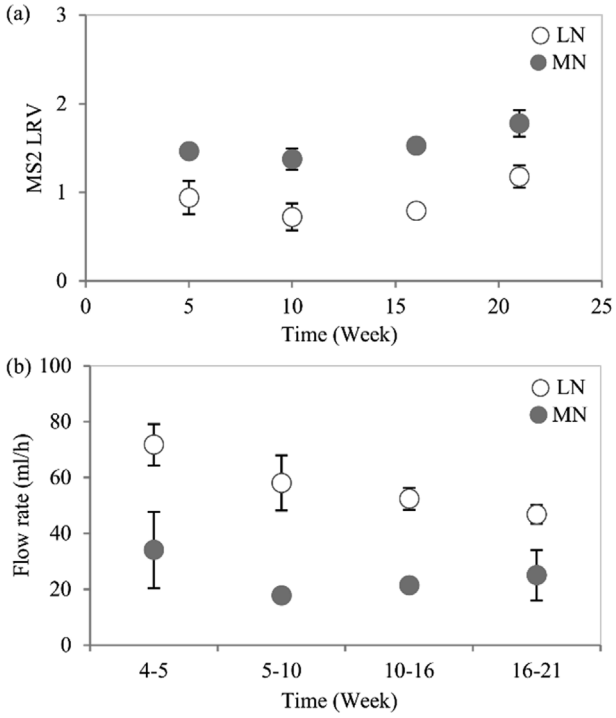

Fig. 4. Relation between CFD (n = 2) run time, (a) MS2 LRV and (b) flow rate. Error bars represent SD.

The TCC of the biofilms increased considerably between weeks 0 and 10 both for MN (> 2 logs) and LN (> 1.5 logs) biofilms (Fig. 5). Similarly, the ATP of the biofilms had a greater increase for MN (2.7 log) than for LN (0.97 log) biofilms (Fig. 5). Between weeks 10 and 21, the TCC and ATP of LN biofilms increased by 0.6 and 0.4 logs, respectively. Meanwhile, the TCC of MN biofilms decreased by 0.3 logs while its ATP increased by 0.2 logs. The increase in ATP, despite reduction in TCC, could be due to the release of ATP from damaged cells or a higher ATP per intact cell, possibly resulting from larger intact cells. So, while LN biofilms slowly continued their growth beyond 10 weeks, MN biofilms stabilized and had slightly lower cell counts. This is consistent with the changes in flow rate values shown in Fig. 4b. Flow rates continued decreasing for LN CFDs beyond week 10 but were relatively stable for MN CFDs.

By combining the results of the HN-5 week biofilm with the previous test as well as the results of the tests after cleaning (see next paragraph), a more inclusive image about continuous loading without cleaning and nutrient concentration of the feed can be projected. ATP measurements provide insight into biofilm biological activity (G. Liu et al., 2013). Measured HN-ATP after 5 weeks was 1 and 3 logs higher than MN and LN ATP, respectively, at 21 weeks (Fig. 5). Also, the ratio between ICC and TCC was higher in 5-week-old HN biofilms (56%) compared to (42% and 8%) for MN and LN in 21-old biofilms (Fig. 5). Even though the TCC of LN biofilms (21 weeks) was comparable to that of MN biofilms (21 weeks) and HN biofilms (5 weeks), ATP levels, ICCs and MS2 LRVs (Figs. 2 and 4) were higher in HN than MN and LN biofilms, accordingly.

These findings show that the initial nutrient concentration of the feed is the key factor in biofilm growth rate, thickness and associated MS2 LRVs rather than the length of the growth period (Donlan, 2002; Pedersen, 1990). Higher nutrient feeds led to the growth of thicker biofilms on CFDs, and therefore probably a greater amount of extracellular polymeric substances (EPS) (Wäsche et al., 2002). The magnitude and properties of produced EPS is tied to assembling microbial community which varies in relationship to growth nutrient concentration (Belkin and Colwell, 2006; Hall-Stoodley et al., 2004). Increased

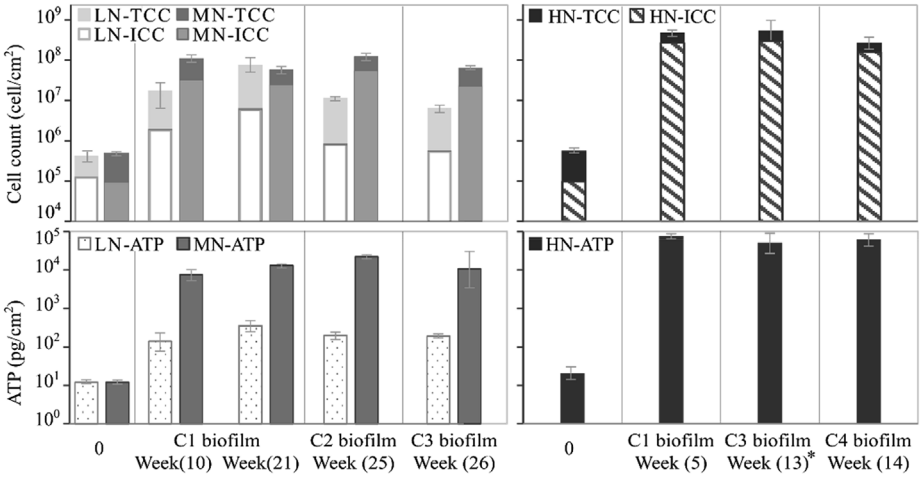

Fig. 5. Biofilm analyses for LN, MN (left) and HN biofilms (right) excluding CFDs cleaned surface sampling at cleaning events (Cn). *C(n) biofilm refers to the surface biofilm grown on the CFDs before the cleaning events (Cn). The age of Cn biofilm is the difference between the experimental weeks stated in X-axis as the cleaning event Cn is considered a reset point (i.e. the text below addressing LN or MN C2 biofilm week 25 and C3 biofilm week 26 are referred to as 4 weeks and 1 week biofilm). *age of HN biofilm at C3 is 4 weeks due to the 4 weeks LN gap after C1.* Error bars represent SD.

EPS facilitates virus uptake due to its sorption capacity, surface charge and hydrophobicity, which are key factors for virus removal (Armanious et al., 2015; Branda et al., 2005; Flemming, 2008; Skraber et al., 2005; Späth et al., 1998; Storey and Ashbolt, 2003, 2001). In addition, biofilms grown under high nutrient feed have a thicker boundary layer and therefore less mass transfer and stronger resistance, leading to lower flow rates (Huisman and Wood, 1974; Wäsche et al., 2002).

3.2. Effect of cleaning the surface

LN and MN CFDs were cleaned for the first time after 21 weeks (C1) and subsequently in weeks 25 (C2) and 26 (C3). MS2 LRVs after cleaning were < 0.5 for C2 and C3 but 0.8 and 1.2 for LN and MN C1, respectively (see Fig. 6). Biofilm age before C1 was the longest (21 weeks), compared to 4 weeks before C2 and 1 week before C3. Thus, biofilm age potentially resulted in greater compactness due the dynamic processes of attachment and detachment over time (Wäsche et al., 2002), which became harder to eradicate only by scrubbing. Biofilm analyses at C1- cleaned surface confirms the presence of biofilm residuals, as TCC and ATP measured: 5.7×10^6 cell/cm^2 (SD 2×10^6), 38 ± 7 pg/cm^2 for LN-CFDs and 2.8×10^6 cell/cm^2 (SD 5×10^5), 145 ± 7 pg/cm^2 for MN-CFDs. This confirms that the biofilm layer, which is partially removed during a scrubbing event, plays a vital role in the removal of MS2 in CFDs.

Biofilm regrowth and recovery after 4 weeks of reloading was faster for MN CFDs than for LN CFDs. Measured TCC and ATP (Fig. 5- week 25) for MN biofilms at 4 weeks were slightly higher than for the 21 week-old biofilm but lower for LN biofilms. Biofilm regrowth after 1 week reached the same TCC and ATP as the biofilm after 4 weeks for LN CFDs but was lower for MN CFDs (Fig. 5-week 26). MS2 LRVs followed similar pattern to that observed for biofilm regrowth. LN MS2 LRVs were lower for 4-week-old biofilm than 21-week-old biofilm, but higher than 1-week-old biofilm (Fig. 6). For MN, average MS2 LRVs were similar for 21-week- and 4-week-old biofilms but slightly lower for 1-week-old biofilm (Fig. 6). The fast recovery and regrowth of biofilm is probably due to biofilm bacteria retained after C1, which enabled fast recolonisation of the CFD surface with biofilm bacteria (Flemming and Wingender, 2010; Stiefel et al., 2016). It is probable that reloading with feed water provided essential nutrients to deep biofilm layers, creating new channels and pores in the structure and hence accelerating bacterial growth (Flemming and Wingender, 2010; Stiefel et al., 2016).

Early scrubbing of HN CFDs after 5 weeks (C1) reduced the average MS2 LRV by 1.5 logs. Cleaning HN CFDs after 4 weeks of operation with LN (C2) resulted in the same MS2 LRVs as C1 (< 0.3 logs: Fig. 6). Reduction in biofilm MS2 LRVs by cleaning was the highest at C3 (2.4 logs), despite its high absolute value of 1.6 logs (Fig. 6). C4 also reduced average HN MS2 LRVs by 0.8 logs yet retained an average MS2 LRV of 1.9 ± 0.4. MS2 LRVs of C4 is similar to values obtained from HN 5-week-old biofilm, despite the drastic difference in their flow rates

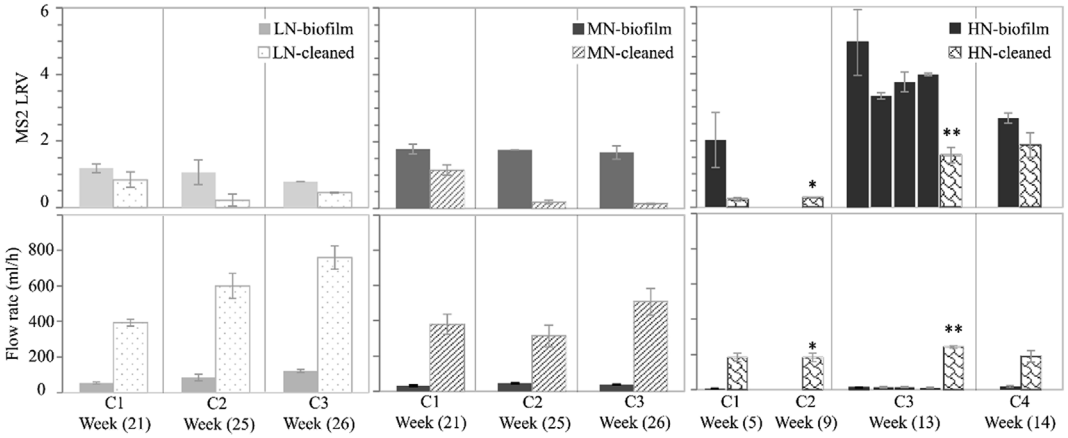

Fig. 6. Effect of scraping the surface on MS2 LRV (top row) and flow rates (bottom row). Left column is LN CFDs, middle column is MN CFDs and right column is HN CFDs. *Cn is the number of the cleaning event. Each cleaning event follows a challenge test with a grown biofilm before it, except for HN CFDs between C1 and C2. The age of Cn biofilm is the difference between the experimental weeks stated in X-axis as the cleaning event Cn is considered a reset point (i.e. the text below addressing LN or MN C2 biofilm week 25 and C3 biofilm week 26 are referred to as 4 weeks and 1 week biofilm). *HN CFDs were fed with LN water for 4 weeks before C2. **before C3 biofilm was grown for 4 weeks and challenged weekly.* Error bars represent SD.

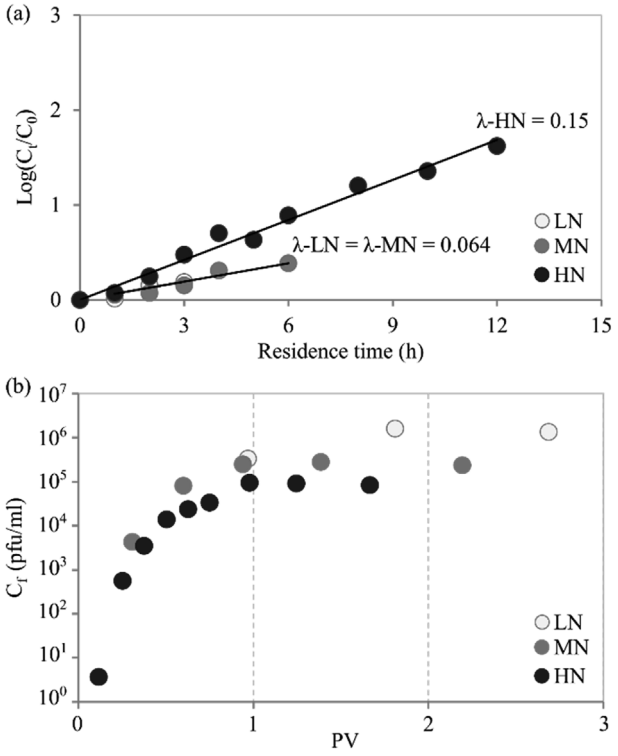

Fig. 7. Stage 1 MS2 concentrations in supernatant and filtrate *((a) MS2 LRV in challenge water residing on top of LN, MN, HN biofilms (b) MS2 filtrate concentration (C_f) after passing through CFDs with LN, MN and HN biofilms).*

(Fig. 6) and residence time. TCC at C4 was 2.1 × 10^7 cell/cm^2 (SD: 9 × 10^6), and ATP was 1550 ± 354 pg/cm^2, which is 1.3 and 2 logs lower than that of the 5-week-old HN biofilm shown in Fig. 5. According to Nickels et al. (1981), manual brushing of a fouled surface eliminates the majority of the biomass, but a community remains that excretes more EPS by 2–3 fold compared to the preceding community. Thus, it is possible that shift in community by selection due to repetitive cleaning led to higher MS2 LRVs, even with reduced biomass and high flow rates.

Interestingly, reloading HN CFDs after C2 with HN feed water for 1 week increased the LRV significantly, reaching 5 ± 1. The unprecedented high removal after C2 persisted for a month throughout 4 challenge tests (Week 9 to 13; Fig. 6), achieving an average MS2 LRV of 4. TCC and ATP measurements at the end of this period (4 weeks) were similar to other HN biofilm measurements (Fig. 5-week 13). In fact, ATP was slightly lower at the 4 weeks biofilm (post LN) than 5- or 1-week-old biofilm. Thus, the current biofilm analyses provide no explanation for the high MS2 LRV. It is possible that the period using LN feed introduced an alteration in the base community retained after cleaning (C1) that persisted throughout the month and allowed for rapid growth of specific biofilm bacteria on the filter surface. However, repeating the procedure by cleaning (C3) the CFD and reloading with HN for 1 week increased LRVs to 2.7 (Fig. 6, Week 14), below the previously achieved 4 LRV.

A potential observation of interest was that the dissolved oxygen measurements of supernatants during the initial operation of LN, MN and HN CFDs were 8.15 ± 0.05, 4.25 ± 0.25 and 0.4 ± 0.18 mgO_2/l respectively, showing evidence of anoxic conditions in HN CFDs. Switching to LN feed water may have diversified the biofilm base community or induced bacteria to shrink and adopt a spore-like state, awaiting conditions that were suitable for active growth (Hall-Stoodley et al., 2004). Yet, no measurements were conducted to verify this hypothesis.

HN cleaning events (C1 to C4; Fig. 6) did not restore the flow rate to initial values. It is possible that build-up of irreversible (deeper, internal) clogging by biofilm did not allow for the same increase of flow rate after repeated scrubbing events observed in MN and LN CFDs. Repeated cleaning events increased flow rates for LN and MN CFDs, yet independent of achieved MS2 LRVs (Fig. 6). The correlation between flow rates and MS2 LRVs of cleaned CFDs was weak and insignificant (r^2 = 0.2 and p = 0.08). Correlation was also weak but significant for biofilm amended CFDs (r^2 = 0.4, p = 0.0009). Thus, reduced flow rates do not necessarily indicate higher MS2 LRVs. On the other hand, flow rates were clearly correlated to biofilm TCCs (r^2 = 0.7, p = 5 × 10^{-8}). TCC and ATP had only moderate, yet significant correlation with MS2 LRVs (r^2 = 0.5, p = 6 × 10^{-5} and r^2 = 0.5, p = 0.0001, respectively). This emphasises the effect of biofilm growth on changing flow rates and MS2 LRVs.

Flow rates reduction in CPFs has been linked to pore fouling with particulate matter, organic build-up and inorganic precipitation (i.e.$CaCO_3$ or insoluble iron) (Brown and Sobsey, 2006b; Farrow et al., 2014; van Halem et al., 2009b). MS2 LRVs have been found to increase when flow rate decreased (Farrow et al., 2014; van Halem et al., 2017), although statistical significance and biofilm role were not factored in the assessment. In slow sand filtration, were biofilm growth is an essential element for the treatment process, Jenkins et al. (2011) observed that longer contact time (lower filtration rates) allowed for a higher attachment of virus to sand, hence higher LRVs. Schijven et al. (2013) proved experimentally and through a mathematical model that microorganism removal is best determined by biofilm (Schmutzdecke) age and temperature, rather than flow rates. This aligns with our observation that virus LRVs are not affected by flow rate but by biofilm growth, which in term increases residence time and reduces flow rate.

However, the flow rate plays an important role in terms of practicality and application. Slow performance of the filters can lead users to abandon using the filter (Casanova et al., 2012). The manufacturer recommends flow rates range within 1–3 L/h (Rayner et al., 2013a; Salvinelli and Elmore, 2015). If the conversion between the flow rates of CPF full unit and CFDs flow rates is used, this is equivalent to 24–72 ml/h in the CFD, since 36 ml/h in the CFD corresponds to approximate 1.5 l/h (Oyanedel-Craver and Smith, 2008). Flow rates observed throughout HN- biofilm CFD are below the lower limit (24 ml/h) which hampers the applicability of the CFDs. CFDs with LN biofilm had flow rates > 85 ml/h, while CFD with MN biofilm approximately yielded 40 ml/h, except for 21 weeks old biofilm which had 55 and 31 ml/h, respectively (Fig. 6). Thus, there's potential for application of CFP's with LN and MN water feed with sufficient flow, while benefiting from improved virus LRVs. This is not the case for HN filters, unless cleaned.

3.3. The fate of phages in the system

Depending on the CFD flow rates, challenge water resided on top of the CFDs for 3–12 h. Fig. 7a shows that the decrease in MS2 concentration in the feed water of LN, MN and HN CFDs were λ: 0.064, 0.064 and 0.15 log/h respectively. The faster inactivation rate for HN biofilms can be due to the additional strong attachment of MS2 to the HN CFD surface biofilm (Schijven and Hassanizadeh, 2000). Since water used in all challenge tests is LN water spiked with MS2 phages, the difference between inactivation rates is only attributed to the behaviour of the surface biofilms grown under different nutrient conditions.

The concentration of phages in the filtrate increased over time/pore volume (Fig. 7b). After 1 pore volume (PV), the MS2 concentration in MN and HN filtrate did not increase any further, while in the LN filtrate there was still some increase following 1 PV. This confirmed our approach to start filtrate sampling after the first PV to determine the LRV in challenge tests. Theoretically, the first PV of filtrate should be free of phages since the pores are filled with the previous MS2-free nutrient feed water. However, in reality, CFDs do not have an ideal plug flow.

Table 1
Mass balance of MS2 phages.

	t_1	t_2	N_i		N_f		N_b		N_d		Unrecovered
Unit	(h)	(days)	pfu	%	pfu	%	pfu	%	pfu	%	(%)
LN	3	1	2.7×10^9	100	3.6×10^8	12.4	2.2×10^1	< 0.1	4.6×10^7	0.5	87.1
MN	6	8	2.0×10^9		6.4×10^7	3.3	2.2×10^6	0.1	2.1×10^6	0.1	96.5
HN	12	22	6.7×10^8		1.2×10^7	1.8	4.1×10^6	0.6	1.6×10^6	0.2	97.4

The phages measured in the filtrate through the first PV confirm the existence of preferential flow paths in the CFDs.

Since the duration and MS2 inactivation rate of Stage 1 varied per nutrient type (see t_1:Table 1), the amount of phages entering the CFDs (N_i) varied as well. N_i is calculated as average = ($\sum_0^{t1} C_t$ * average V_{sup}) to exclude the effect of residence time, temperature and other processes taking place in the supernatant.

Table 1 shows that most of the phages in the feed (N_i) were removed by the biofilm/filter, with only 2–12% passing through the biofilm and filter (N_f) during the challenge phase. After the MS2 challenge was stopped, MS2 phages did survive in the biofilm (N_b); some detached and passed the filter into the filtrate (N_d) for up to 22 d in HN CFDs. Σ N_f + N_d + N_b is the total number of recovered phages, which varied between 2.6 and 12.9%. The highest unrecovered values were in HN-CFDs (97.4%) followed by MN (96.5%) and LN (87.1%) CFDs. It is possible that unrecovered phages were inactivated inside the biofilm; however, it is not possible to quantify the inactivation rate or temperature effect on MS2 inside the biofilm. Also, potential low recovery efficiency of the swabbing method (Ismaïl et al., 2013) may have influenced the recovery rate of MS2 phages.

4. Conclusion

The WHO standard for HWTS technologies recommends ≥3 LRV for protective technologies and ≥5 LRV for the highest protection from viruses (WHO, 2011). Under stable operational conditions, the CFDs achieved MS2 LRVs ranging between 0.9 ± 0.2 for LN, 1.6 ± 0.2 LRV for MN and 2.4 ± 0.5 LRV for HN biofilms. Cleaning the surface by scrubbing led to partial or total loss in achieved LRVs, indicating the importance of this biological top layer to MS2 LRVs. Overall, repeated scrubbing resulted in a faster recovery of biofilms and associated log reductions. Recovery of MS2 from biofilm swabs confirmed the role of CFD biofilms in virus removal, although the MS2 LRV did not reach the WHO protective standard of 3 LRV.

Biofilm formation on CPFs is inevitable and, as demonstrated, it positively contributes to virus safety of drinking water for CPF users. Further research is required to understand the mechanisms of interaction between virus and biofilms, supported by the unprecedented high LRV > 4 LRV achieved after changing feed conditions. Besides, future studies should factor in biofilm growth in evaluating virus removal capacity of CPFs and also in recommending cleaning practices for CPF users.

Declaration of interests

The authors declare no conflict of interest.

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