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Treatment of biofilm formation in irrigation lines in zero liquid discharge cultivation systems

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Abstract

Biofilm formation in irrigation systems can be problematic, as it can cause clogging of drippers or sprinklers and therefore unequal supply of nutrient solution to the crop. In addition to that, a biofilm can be a nesting place for pathogenic microorganisms which diminishes the effect of central irrigation water disinfection. Several commercial products are available to prevent biofilm formation or to remove an existing biofilm, but not all are suited for use in hydroponic cultivation with reuse of drain water, especially in cases in which no drain water can be discharged (goal for Dutch greenhouse horticulture for 2027). An installation and a protocol were developed to test the effectiveness of technologies and strategies suitable for use in zero liquid discharge cultivation systems for prevention of biofilm formation and removal of existing biofilms. The installation consists of three parallel systems and mimics conditions in a tomato greenhouse for temperature, nutrient solution composition, irrigation schedule and system length. Biofilm formation is measured in a biofilm monitor, from which rings of irrigation lines could be sampled and analysed for presence of biomass from living cells. Two chemical treatments (Oxyl-PRO S silver stabilised hydrogen peroxide and in situ produced SureFlow chlorine dioxide) and three physical treatments (electro-magnetic treatment, ultrasound treatment and antibacterial irrigation lines) were tested. The chemical solutions were able to remove existing biofilms and to prevent new biofilm formation, whereas the physical treatments were less effective or did not show any effect at all.

Keywords: greenhouse horticulture, hydrogen peroxide, chlorine dioxide, electromagnetic treatment, ultrasound, antibacterial material

INTRODUCTION

In the drinking water industry, the prevention of biofilm formation is of major importance, as pathogenic microorganisms can survive, grow and disperse from the biofilm (van der Kooij et al., 2003). Planktonic microorganisms can attach to a surface (like the insides of a pipeline) under the right circumstances, forming a slimy layer of extracellular polymeric substances protecting the cells against harsh circumstances. In every drinking water distribution system, measures are taken to prevent the formation of biofilms, e.g., by distributing biologically stable drinking water and by using materials with a low potential for biofilm formation (van der Kooij et al., 2003). These measures need to ensure the chemical and microbial quality of the tap water, without compromising the chemical and microbial safety at the end-users. Formation of biofilm will always occur on wet surfaces, especially when plenty of nutrients, a high temperature and low water velocities are present.

In irrigation lines in horticulture, other problems related to biofilm formation may occur: drippers and sprinklers may be clogged by flocks of biofilm detached from the surface, causing heterogeneity in supply of nutrient solution to plants along an irrigation line (Yan et al., 2009). Such flocks occur naturally due to growth of mature biofilms, but can also occur due to (rapid) removal of biofilm. Removal of biofilm is typically done either by mechanical cleaning (high flow rate, rapid air bubbles, ultrasonic vibrations), physical treatment of the

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water (increasing solubility) or chemical cleaning (cleaning agents). Dutch greenhouse horticulture is working towards a zero liquid discharge cultivation by 2027 (van Os et al., 2019). These cultivation systems have another complication for biofilm treatment: all products added to the irrigation system need to be taken up by the crop, naturally degraded to safe components or actively removed to avoid accumulation. This is an important restriction, especially in situations where irrigation lines used to be cleaned chemically.

In this article the results are presented of a study on methods for removal of an existing biofilm, or for prevention of biofilm formation in new irrigation lines. Methods were selected based on their potential effect on biofilm formation and their suitability to be used in zero liquid discharge greenhouse cultivation systems.

MATERIALS AND METHODS

Experimental set-up

1. Test system.

A test system was designed and built (Figure 1) representative for a greenhouse drip irrigation system, in which the effect of treatments on biofilm formation could be measured in a controlled environment. A system pump sent water from a buffer tank (500 L, 60% filled) into the distribution system at 450 L h^{-1}, matching the water velocity in commercial greenhouse systems. The distribution system consisted of an implementation point for technologies, a pulse generating flow meter, 50 m of rolled irrigation lines including drippers, an analogue flow meter and a biofilm monitor, before returning to the buffer tank. The biofilm monitor has been developed to determine the biofilm formation potential of drinking water (van der Kooij et al., 1997). It consists of a vertical glass column filled with cylinders of test material (rings) on top of each other. The water flow is equally split over two columns (220 L h^{-1} each) in the biofilm monitor to enable duplicate biofilm measurement. The installation consisted of three equal parallel systems and was placed in a climate chamber at 24°C, without daylight.

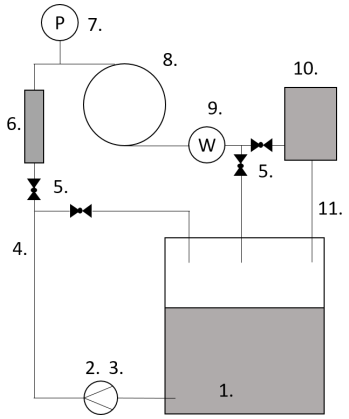

Figure 1. Overview of the test system: 1) buffer tank (500 L); 2) self-priming pump; 3) time/pulse relais; 4) pipe work EPDM PVC 32 mm; 5) Y-valve hand controlled EPDM PVC-U; 6) insertion point for technologies; 7) manometer; 8) irrigation pipeline 50 m, 16 mm LDPE white/black, dripper Kameleon High 2 L h^{-1}; 9) analogue water meter; 10) biofilm monitor; 11) Tricoflex soft & flex 12.5 mm inner diameter.

2. Test water composition.

The buffer tank was filled with 300 L of tomato nutrient solution (Table 1). Source water was a UV-disinfected mix of rain water and reverse osmosis treated well water (ratio depending on the availability of rain water). Nutrients were added from a 100× concentrated

stock solution (separated in two to avoid precipitation). This recipe resulted in an EC of 1.9 mS cm^{-1}, pH 5.5 was achieved by adding YaraTera Substrafeed Baskal (a mixture of potassium carbonate and potassium hydroxide) for a pH rise, or nitric acid for a pH reduction.

Table 1. Concentration of nutrients and trace elements in the nutrient solution, standard nutrient solution for tomato cultivation (De Kreij et al., 1999).

NH_4	K	Ca	Mg	NO_3	SO_4	H_2PO_4	Fe	Mn	Zn	B	Cu	Mo
Concentration (mmol L^{-1})							Concentration (µmol L^{-1})					
1.0	6.5	2.75	1.0	10.75	1.5	1.25	15	10	4	20	0.75	0.5

A bacterial inoculum (2 L) was collected from a drain collection point at Wageningen University & Research in Bleiswijk, homogenised, split in doses of 20 mL and stored at 4°C under dark conditions. To each test system one dose of bacterial inoculum was added together with 10 µg C L^{-1} sodium acetate (CAS nr. 127-09-3, 1 g in 5000 mL MilliQ water, sterilised for 15 min at 121±3°C, stored at 5±3°C for at most 6 months) as carbon source.

3. Protocol.

Treatments for prevention or removal of biofilm formation were installed by the technology supplier. The three parallel test systems were cleaned according to a cleaning protocol (see section 4) and the tanks were filled with nutrient solution. The columns of the biofilm monitor were filled with 12-14 disinfected rings made from LDPE irrigation pipe (17±2 mm) alternating with disinfected glass rings, to prevent flotation of the LDPE rings. The rings were placed in the biofilm monitor using sterile gloves and a stainless steel wire with a hook (disinfected with a Bunsen burner). The water was pumped through the test systems following a spring/summer irrigation schedule for tomato, in 12-min cycles (2 min recirculation, 10 min stagnancy) during daytime, i.e., between 8:00 and 17:00, automated with a timer. During night time the water remained stagnant. The flow rate through the system was 450 and 225 L h^{-1} through each of the columns of the biofilm monitor.

Each test run consisted of comparing a reference system with two different treatments. Furthermore, two different types of test runs were performed: prevention of biofilm formation and removal of an existing biofilm (curative). In the preventive test runs the treatments started directly at the start of the test run (day 0) and ran until day 8. In the curative test runs, the three parallel systems were treated as reference systems for the first 6 days to allow a biofilm to form, before the two treatments started at day 6 and ran until day 10 (end). All treatments were tested in duplicates (in different parallel systems to avoid systematic errors in test results (Table 2), in both preventive and curative test runs).

Table 2. Overview of test runs and treatments.

Test run	Test type	System 1	System 2	System 3
1	Curative	Elec. magn. reson. fields	Oxyl-PRO S (H_2O_2)	Control
2	Curative	Control	Ultrasonic waves	Elec. magn. reson. fields
3	Curative	Oxyl-PRO S (H_2O_2)	Control	Ultrasonic waves
4	Preventive	Control	Oxyl-PRO S (H_2O_2)	Antibact. irr. lines[a]
5	Preventive	Ultrasonic waves	Control	Oxyl-PRO S (H_2O_2)
6	Preventive	Control	Elec. magn. reson. fields	Antibact. irr. lines
7	Preventive	Elec. magn. reson. fields	ClO_2	Control
8	Preventive	Control	Ultrasonic waves	ClO_2
9	Curative	ClO_2	Control	Control[b]
10	Curative	Antibact. irr. lines[c]	ClO_2	Control

[a]Material without antibacterial properties was used.
[b]An additional control treatment was applied with 80 µg C L^{-1} of sodium acetate.
[c]An extended preventive test run of 30 days was performed for the antibacterial irrigation lines.

4. Cleaning protocol.

Before the start of each test run, the test system was cleaned and disinfected. In short, the buffer tank was filled with 300 L clean water and 3 L of unstabilised hydrogen peroxide (50% concentrate, no additives, Cindro), resulting in a 5 g L^{-1} H_2O_2 solution. The water was circulated through the systems according to the regular irrigation schedule. After 24 h the hydrogen peroxide solution was pumped from the system and flushed with clean water until no residual H_2O_2 could be measured. The glass rings from the biofilm monitor were disinfected with a similarly prepared H_2O_2 solution for 4-6 h. These rings and the new LDPE rings were rinsed with flowing tap water for 1 h, to stand in tap water for 24 h and finally flushed with flowing tap water for 1 h.

Analyses

Water was sampled from stainless steel taps at the bottom of the glass pipes of the biofilm monitor and were sterilised with a Bunsen burner and cooled down with water from the system, before taking a sample of 250 mL in a sterile sampling bottle. To collect biofilm from the monitor, the system pump was switched off. After that, 10 mL of sterilised tap water (tap water with 1 mL of NTA (nitrilotriacetic acid) solution (50 g L^{-1}) sterilised for 15 min at 121±3°C) was added to a test tube, the water level in the biofilm monitor was lowered and the top LDPE ring was collected with a sterilised (Bunsen burner) stainless steel wire and placed in the test tube. The test tubes with the LDPE rings were filled with 10 mL sterilised tap water and placed in an ultrasound water bath (Eumax, 40 kHz, 50 W) for 2 min, to mechanically remove the biofilm from the rings. The water from the test tubes was poured into 50 mL sterile greiner CELLSTAR centrifuge tubes, and again 10 mL of fresh sterilised tap water was added to the test tubes with the rings. This procedure was repeated until 40 mL of sterilised tap water with removed biofilm was collected in the centrifuge tube. The height of the LDPE ring was measured to calculate the amount of ATP cm^{-2} of irrigation line.

With a Luminultra QGA test kit (Quench-Gone Aqueous, ATP detection limit: 0.1 pg mL^{-1}) 20 mL of the collected solution was analysed for the presence of ATP from living cells from the biofilm, according to manufacturer's instructions. In short: the sample water was filtered (Quench Gone syringe filters; DIS-SFQG-25) to collect complete cells on the filter and to remove dissolved ATP from broken cells. Subsequently 1 mL of LuminUltra UltraLyse was forced through the filter membrane to breakdown the cell walls of the retained cells. The filtrate was collected and mixed with 9 mL LuminUltra UltraLute buffer. Finally, 100 µL of this solution was added to 100 µL LuminUltra Luminase enzyme and luminescence was measured with the LuminUltra PhotonMaster. This yielded a certain amount of ATP (pg cm^{-2} of irrigation line). ATP from living cells in the nutrient solution was also analysed with the QGA test kit, with 50 mL of collected water, leading to an amount of ATP in pg mL^{-1}.

The concentration of hydrogen peroxide in the nutrient solution was measured using Quantofix peroxid 0-25 mg L^{-1} test strips. ClO_2 was measured using a Palintest ClO_2+ meter, total oxidants were measured in the treatment with ClO_2 by titration, summarising the concentrations of ClO_2, chlorite and chlorate (a disinfection by-product of chlorine dioxide).

Treatments

Silver stabilised hydrogen peroxide (Oxyl-PRO S, Cindro; 50%) was added to the nutrient solution to an initial concentration of 40 mg L^{-1} (20 mg L^{-1} H_2O_2) before the irrigation lines by a pulse pump; a pulse each 4 L of water. Concentration of H_2O_2 was frequently determined manually, aiming at a concentration at the biofilm monitor of 20 mg L^{-1}. Dosing was switched off when the desired concentration was reached and switched on again at lower concentrations. The aimed concentration of 20 mg L^{-1} H_2O_2 is applied by Cindro to slowly remove an existing biofilm, to prevent release of flocs in the irrigation lines.

Chlorine dioxide (SureFlow, CH_2O) was produced on site by combining hydrochloric acid and sodium chlorite. A dosing pump was connected before the irrigation lines to reach a concentration of 0.75 mg L^{-1} at the biofilm monitor, manually controlled. The pH of the nutrient solution was checked and corrected if necessary by adding Baskal (YaraTera Substrafeed Baskal).

Antibacterial irrigation lines (MVP-Starmaker) produced from LDPE with a zinc-additive replaced the regular irrigation lines in the test system. As the rings from this material did not fit the biofilm monitor, samples for biofilm analyses were taken by disinfection of the outside of the irrigation lines themselves, using 70% ethanol, and cutting a piece from the material. After that the ATP analyses in biofilm were performed as described earlier.

Electromagnetic resonance fields (AQUA4D, Planet Horizons Technologies; <20 mT) were dispersed in the water by a flow through reactor right after the system pump. The treatment unit was in hydraulic continuity with the water in the biofilm monitor and is continuously switched on. The test system was grounded and equipment was installed to remove stray currents.

Low frequency ultrasonic waves (SonoPure, PureBlue; 25 kHz) were applied to the water just before the irrigation lines, to propagate through the water to reach the monitor.

RESULTS AND DISCUSSION

To examine if a consistent biofilm could be produced in the three parallel systems and in consecutive test runs the initial biomass in all test runs at day 0 and biomass at day 6 in curative test runs was compared (Figure 2). Two-way ANOVA showed a significant test run effect ($P<0.001$), but no significant difference between systems. There was no significant interaction ($P=0.622$) between test run and system. After six days of development, differences between individual test runs became visible but this did not give significant test run or systems effects and no significant interaction between these factors either ($P<0.05$). Therefore, a fair comparison can be made between treatments and control in a test run. Differences between test runs can be explained by variations in source water or nutrient composition, or variations in the effectivity of the cleaning protocol.

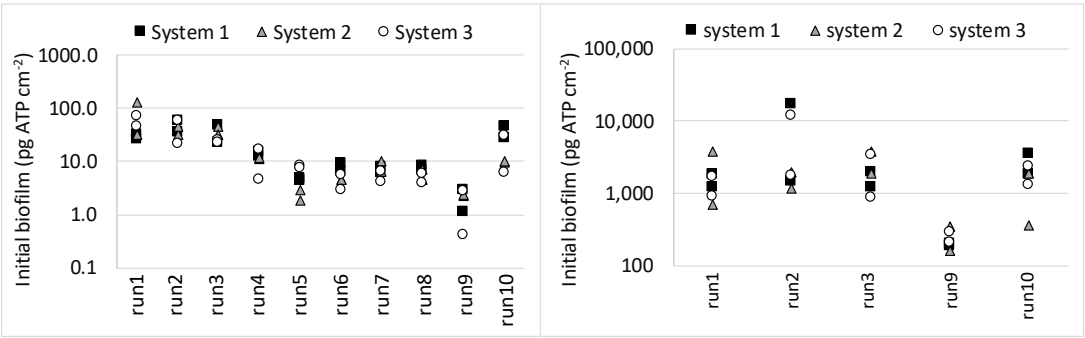

Figure 2. Initial biomass in biofilm at day 0 in all test runs (left) and at day 6 in curative test runs (right).

Maximum biomass concentrations were reached at day 6/7, after which a stabilisation or a small decrease was observed. To test the hypothesis that this was caused by a lack of carbon source, an additional test was performed on the effect of addition of extra sodium acetate (80 µg L^{-1}). The growth curve of the control system and the system with the additional carbon source treatment were not significantly different after 10 days. In this test, possible limitation by dissolved oxygen was not observed either. To check conformity of levels of biofilm biomass in the test systems and irrigation lines in commercial greenhouses, a triplicate measurement of biofilm biomass was done from an irrigation line in a real greenhouse with an untreated irrigation system of 1 year old. This system had an average biofilm biomass of 71 pg ATP cm^{-2}.

Figure 3 shows the biofilm biomass for the preventive (left) and curative (right) treatments for respectively hydrogen peroxide (A), chlorine dioxide (B), electromagnetic resonance fields (C), ultrasound (D) and antibacterial irrigation lines (E).

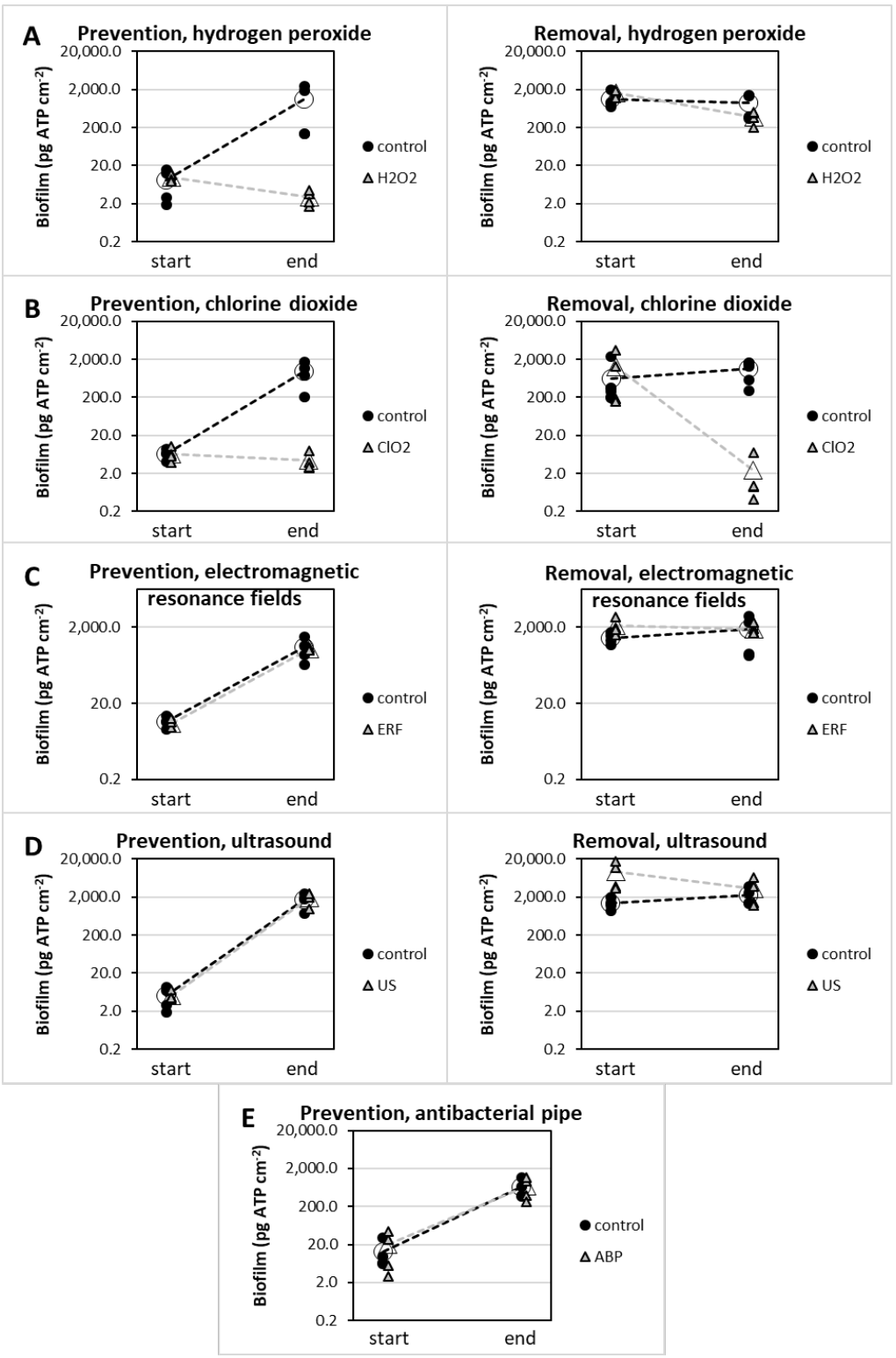

Figure 3. Concentration of biomass in biofilm during preventive (left) and curative (right) test runs in pg ATP cm^{-2} for treatment with silver stabilised hydrogen peroxide (A), chlorine dioxide (B), electromagnetic resonance fields (ERF, C), ultrasonic treatment (US, D) and antibacterial irrigation lines (E), compared to untreated control.

Treatment with 20 mg L^{-1} Oxyl-PRO S (Figure 3A) showed a significant preventive effect on biofilm formation ($P<0.001$). With the same dosage (Figure 4), no significant curative effect ($P=0.106$) was seen within the test period of five days. The measurements of hydrogen peroxide concentration of run 3 show that the concentration clearly drops during the night and increases during the day. A higher dosage or longer exposure time would be needed to demonstrate a curative effect on the biofilm biomass concentration, since in practice dosage would be continuous for the whole time of operation. The effect of the coated silver nanoparticles in this product in zero liquid discharge systems is yet unknown and was not part of this research.

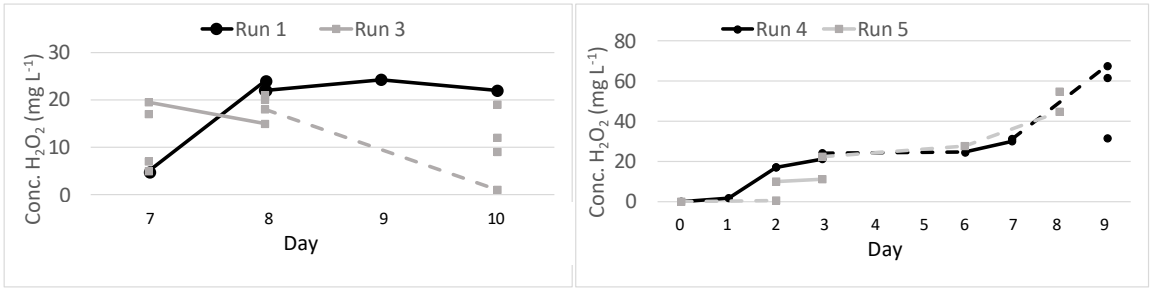

Figure 4. Measured concentration profile of H_2O_2 in preventive (left) and curative (right) test runs. Measurements at consecutive days are connected with a line, two measurements with a day (or two) in between are connected with a dotted line and measurements during one day are not connected.

Treatment with 0.5-0.7 mg L^{-1} chlorine dioxide significantly ($P<0.001$) prevented the formation of biofilm in clean irrigation lines (Figure 5B, left). The technology showed also capable to remove an existing biofilm (Figure 5B, right), as the concentration of biomass in the biofilm dropped significantly ($P<0.001$) compared to the control after treatment start-up, at a concentration of 0.7 mg L^{-1}. In crops like cucumber, zucchini and egg-plant, the reaction by-product chlorate could accumulate to maximum European limit values in the fruit, depending on the concentrations of ClO_2 in the water. In tomato, sweet pepper and floricultural products this is not an issue (W. Vijverberg (CH_2O), 2020, pers. commun.).

Application of electromagnetic resonance fields (ERF) did not show a significant ($P=0.86$) effect in the prevention of biofilm formation compared to the untreated control (Figure 3C, left), as treatment and control follow the same development. An existing biofilm was not significantly ($P=0.47$) removed by application of ERF (Figure 3C, right).

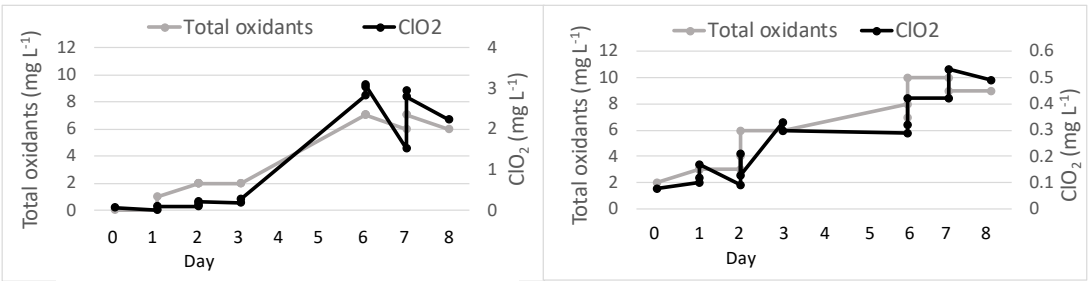

Figure 5. Measured concentration profiles of ClO_2 and total oxidants (ClO_2, chlorite and chlorate) of preventive test runs 7 (left) and 8 (right).

Biofilm formation in clean irrigation lines was not significantly ($P=0.66$) slowed down by application of ultrasound (US) waves compared to untreated control (Figure 3D, left). Also, in the curative tests no significant ($P=0.008$) effect of ultrasound was found (Figure 3D, right), although high intensity ultrasonic waves are known to be able to remove both sessile and

planktonic bacteria in water by cavitation (Broekman et al., 2010). We hypothesise that the long distance and line bends between the point of application and irrigation line were the reasons the technology did not work. Intensity and frequency should also be evaluated.

The applied antibacterial irrigation lines showed the same trend in biofilm formation as the control treatment, so no significant (P=0.97) preventive effect was seen (Figure 3E). In the second test run the test period was extended to 30 days, as it was hypothesised that the effect would appear after longer contact with the material. However, measurements did not show an effect of the longer contact time.

CONCLUSIONS

It was shown that the developed system and protocol were capable of producing a reproducible biofilm in consecutive test runs, without significant differences between the three parallel systems. This allowed a fair comparison between treatments and the untreated control in a test run. Of the tested technologies, chlorine dioxide (SureFlow, CH_2O) proved to be most effective in both preventive and curative treatment of biofilm formation at the tested concentration of <0.7 mg L^{-1}. Oxyl-PRO S silver stabilised hydrogen peroxide (Cindro) prevented the formation of a new biofilm in clean irrigation lines (20 mg H_2O_2 L^{-1}), but did not show a significant effect on existing biofilms at this concentration and timescale. Further research needs to show the effect of prolonged treatment or an increased concentration. Ultrasonic treatment (25 kHz, SonoPure, PureBlue), electromagnetic resonance fields (<20 mT, Aqua4D, Planet Horizons Technologies) and an antibacterial irrigation line with a zinc additive (MVP-Starmaker) did not show an effect on prevention of biofilm formation or removal of an existing biofilm.

ACKNOWLEDGEMENTS

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Literature cited

Broekman, S., Pohlmann, O., Beardwood, E.S., and de Meulenaer, E.C. (2010). Ultrasonic treatment for microbiological control of water systems. Ultrason Sonochem *17* (*6*), 1041–1048 https://doi.org/10.1016/j.ultsonch.2009.11.011. PubMed

De Kreij, C., Voogt, W., Van den Bos, A.L., and Baas, R. (1999). Bemestingsadviesbasis substraten. PPO nr. 169. https://edepot.wur.nl/218455.

van der Kooij, D., Vrouwenvelder, J.S. and Veenendaal, H.R. (1997). Bepaling en betekenis van biofilmvormende eigenschappen van drinkwater. H2O *30* (*27*), 767–771.

van der Kooij, D., Vrouwenvelder, J.S., and Veenendaal, H.R. (2003). Elucidation and control of biofilm formation processes in water treatment and distribution using the Unified Biofilm Approach. Water Sci Technol *47* (*5*), 83–90 https://doi.org/10.2166/wst.2003.0287. PubMed

van Os, E.A., Beerling, E.A.M., Blok, C., Janse, J., Leyh, R., Van Ruijven, J.P.M., Van der Staaij, M., and Kaarsemaker, R. (2019). Zero discharge of nutrients and pesticides to the environment in hydroponic production. Acta Hortic. *1266*, 443–450 https://doi.org/10.17660/ActaHortic.2019.1266.61.

Yan, D., Bai, Z., Rowan, M., Gu, L., Shumei, R., and Yang, P. (2009). Biofilm structure and its influence on clogging in drip irrigation emitters distributing reclaimed wastewater. J Environ Sci (China) *21* (*6*), 834–841 https://doi.org/10.1016/S1001-0742(08)62349-9. PubMed