Biofilm structure and its influence on clogging in drip irrigation emitters distributing reclaimed wastewater

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Abstract

Using reclaimed wastewater for crop irrigation is a practical alternative to discharge wastewater treatment plant effluents into surface waters. However, biofouling has been identified as a major contributor to emitter clogging in drip irrigation systems distributing reclaimed wastewater. Little is known about the biofilm structure and its influence on clogging in the drip emitter flow path. This study was first to investigate the microbial characteristics of mature biofilms present in the emitters and the effect of flow path structures on the biofilm microbial communities. The analysis of biofilm matrix structure using a scanning electron microscopy (SEM) revealed that particles in the matrix of the biofilm coupled extracellular polysaccharides (EPS) and formed sediment in the emitter flow path. Analysis of biofilm mass including protein, polysaccharide, and phospholipid fatty acids (PLFAs) showed that emitter flow path style influenced biofilm community structure and diversity. The correlations of biofilm biomass and discharge reduction after 360 h irrigation were computed and suggest that PLFAs provide the best correlation coefficient. Comparatively, the emitter with the unsymmetrical dentate structure and shorter flow path (Emitter C) had the best anti-clogging capability. By optimizing the dentate structure, the internal flow pattern within the flow path could be enhanced as an important method to control the biofilm within emitter flow path. This study established electron microscope techniques and biochemical microbial analysis methods that may provide a framework for future emitter biofilm studies.

Key words: emitter clogging; drip irrigation; biofilm; microbial community; phospholipid fatty acid; reclaimed wastewater; scanning electron microscopy

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Introduction

As one of the current most effective water-saving irrigation technologies, drip irrigation is getting more attention and playing an important role in modern agriculture. It has been used as a wastewater treatment and distribution alternative that offers solutions at where other soil treatment systems are inappropriate due to a seasonally high water table, a shallow dense soil layer, vegetative cover or space constraints, or other site limitations (Rowan, 2004). Emitter clogging has been identified as one of the most important concerns for drip irrigation systems. Clogging is closely related to the quality of the irrigated water and the structure of the emitter flow path, and occurs as a result of multiple physical, biological and chemical factors (Gilbert *et al.*, 1981; Pitts *et al.*, 1990; Adin and Sacks, 1991; Coelho and Resende, 2001).

Due to the growing utilization of drip irrigation for wastewater applications, emitter anti-clogging capability

is critical. The complex chemistry of wastewater supports the rapid growth of several species of algae and bacteria resulting in slime-like gelatinous deposits of amorphous shape. Particles of definable shape were found in the matrix of the gelatinous substance (biofilms) which formed sediment in the emitters, and caused serious emitter blockage (Adin and Sacks, 1991). More than 90% of the clogging composition included biological species and the clogging process was usually initiated by bacterial biofilms (Gilbert *et al.*, 1981; Taylor *et al.*, 1995). Although the clogging materials are mainly suspended particles, some of the biofilm bacterial species may also cause emitter clogging through the precipitation of iron, manganese and sulfur minerals dissolved in reclaimed wastewater (James, 1988; Pitts *et al.*, 1990; Şahin *et al.*, 2005).

Microbial cell that grow in association with surfaces often form biofilms, microorganisms immobilized and embedded within an organic polymer matrix (Liu *et al.*, 1994; Kristina *et al.*, 2007; Strathmann *et al.*, 2002). The biological species in the biofilm excrete a protective mass

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of extracellular polysaccharides (EPS), a wide group of polymers that can be highly attached to the cell surface or released as extracellular slime in the surroundings of the cell (Knoshaug *et al.*, 2000). The EPS entraps suspended particles, helps build the three-dimensional matrix structure and poses a potential for clogging the emitter flow path. The hydraulic properties of varying emitter flow path boundaries might also influence the biofilm dimensional structure and microbial accumulations. Hydrodynamic stress influenced the biofilm phenotypes and the flow regime significantly influenced the biofilms architecture (Pereira *et al.*, 2002). The complex EPS structures provide the microbial cells with an environment of stability and resistance to disinfection procedures, making them difficult to eliminate once established (Sutherland, 2001; Pitts *et al.*, 1990).

Several groups of investigators suggested examining the diversity of these biofilms to identify bacteria involved in emitter clogging, but valuable correlative literature is very rare. The purposes of the present study were: (1) to analyze microbial community and biofilm structure within the emitter flow path, (2) to measure the biofilm biomass in emitters with different structural dimensions. This study provides a better understanding of biofouling and emitter clogging to promote the development of affective methods for clogging prevention.

1 Materials and methods

1.1 Experimental design

The tests were conducted from August to December 2007 in experimental hall of China Agricultural University, Beijing. Six 10-m lines of irrigation tubing, each differing in the nominal discharges and flow path structures were used (Fig. 1). The emitter models tested were selected based on regional availability and are representative of the current technology. Table 1 summarizes the characteristics of each emitter. The experimental setup consisted of 30 emitters of each type placed inline 0.3 m apart. Other components included an 80-mesh weave wire nylon screen filter, a 120-mesh disk filter, and two pressure regulator valves (Fig. 2).

Reclaimed wastewater was provided by the Gaobeidian Wastewater Treatment Factory in Beijing, China. The physical, chemical, and microbiological parameters of the reclaimed wastewater characteristics are shown in Table 2. Reclaimed wastewater was delivered to the drip tubing from a dosing tank (50 m³).

Fig. 1 Flow path structures of emitters.

Fig. 2 Experimental layout of drip irrigation system with reclaimed wastewater application. (1) dosing tank; (2) 80-mesh screen filter; (3) pump; (4) pressure regulator valve; (5) 120-mesh disk filter; (6) pressure gauge; (7) laterals with emitters.

1.2 Emitter performance

Because of the close placement of the emitters, friction losses along the dripline were negligible and the pressure along the lateral was considered essentially constant. Therefore, the discharge variations are attributed only to the degree of clogging. To evaluate emitter performance discharge measurements were taken at the beginning of experiment and then taken periodically throughout 360 h

Table 1 Characteristic of the dripper emitter

Serial number	Φ (mm)	W (mm)	D (mm)	L (mm)	a (mm)	h (mm)	Place	Flow path structure
A	16	1.13	0.80	320	1.21	1.02	Spain	II
B	16	0.80	0.81	102	2.00	0.81	China	IV
C	16	0.98	1.09	240	1.47	1.16	Israel	I
D	16	1.46	0.95	300	1.23	3.08	China	III
E	16	1.10	1.03	350	1.24	1.20	Korea	V
F	16	1.13	0.89	360	1.60	1.71	China	II

Φ : pipe diameter; W : width of flow path; D : depth of flow path; L : length of flow path; a : dentation space; h : dentation height.

of irrigation (2 d operation at 6 h/d and 1 d off). Samples of individual emitter discharge rate measurements were used to evaluate the different lines in the system. Emitter discharge rates were determined by measuring individual flows from 16 randomly selected emitters over a period of 10 min and computing the flow rate ($\bar{q}$) and distribution uniformity (DU) using a 100-mL graduated cylinder. This procedure was repeated every 10 d during the experiment.

1.3 Biofilm structure

Biofilms developed on emitter internal polyethylene structures and were collected from the emitter flow path after 360 h of intermittent irrigation. Random samples were collected from each type of emitter. In the laboratory biofilms were detached from emitter flow paths by cutting open the samples, placing them in 150 mL of sterile water, adding 5 g of sterile glass beads and shaking for 2 h. The resulting solution was poured into a sterile graduated glass cylinder. Minna *et al.* (2004) demonstrated that this technique removes 98% of biofilm cells, as determined by the plate count technique.

Emitter flow paths covered with biofilms were observed using environmental scanning electron microscope (ESEM, Quanta200, FEI, USA). Prior to ESEM observations, biofilm samples were fixed with glutaraldehyde (2.5% in 50 mmol/L sodium cacodilate). Upon fixation, samples were incubated in the dark in an osmium tetroxide solution (1% in 50 mmol/L sodium cacodiolite), dehydrated using an ethanol/water gradient (25%–100%) and finally critical point dried. The samples were sputter-coated with gold and examined with ESEM at 10 kV. ESEM observations were documented through the acquisition of at least 5 representative microphotographs in 3 fields of each position of flow path.

Extraction of biofilm EPS was carried out according to the methods by Frlund *et al.* (1996). The biofilm suspension was centrifuged at 2000 $\times g$ for 15 min at 4°C. The biofilm sediment were resuspended using a buffer consisting of (mmol/L): Na₃PO₄, 2; NaCl, 9; NaH₂PO₄, 4; and KCl, 1; at pH 7.0. The total EPS was extracted by heating at 80°C for 1 h and harvested by centrifugation for 15 min at 12000 $\times g$ and 4°C. Total polysaccharides were determined according to phenol-vitriol method (Leonardos and Lucas, 2000). The absorbance of the reaction mixture was then measured at 490 nm using the UV-2802 spectrophotometer (UNICO, USA). Glucose was used as a standard. The Lowry method was employed for total protein determination in the EPS (Raunkjær *et al.*, 1994). Bovine Serum Albumin (BSA) was used as a standard.

1.4 Phospholipid fatty acid analysis

The biofilm microbial community was analyzed for phospholipids fatty acids (PLFAs). This method provides an unbiased view of the complex microbial communities and viable biomass found in environmental samples such as soils (Ratcliff *et al.*, 2006), sediments (Ringelberg *et al.*, 1997), the rhizosphere and phyllosphere of plants (Xue *et al.*, 2005; Zhang *et al.*, 2008), indoor air (Saraf *et al.*, 1997), and drinking water systems (Smith *et al.*, 2000;

Minna *et al.*, 2004). It is based on the assumption that phospholipids make up a relatively constant proportion of the cell biomass and that fatty acid variation among taxonomic groups provide markers which can be used to interpret community-level profiles. Since different subsets of microorganisms have different PLFA compositions, the PLFA pattern of an environmental sample should reflect the microbial community composition.

PLFAs were extracted using a modified procedure described by Pennanen *et al.* (1999). In summary, biofilms washed from the surface of the emitter flow path were transferred to a glass tube. Lipids were extracted by a one-phase chloroform, methanol and 0.15 mol/L citrate buffer extractant (1:2:0.8, V/V/V) by shaking 4 h at 20°C (White *et al.*, 1979). The solvents were separated by centrifuging (4000 $\times g$) and volumes were measured. The total lipid extract was fractionated into neutral, glyco- and polar (phospho-) lipids by silicic acid chromatography. A glass column (height, 120 mm; inner diameter 8 mm) was packed with silicic acid (100 mesh size) activated at 100°C for 2 h and washed with chloroform. The lipid extract was applied in chloroform to the top of the column. Neutral lipids were eluted with 5 mL of chloroform, glycolipids with 10 mL of acetone, and phospholipids with 10 mL of methanol (Minna *et al.*, 2004; Xue *et al.*, 2005). The methyl ester of nonadecanoic acid (19:0) was added as an internal standard immediately before the separated phospholipids fatty acid was transesterified into fatty acid methyl esters using a mild alkaline methanolysis reaction. Fatty acid methyl esters (FAMES) were analyzed using a GC-MS system (Hewlett Packard HP 6890, GMI, USA) equipped with a HP-5 capillary column (60 m $\times$ 0.32 mm). Total quantity (nmol/mm³) was determined using methyl esters of nonadecanoic acid as an internal standard. PLFAs were named by standard nomenclature.

1.5 Data analysis

Data processing and analysis included the assessment of emitter performance using the average discharge ($\bar{q}$) and the distribution uniformity (DU).

DU provides a measure of the variability of discharge along each lateral. It was computed using Eq. (1) proposed by Keller and Karmeli (1975).

$$DU = \left(\frac{q_{25\%}}{\bar{q}} \right) \times 100\% \quad (1)$$

where, $q_{25\%}$ (L/h) represents the average emitter discharge of the lowest quartile and $\bar{q}$ (L/h) represents the overall average emitter discharge for a given lateral.

Also the biofilm EPS, including proteins and polysaccharides, was transformed to milligram per unit volume. Fatty acids with C14 to C18 carbon chain lengths were used to analyze microbial community structure. Fatty acids were transformed to mole per unit volume (mol/mm³). PLFA data were reported as means together with standard error of the mean (SE).

2 Results

2.1 Water quality

The water sample parameters of the dosed reclaimed wastewater are illustrated in Table 2. The irrigation water contained a relatively high concentration of iron (0.78–1.04 mg/L), which could precipitate and severely clog the filter element and flow path.

While it has been reported that pH may not have a direct impact on clogging, it could accelerate the chemical reactions or biological growth involved in clogging (Nakayama and Bucks, 1991; Dehghanisani *et al.*, 2004). According to Nakayama and Bucks (1991), a pH < 7.0 can promote slight clogging, a pH of 7.0–8.0 cause medium clogging, and > 8.0 (as recorded for the samples in this study) can lead to severe clogging. The risk of clogging due to another chemical clogging factor TDS level was medium (Nakayama and Bucks, 1991). The total suspended solids (TSS) ranged from 33.6 to 101.4 mg/L and the disk filter was cleaned every 3 d during the irrigation season. According to the results of water sample testing, the classification of potential biological clogging was difficult to identify.

The water temperature, measured at the middle of the each drip line, reached the highest of 29°C in the middle August and the lowest of 8°C in early December. During the irrigation period, the initial temperature increase promoted the plankton growth which resulted biomass accumulation. The abrupt temperature drop in early October (about 14°C) might have decreased the plankton levels and reduced the biofilms.

2.2 Emitter performance

It appears that suspended particles combining with microbial slime were the main cause of the slowly progressive emitter clogging occurred during the irrigation season. A clogged emitter sample (emitter F) showing biofilm formation in the flow path is shown in Fig. 3.

Changes in the emitter discharges and DU due to clogging are shown in Fig. 4. As indicated in Fig. 4a, an overall discharge reduction occurred for each of the emitter types over the course of the experiment. A gradual reduction followed by a partial recovery was observed in all types of emitter. The discharges dropped rapidly during the first 200 h, recovered slightly over the next 50 h and continued to decrease through the end of irrigation. After

Fig. 3 Clogging in an emitter flow path (emitter F).

360 h of irrigation with reclaimed wastewater, the emitters' average flow reduction ranged from 72.2% to 14.4% with an average value of 34.7%. The results on variation in the discharge of the six different types of drip irrigation emitters showed the highest discharge reduction in emitter F (72.2%) followed by that in emitter D (36.4%) as the irrigation season advanced. Emitters C and B resisted clogging and performed better with only 14.4% and 23.4% reduction (< 25%).

The DU values of the six emitter types were 82.3%, 86.1%, 95.0%, 87.5%, 86.4%, and 57.2% after 360 h irrigation (Fig. 4b). Classification of uniformity of distribution values is as follows (ASAE, 1988; Cararo *et al.*, 2006): 94 to 100 excellent, 81 to 87 good, 68 to 75 fair, 56 to 62 poor, and below 50 unacceptable. According to this standard, Emitter C maintained excellent uniformity while emitter F was the worst.

2.3 Biofilm structure in emitter flow path

The investigation of the biofilm structure in emitter flow path was assisted by SEM. The SEM micrographs of biofilm developed over 360 h in the flow paths of emitter F were inspected by direct visualization (Fig. 5). The biofilm surface appeared coarse and slimy (Figs. 5b and 5c), that might be attributed to the mixture of a bacterial layer and clay. The micrographs also revealed the existence of a greater amount of bacterial cells within the biofilm matrix. The bacteria coupling EPS built microcolonies with a complex three-dimensional architecture. Particles were also found trapped in the matrix of the biofilm forming sediment in the emitter flow path. A higher megascopic ratio picture of biofilm presented visual detail of the netted coupling structure (Fig. 5d).

2.4 Microbial community structure

The total amount of PLFAs can be used to indicate total microbial biomass. Analysis of PLFA profiles of the six emitter types was carried out in triplicates. Figure 6 shows that the flow path structure had a significant impact on total PLFA concentration in the emitter. After 360 h irrigation, the highest PLFA concentration (180.6×10^{-3} nmol/mm³) occurred in emitter F and the lowest concentration (7.74×10^{-3} nmol/mm³) in emitter C. This study found PLFAs 14:0, 15:00, 16:0, 16:1 ω 7c, cy17:0, i17:0, 17:0, 18:1 ω 11t, 18:1 ω 9c, 18:2 ω 6, 9t and 18:0 present in emitter flow paths. The PLFAs 14:0, 16:0, 18:1 ω and 18:0 were the main bacterial PLFAs, and were found to be the only PLFAs in emitters B and C. Fungi and algae are also considered as important factors in emitter biological clogging (Dehghanisani *et al.*, 2004), but in this experiment fungal PLFA profiles indicated there was fungal PLFA (18:2 ω 6, 9) present only in emitter D (3.89×10^{-3} nmol/mm³) and F (2.32×10^{-3} nmol/mm³).

Table 2 Characteristics of reclaimed wastewater in the dosing tank

Fe (mg/L)	pH	SO ₄ ²⁻ (mg/L)	HCO ₃ ⁻ (mg/L)	COD (mg/L)	BOD ₅ (mg/L)	TSS (mg/L)	TDS (mg/L)	<i>E. coli</i> (100 mL)
0.78–1.04	8.04–8.21	52.2–62.4	204–222	26.4–31.8	16.7–27.1	33.6–101.4	542–567	≥ 2400

TSS: total suspended solids; TDS: total dissolved solids.

Table 3 shows the amount of total proteins, total polysaccharides and total EPS found in the biofilms. The results were established per flow path volume that provided the amount of EPS, causing accurate comparative measures.

The chemical composition of the EPS matrix was found to be very heterogenous. Polysaccharides and proteins are usually the major EPS components with a protein to polysaccharides ratio range 0.2–5 (W/W) (Frløund *et al.*, 1996). In this study, the ratio of protein to polysaccharide ranged from 0.36 to 0.70 and there were no clear differences among the six emitters in total proteins. Emitter F had the highest total polysaccharide content.

2.5 Influence of biomass accumulation on emitter anti-clogging performance

Microorganisms can grow and proliferate within the pipeline network, develop into a biomass and clog almost any component of the drip irrigation system (Nakayama and Bucks, 1991; Ravina *et al.*, 1992). Suspended solids are likely to stick to the biological slime (biofilm) and agglomerate inside the emitters. In an attempt to establish a correlation between biofilm biomass and emitter clogging, the discharge reduction from the emitter was determined from outlet discharge after 360 h of irrigation. In our study, the discharge reduction indicates emitter clogging, while the PLFAs, total EPS, total proteins and total polysaccharides represent biofilm accumulation in the different emitters. Table 4 shows the relationship between the total amounts of PLFAs (T_{PLFA}), total EPS (T_{EPS}), total proteins (T_{Pr}), total polysaccharides (T_{Po}) and discharge reduction (D_r). It was verified that the discharge reduction provides a very good way to infer live biomass (PLFA) ($R^2 = 0.986$), whereas the total EPS and total polysaccharides provides a slightly worse correlation coefficient ($R^2 = 0.773$, $R^2 =$

0.778, respectively).

3 Discussion

The characteristics and behaviors of biofilms have a significant impact on many engineering system. The literature is deficient in its descriptions of biofilm structure in drip emitter flow paths. This study was the first attempt to assess the biofilm structure in the emitter flow path and the impact of path structure on the microbial community using biochemical approaches. The structure of the microbial biofilm in the emitter flow path provides important insight into drip irrigation clogging problems.

After 360 h of irrigation with reclaimed wastewater, there were significant differences in the discharge reduction and distribution uniformity among the six different emitter types. A partial recovery of emitter discharge occurred for all emitter types in the middle of October after approximately 250 h of operation. This could be due to the weakening of biofilm adhesion properties as a result of the sudden drop in temperature.

It was evident from the SEM micrographs that the bacteria coupling EPS built microcolonies with complex three-dimensional architectural and that sediment

Table 4 Correlation of PLFA, total EPS, total proteins, total polysaccharides and discharge reduction after 360 h of irrigation with reclaimed wastewater

Biofilm characteristic	Correlation	
PLFA	$T_{PLFA} = -315.37D_r + 265.44$	$R^2 = 0.986$
Total EPS	$T_{EPS} = -24.17D_r + 46.56$	$R^2 = 0.773$
Total proteins	$T_{Pr} = -1.71D_r + 12.06$	$R^2 = 0.329$
Total polysaccharides	$T_{Po} = -22.46D_r + 34.49$	$R^2 = 0.778$

Biomass and EPS over discharge reduction at significance of 5% by *F* test.

Fig. 4 Emitter average discharge variation (a) and distribution uniformity variation of emitters (b) over irrigation time compared with initial discharge.

Table 3 Biofilm EPS chemical components of six different emitters

Biofilm chemical components ($\times 10^{-3}$ mg/mm ³)	Emitter A	Emitter B	Emitter C	Emitter D	Emitter E	Emitter F
Total proteins	10.29 $\pm$ 1.57	10.95 $\pm$ 0.77	10.16 $\pm$ 1.02	11.45 $\pm$ 1.61	11.20 $\pm$ 3.10	11.61 $\pm$ 1.34
Total polysaccharides	20.47 $\pm$ 3.49	16.21 $\pm$ 0.92	14.43 $\pm$ 1.01	23.07 $\pm$ 2.00	16.39 $\pm$ 2.18	28.01 $\pm$ 4.83
Total EPS	30.76	27.16	24.59	34.52	27.59	39.62

Total EPS: total proteins + total polysaccharides.

Fig. 5 SEM micrographs of biofilm in emitter flow path after 360 h of irrigation with reclaimed wastewater. (a) 500×; (b) 1500×; (c) 2000×; (d) 8000×.

composed of a mixture of tiny particles and bacteria adhered to the corners of emitter flow paths (Fig. 5). SEM images are useful for describing biofilm morphotypes and estimating biofilm dimensional architecture (Horath *et al.*, 2006). Geostatistical analysis has also been utilized in examining the morphologies of biofilms (Yang *et al.*, 2000). The matrix structure of particles and bacterial in an emitter provides evidence to the ability of biofilms to induce clogging. Some researchers have already shown the fouling material composition in other parts of the irrigation system using SEM, including filter screens (Adin *et al.*, 1989), drip laterals (Gilbert *et al.*, 1979) and others described particle distribution in emitter flow paths (Adin and Sacks, 1991). Our study clearly focused on the drip emitter biofilm matrix structure using electron microscope technology.

The varying amounts of total proteins and polysaccharides were arguably related to the stress imposed by the flow conditions on the resultant bacterial physiology (Simões *et al.*, 2007). Emitter F had the highest content of total proteins and total polysaccharides per unit area, and the highest EPS content which potentially offers protection against antibiotics and resistance to antimicrobial agents and disinfection procedures (Sutherland, 2001; Pitts *et al.*, 1990). Chlorine disinfection is a common and low-cost

treatment to control drip system fouling (Tajrishy *et al.*, 1994), and high EPS content might decrease the effect of chlorine procedures.

The composite PLFA profiles provided an indication of biofilm microbial biomass as well as community structure. Microbial biomass can be predicted by summation of the mass of all detectable PLFAs (Ben-David *et al.*, 2004). Total PLFAs, especially bacterial PLFAs, were significantly different between all six emitter types (Fig. 6). PLFA analysis indicated that the flow path structure, specifically the dentate design, affected the microbial community. The influence of flow regime on biofilm the microbial community was clearly shown. According to the results, the diversity of the major PLFAs in the biofilms was low (14:0, 16:0, 18:1 ω , 18:0), but emitter biofilms with a greater amount of PLFAs experienced a higher degree of clogging. Although not all the emitter biofilms contained the detectable fungi PLFAs, emitters D and F with fungi (18:2 ω) exhibited the worst anti-clogging performance. Fungi and algae were still considered as potential clogging factors in each emitter type.

Total PLFAs was strongly correlated with emitter discharge reduction. Biofilm activity might be a good indicator for predicting degree of emitter clogging but more research work is needed including seasonal experiments.

Fig. 6 Total PLFAs in emitter flow path after 360 h of irrigation with reclaimed wastewater. Error bar represent the standard error of the mean ($n=3$).

Future studies will investigate the relationship between biomass accumulation and emitter clogging in other emitter types and further analyze microbial community diversity using biochemical methods. A better understanding of how microbial communities respond the emitter clogging will be pursued.

4 Conclusions

This study is the first using PLFA and SEM to investigate the biofilm structure and the effects of path boundary conditions on the emitter flow path microbial community. An advantage of PLFA analysis is that it can provide quantitative insight into makeup of the microbial community. The majority of the microbial communities in emitter flow paths irrigated with reclaimed wastewater contained PLFAs 14:0, 16:0, 18:1 ω , 18:0. In addition, SEM analysis can generate high visibility and reveal the nature of the biofilm structure and composition. Particles were found in the matrix of the bacteria coupling EPS and forming sediment in the emitter flow path. This study provided a framework for future dripper biofilm studies.

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