Response of simulated drinking water biofilm mechanical and structural properties to long-term disinfectant exposure

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Supporting information

10 PAGES

5 FIGURES AND 2 TABLES

Sphere Probe Preparation

The sphere probe used in this study was built by adhering a silica sphere into the tipless cantilever (Calibrated Spring Constant=0.6-1.2 N/m, Mikromasch, Lady's Island, SC). The probe building process was carefully performed using an inverted microscope mounted with a micromanipulator. The silica spheres were washed by repeating 3 times the process of dispersing these spheres in DI water, centrifuging, and removing supernatant. Then the spheres were dispersed in DI water again. 100 μl of this sphere solution was dropped on a mica wafer (diameter = 10 mm) and dried in the air to form a monolayer of spheres onto the mica surface. The mica wafer was then placed in a glass slide fixed in the microscope stage. Before the probe making process, a drop of the UV glue was also added on the same glass slide. To avoid probe contamination caused by directly dipping the tipless cantilever to the large area of UV glue, a waste AFM tip was used to dip in the UV glue and touch on the clean glass slide area several times to create a very small area of glue drop using the micromanipulator. In the probe making process, the micromanipulator moved the tipless cantilever to the small glue drop area and carefully daubed a very small amount of glue to the very tip of cantilever. After that, the cantilever moved on top of the mica wafer and adhered one sphere using the micromanipulator. The UV glue between the cantilever and the sphere was cured under UV light for one hour. All the AFM sphere probes were cleaned in an UV-Ozone generator for one hour.

Examination of AFM Probe Contamination

The contamination of the AFM probe was checked by measuring a normal force-distance curve on a clean glass slide in water. If the AFM probe would be contaminated by residual biofilm, the retraction force curve would exhibit adhesion (Figure S5a). When this occurred, a new probe was used. If no adhesion force was observed in the retraction force curve (Figure S5b), the probe was considered to be suitable for further use. Each AFM probe was typically used to conduct approximately 100-500 indentation measurements before contamination was detected.

Statistical analysis

OriginPro 9.0 (OriginLab Corporation, Northampton, MA) was used for statistical analysis of the AFM, OCT, and CLSM results. For comparing the biofilm Young's modulus distributions obtained by AFM, Kolmogorov-Smirnov tests were conducted between two examined distributions. For biofilm thickness and roughness results obtained by OCT, t-tests were used to compare the biofilm structures at different times or under different treatment conditions. T-tests were also conducted for comparing protein/polysaccharide ratios obtained by CLSM.

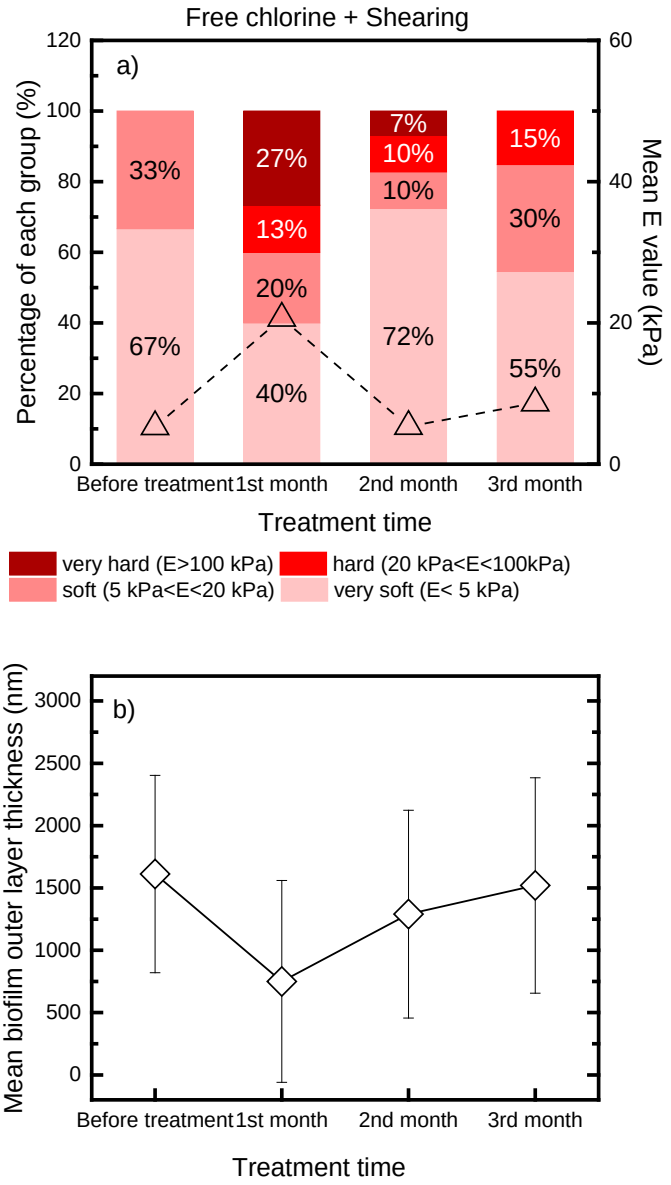

Figure S1 a) The percentage stacked bar and b) the outer layer thickness of biofilms during the 3 months of free chlorine treatment under stirring condition. The red line in Figure a) shows the mean value of *E* at each time point.

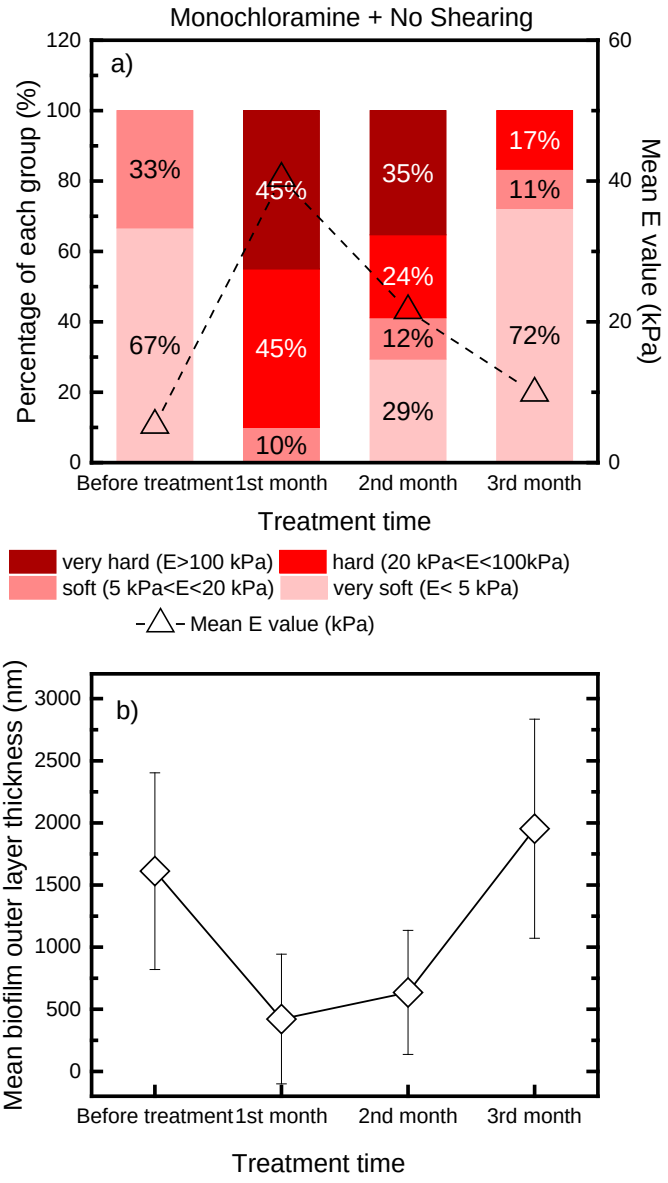

Figure S2 a) The percentage stacked bar and b) the outer layer thickness of biofilms during the 3 months of monochloramine treatment under no stirring condition. The red line in Figure a) shows the mean value of *E* at each time point.

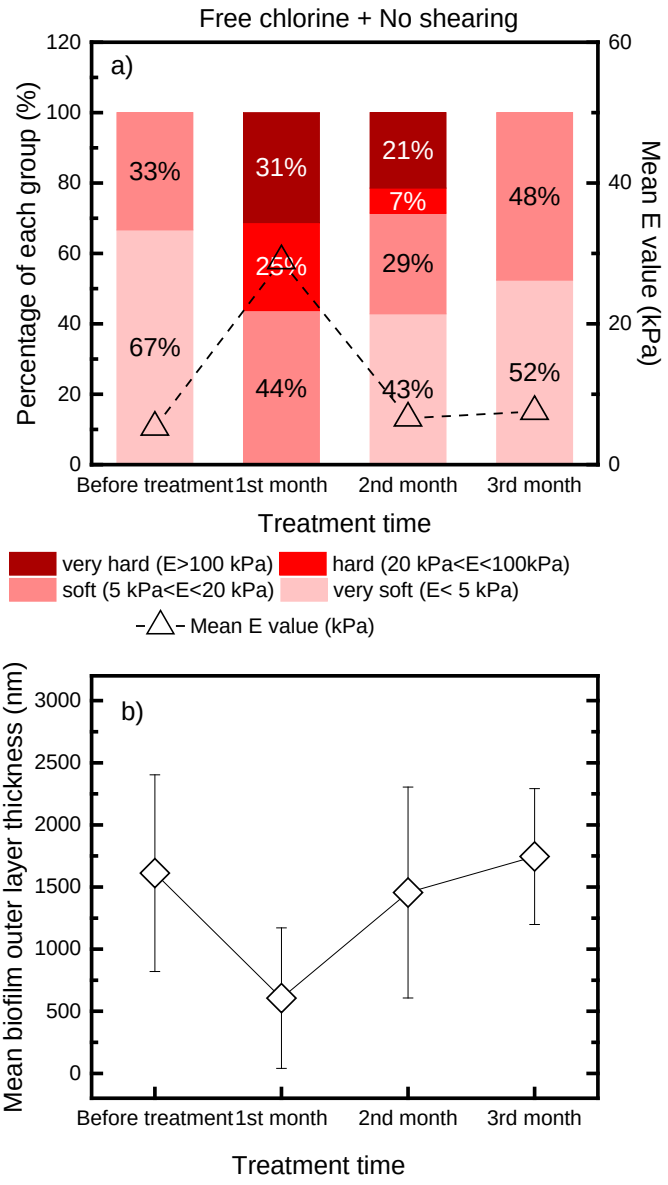

Figure S3 a) The percentage stacked bar and b) the outer layer thickness of biofilms during the 3 months of free chlorine treatment under no stirring condition. The red line in Figure a) shows the mean value of *E* at each time point.

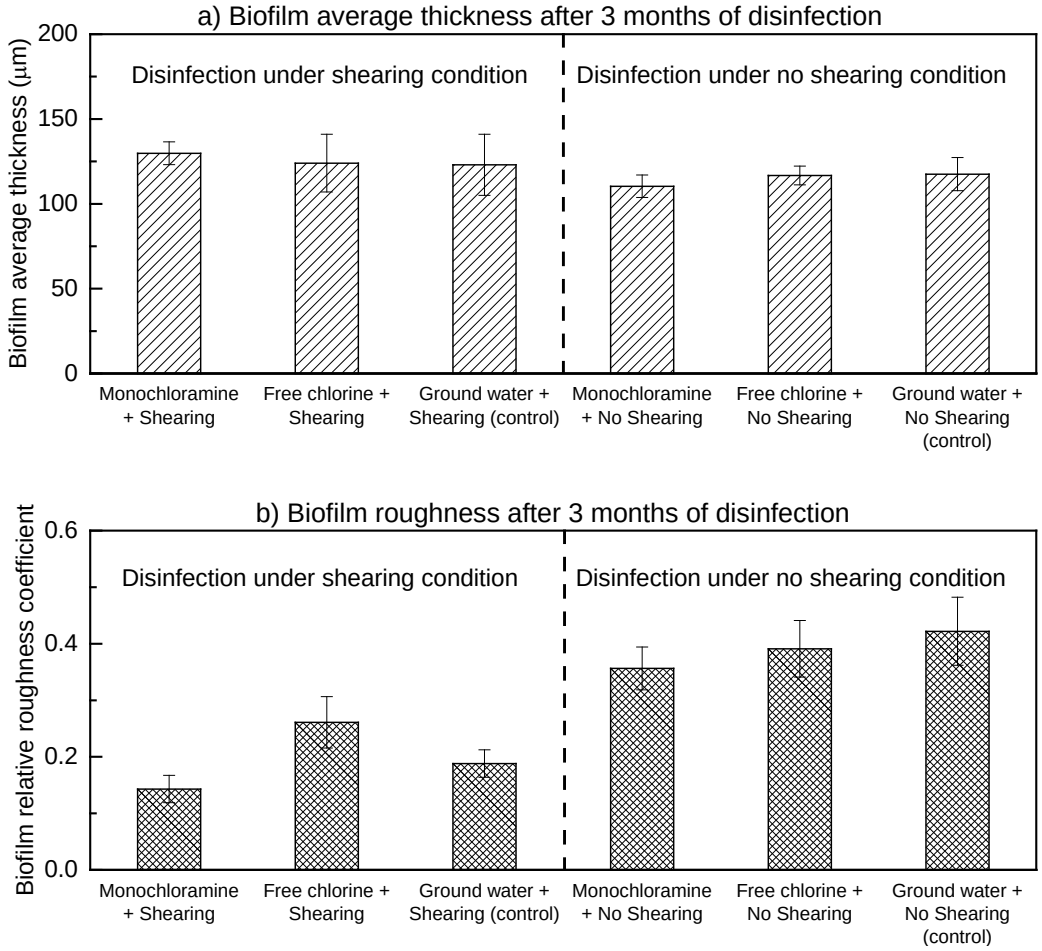

Figure S4 a) Average thickness and b) relative roughness coefficient of biofilms after 3 months of exposure to monochloramine, free chlorine, and groundwater without disinfectant under shearing and no shearing conditions, respectively. The average thickness and roughness were calculated from 20 randomly selected OCT images.

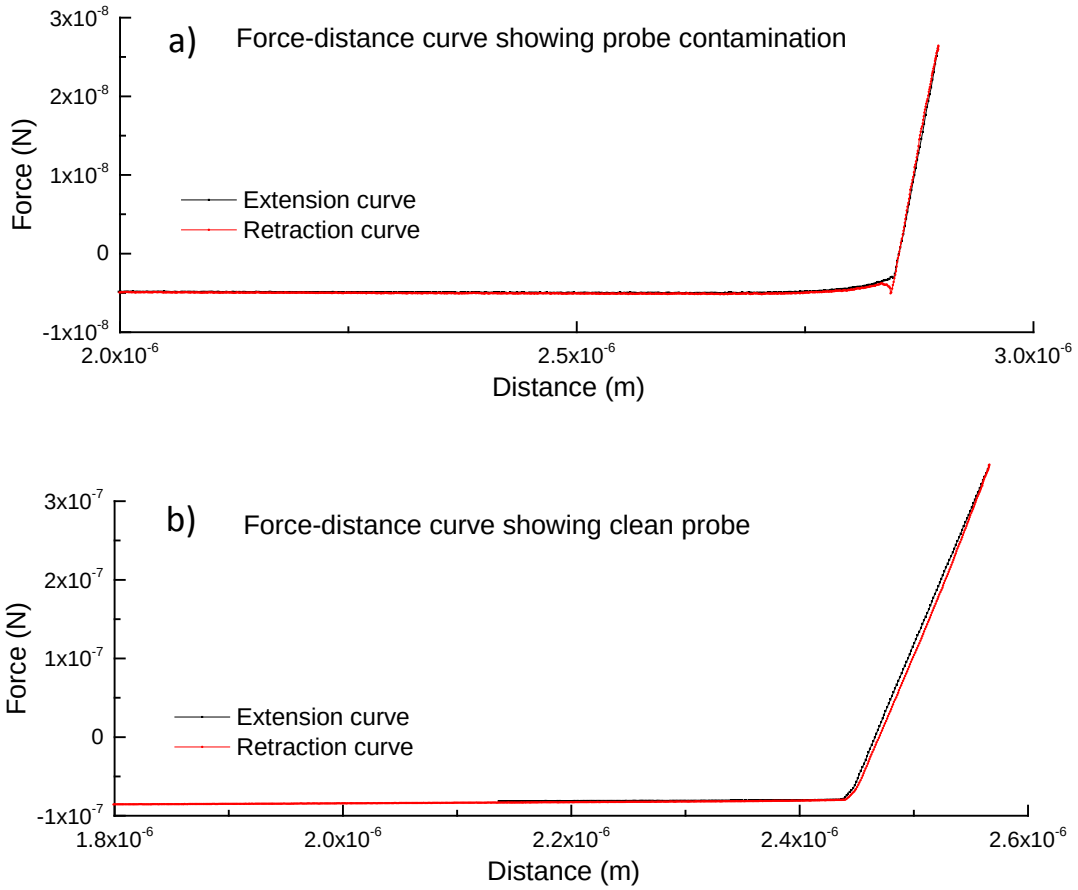

Figure S5 Calibration force-distance curves obtained in water on clean glass surfaces showing a) probe contamination and b) clean probe without contamination.

Table S1 Change of biofilm average thickness during the disinfectant exposure under different treatment

Disinfectant exposure time	Disinfection treatment conditions					
	Monochloramine + Shear	Free Chlorine + Shear	Groundwater + Shear	Monochloramine + Shear-free	Free Chlorine + Shear-free	Groundwater + Shear-free
Week 0	120±8	120±8	120±8	120±8	120±8	120±8
Week 2	119±6	114±12	116±12	111±15	117±9	114±10
Week 3	110±10	113±8	114±7	107±8	110±15	116±10
Week 4	108±6	107±11	125±11	100±5	103±8	117±11
Week 5	105±6	131±9	124±11	93±6	116±10	113±13
Week 7	120±7	139±8	111±16	105±8	116±8	111±11
Week 9	118±8	135±9	112±11	95±14	113±11	112±7
Week 10	130±8	130±12	124±12	98±7	105±9	106±10
Week 11	130±9	132±11	133±14	109±6	105±9	121±14
Week 13	129±8	127±19	123±18	110±7	117±6	118±10

* The average thickness and standard deviation was calculated from analysis on 20 randomly selected OCT images.

Table S2 Change of biofilm relative roughness coefficient during the disinfectant exposure under different treatment

Disinfectant exposure time	**Treatment conditions**					
	Monochloramine + Shear	Free chlorine + Shear	Groundwater + Shear	Monochloramine + Shear-free	Free chlorine + Shear-free	Groundwater + Shear-free
Week 0	0.25±0.02	0.25±0.02	0.25±0.02	0.25±0.02	0.25±0.02	0.25±0.02
Week 2	0.25±0.05	0.26±0.05	0.35±0.03	0.26±0.04	0.27±0.03	0.39±0.03
Week 3	0.20±0.03	0.29±0.03	0.31±0.03	0.28±0.02	0.27±0.03	0.39±0.04
Week 4	0.15±0.01	0.30±0.03	0.28±0.04	0.31±0.02	0.31±0.06	0.41±0.04
Week 5	0.14±0.02	0.24±0.03	0.28±0.03	0.31±0.03	0.31±0.02	0.40±0.05
Week 7	0.14±0.02	0.22±0.03	0.24±0.03	0.30±0.02	0.32±0.02	0.39±0.03
Week 9	0.16±0.02	0.20±0.02	0.24±0.04	0.34±0.05	0.33±0.04	0.42±0.05
Week 10	0.17±0.02	0.25±0.03	0.18±0.03	0.33±0.04	0.37±0.04	0.39±0.04
Week 11	0.15±0.04	0.24±0.03	0.17±0.03	0.34±0.04	0.36±0.05	0.35±0.03
Week 13	0.15±0.03	0.26±0.05	0.19±0.02	0.36±0.04	0.39±0.05	0.42±0.06

* The average roughness and standard deviation was calculated from analysis on 20 randomly selected OCT images.