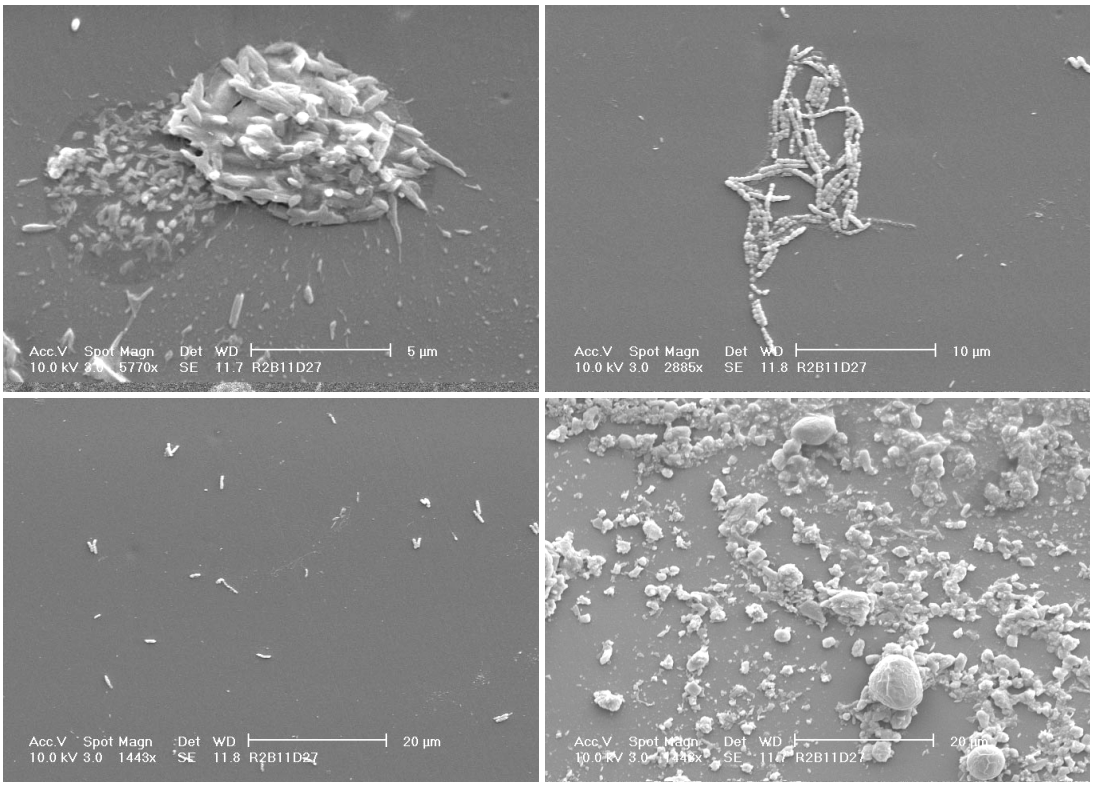


(i) Batch 9 Biofilms treated with 0.5 mg/l Cl_2 + 40µg/l Ag^+

Figure 4.20 Biofilm structure with testing agents (continued)

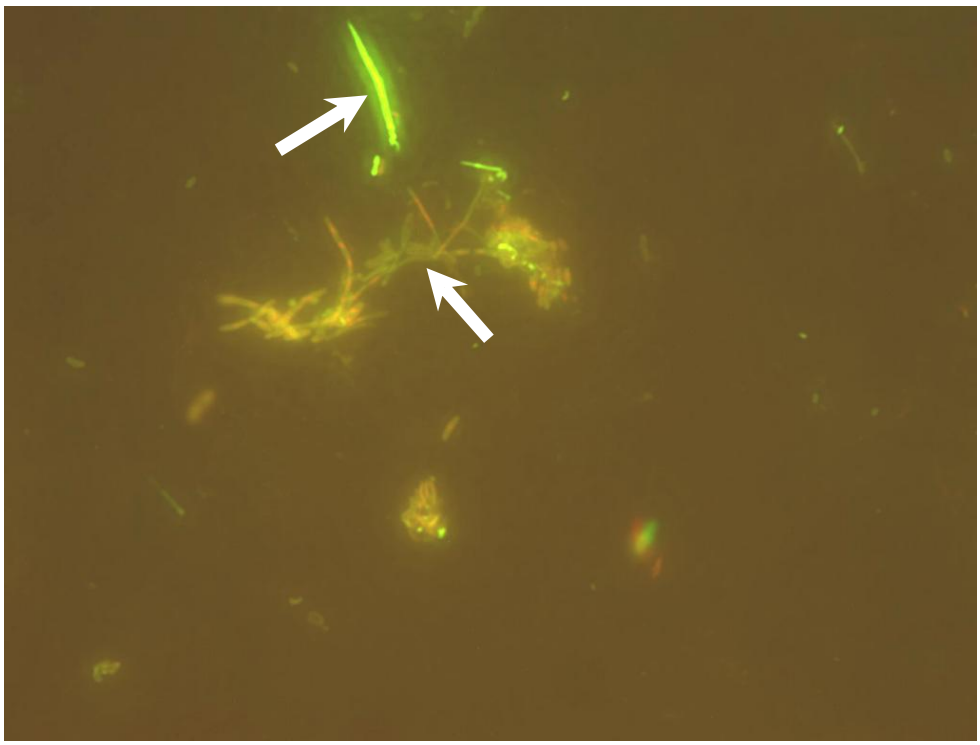


Figure 4.21 Epifluorescence microscopy image of biofilms in control reactor (batch 3 day 26)

Upper arrow: over exposure area (one foreign cell); lower arrow: under exposure area

4.4.3.2 Biofilm physiology

Eifluorescence microcopy with LIVE/DEAD kit was selected and improved to analysis live ratio of bacteria. However, due to the foreign species' invasion (refer to section 4.3.3), there was heavy bias of the live/dead ratio during observation. As noted by Lawrence *et al.* (2002), staining of natural (heterogeneous) assemblages of bacteria with the LIVE/DEAD BacLight probe can give variable results due to differences in cell membrane permeability. During the experimental period, foreign species might reflect much stronger fluorescence than the target species (figure 4.21) and this would influence the analysis. According to above discussion, the further analysis of live ratio was omitted.

4.4.4 Summary

In section 4.4, three testing agents, e.g. free chlorine, monochloramine, and the combination of free chlorine and silver ion, showed various biofilm control abilities in the experimental reactors at different dosages. The free chlorine – silver ion combination showed superiest efficiency among the testing agents both on biofilm disinfection and biofilm removal. SEM pictures illustrated the biofilm structure could be affected by the testing agents: free chlorine could react with EPS, and the cell tended to live individually with less matrix; monochloramine had a less reacting potential which led to a culture with plenty of embedding EPS; the silver ion, which could help detach the cells from biofilm matrix, resulted cell holes on the biofilm mats.

TCC was not very sensitive and accurate in this section and probably due to the relatively low content of the sample. The LIVE/DEAD method for biofilm physiology was unsuccessful and mainly due to the existing of species diversity.

4.5 Biofilm control with high slug dose (HSD) strategies

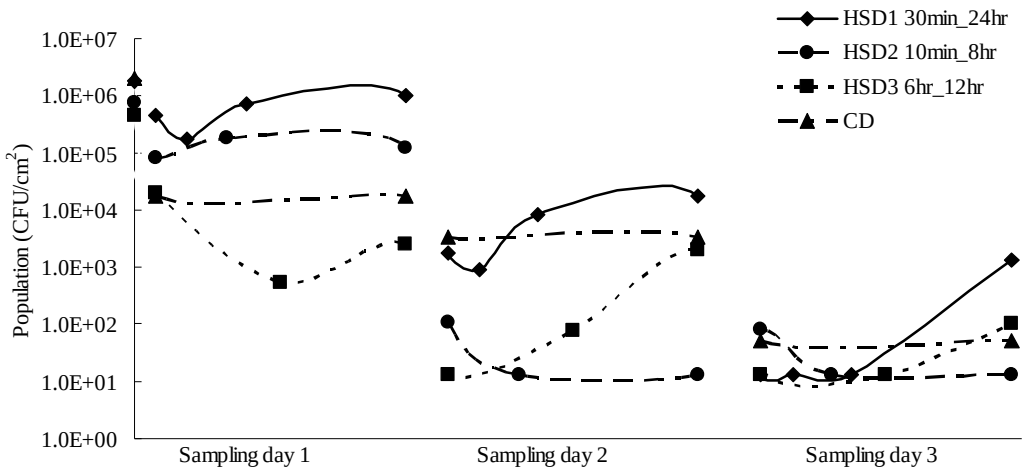
The alternative HSD strategies were applied in the last two batches (batches 10 and 11) to evaluate biofilm control abilities. In this section, the results of three HSD strategies were presented and compared with the one of previous Continuous strategy with equivalent chlorine Dosage (CD) (table 4.13).

Table 4.13 The HSD biofilm control strategies

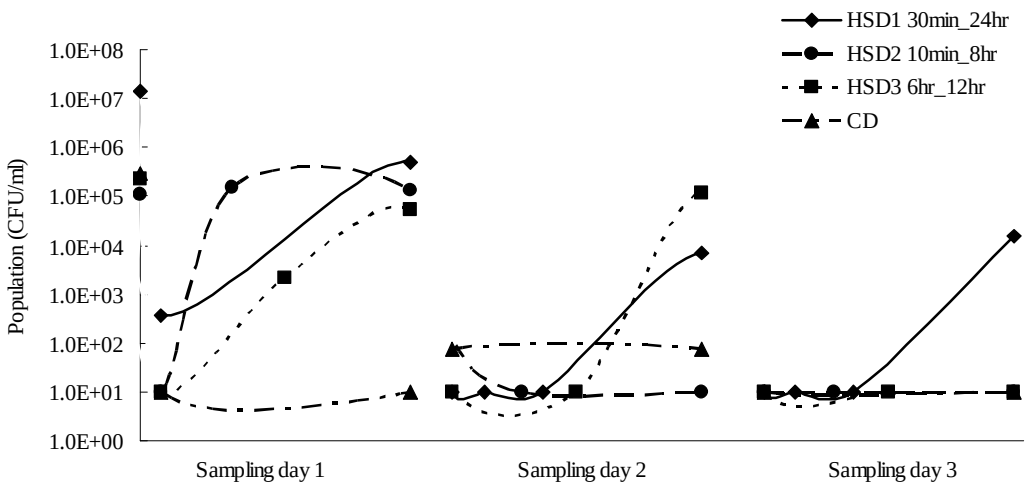
	HSD1	HSD2	HSD3	CD
Dose type	High slug	High slug	High slug	Continuous
Chemical	Chlorine (free)	Chlorine (free)	Chlorine (free)	Chlorine (free)
Dosing duration	30 min	10 min	6 hr	/
period	24 hr	8 hr	12 hr	/
Dosing conc.	48 mg/l	48 mg/l	2 mg/l	1 mg/l
Equivalent continuous dosing conc.	1 mg/l	1 mg/l	1 mg/l	/

4.5.1 Strategies' disinfecting efficiency

Figure 4.22 showed the profiles of bacteria population in the reactors during the batches. Overall, no matter what strategy applied, both biofilm and suspended bacteria had a continuous decrease potential.



(a) Biofilm bacteria



(b) Suspended bacteria

Figure 4.22 Bacteria population treated with testing strategies

Note: Samples were taken throughout the idling phase in each sampling day and the curves presented had eliminated the time scale. Sampling day 1 was the first day when chlorine was applied (the 9th day of the batch), sampling day 2 was the 16th day of the batch, sampling day 3 was the 23rd day of the batch. Detection limit: 1.11 log(CFU/cm^2) for biofilm bacteria; 1.00 log(CFU/ml) for suspended bacteria.

Table 4.14 Biofilm population treated by testing strategies

Strategy	Day 0	Sampling day 1	Sampling day 2	Sampling day 3
HSD1	6.24	5.66 ~ 6.01	2.97 ~ 4.24	<1.11 ~ 3.13
HSD2	5.88	4.90 ~ 5.27	<1.11 ~ 2.04	<1.11 ~ 1.92
HSD3	5.65	2.73 ~ 4.29	<1.11 ~ 3.31	<1.11 ~ 2.01
CD	6.31	4.23	3.53	1.71

Note: Unit – log(CFU/cm^2)
Detection limit – 1.11 log(CFU/cm^2)
Day 0 was the day before testing strategies were started.

For biofilm bacteria (table 4.14), at sampling day 1, the decreases of the HPC were correlated with the exposure periods: the CD and HSD3 strategies were more successful than HSD1 and HSD2 strategies. Since sampling day 1 was the next day after chlorine was applied, the dosing duration, not the dosage, seemed to be more important for the initial disinfection of intact biofilms. At sampling day 2, though the CD strategy could control the population, the superior of the HSD strategies began to emerge. The population decreased with HSD1 strategy was similar with CD strategy, whereas the population decreased with HSD2 and HSD3 strategies were about 2 log lower. After 2 weeks, all the populations with HSD strategies touched the detection limit at sampling day 3, however, a certain range of recovery occurred during the interim. HSD1 strategy, with the longest idling period, resulted the highest population recovery at the end of each period; and the HSD2 and HSD3 with relatively shorter idling periods resulted less recovery.

Table 4.15 Suspended population treated by testing strategies

Strategy	Day 0	Sampling day 1	Sampling day 2	Sampling day 3
HSD1	7.16	2.56 ~ 5.68	1.00 ~ 3.85	1.00 ~ 4.18
HSD2	5.05	1.00 ~ 5.19	1.00 ~ 2.04	1.00
HSD3	5.35	1.00 ~ 4.71	1.00 ~ 5.08	1.00
CD	5.48	1.00	1.88	1.00

Note: Unit – log(CFU/ml)
Detection limit – 1.00 log(CFU/ml)
Day 0 was the day before testing dose strategies were started.

For suspended bacteria (table 4.15), all the HSD strategies and CD strategy could achieve satisfactory disinfecting efficiencies. All the suspended population decreased to lower than 10 CFU/ml (detection limit) sooner or later during the experimental period. Only the HSD1 strategy got a 4.18 log recovery in the sampling day 3, mainly due to the long idling period (23.5 hr). There were no significant suspended population recoveries with other strategies, whose dosing-off period could be up to 7.7 hr.

4.5.2 Strategies' biofilm removal efficiency

4.5.2.1 Biofilm coverage

Figure 4.23 showed the biofilm coverage profiles with the testing strategies and table 4.16 gave the details data. A decreasing coverage potential could be found over the time, which indicated that these strategies had the ability to detach the biofilms from the slide surface.

Table 4.16 Biofilm coverage of the samples treated with testing strategies

	Day 0	Sampling day 1		Sampling day 2		Sampling day 3	
		Start	End	Start	End	Start	End
HSD1	6.73±4.62	9.96±5.05	3.78±1.91	2.11±1.71	2.69±2.26	1.21±0.67	1.71±2.66
HSD2	6.15±2.71	9.63±6.90	12.66±5.93	3.92±3.27	3.29±2.60	4.22±1.98	5.28±2.42
HSD3	7.08±5.17	5.84±4.95	5.91±3.86	0.52±0.38	1.68±1.14	2.70±2.31	1.52±1.55
CD	13.80	5.28		4.79		2.07	

Note: Unit – biofilm coverage percentage (%);
"Start" and "End" denoted the start and end of non-dosing phase, respectively;
Day 0 was the day before testing strategies was started.

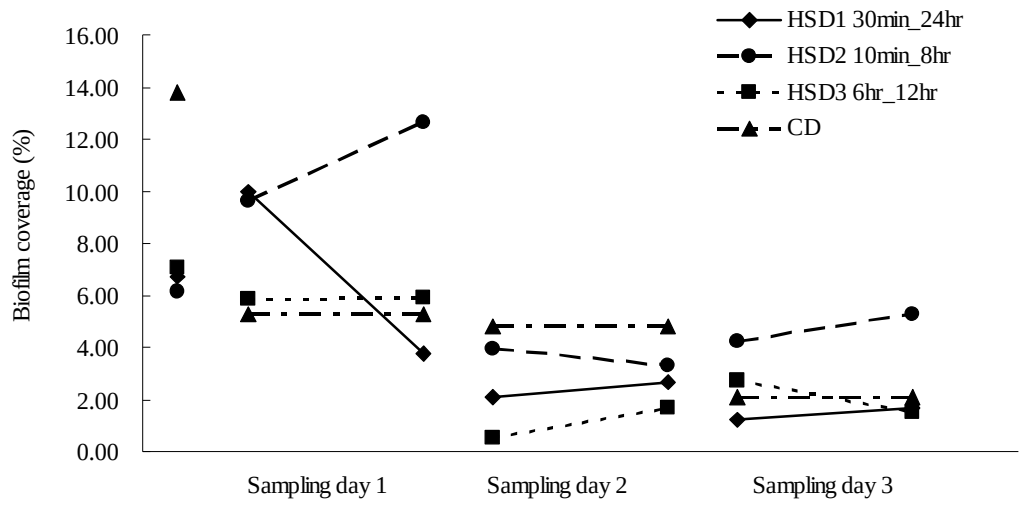


Figure 4.23 Biofilm coverage treated with testing strategies.

Note: Samples were taken throughout the idling phase in each sampling day and the curves presented had eliminated the time scale. Sampling day 1 was the first day when chlorine was applied (the 9th day of the batch), sampling day 2 was the 16th day of the batch, sampling day 3 was the 23rd day of the batch.

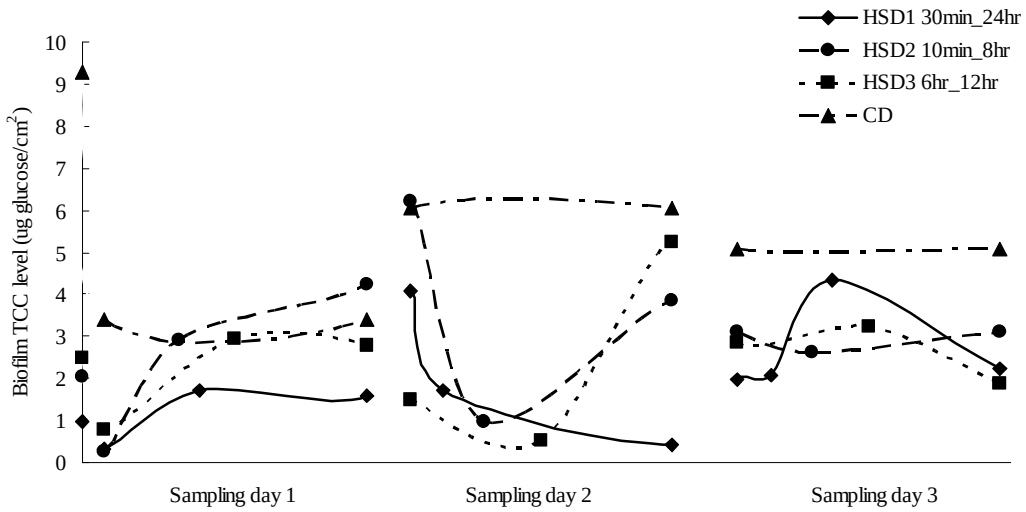


Figure 4.24 Biofilm TCC level treated with testing strategies

Note: Samples were taken throughout the idling phase in each sampling day and the curves presented had eliminated the time scale. Sampling day 1 was the first day when chlorine was applied (the 9th day of the batch), sampling day 2 was the 16th day of the batch, sampling day 3 was the 23rd day of the batch.

At sampling day 1, HSD1 and HSD 2 strategies resulted a sudden increase of biofilm coverage (from 6.73% to 9.96% and from 6.15% to 9.63%, respectively) at the dosage stop point and the coverage fluctuation were more poignant than the counterparts with HSD3 and CD strategies. At sampling day 2, the fluctuations of biofilm coverage with all strategies were insignificant. At sampling day 3, HSD1, HSD3, and CD strategies could control the biofilm coverage at 1.21 – 2.70%, and the difference among them was insignificant. However the HSD2 strategy only inhibited the biofilm coverage at 4.22 – 5.28%, which presented a poorer efficiency.

4.5.2.2 Total carbohydrates content (TCC)

Figure 4.24 showed the biofilm TCC level profiles with the testing strategies and table 4.17 gave the detail data.

Table 4.17 TCC level of the biofilm samples treated with testing strategies

Strategy	Day 0	Sampling day 1	Sampling day 2	Sampling day 3
HSD1	0.96	0.32 ~ 1.70	0.42 ~ 4.07	1.97 ~ 4.34
HSD2	2.04	0.25 ~ 4.24	0.96 ~ 6.23	2.61 ~ 3.12
HSD3	2.48	0.79 ~ 2.95	0.52 ~ 5.25	1.87 ~ 3.22
CD	9.30	3.40	6.04	5.07

Note: Unit – μg glucose per cm^2

Day 0 was the day before testing strategies were started.

From TCC results, it could be seen that the potentials were not as clear as those in biofilm coverage or HPC results and the behaviors of TCC with each HSD strategies were similar: The average of TCC levels at sampling day 1 were similar with day 0; At sampling day 2, all the TCC shifted to a higher level compared with sampling day 1 and the fluctuations were also significant; At sampling day 3, the TCC level slightly decreased and the fluctuations were kept in a narrow range. For

CD strategy, the TCC level also followed the trend mentioned above except for the fluctuation.

The above phenomena indicated that the first high slug dose (whichever HSD strategies) had little effects on the carbohydrate levels of the biofilms (even TCC at the starting points of sampling day 1 decreased, the recoveries were achieved soon), but after about 1 week of treatment, the biofilms were stimulated to produce more carbohydrate to avoid the chemical detachment. However, after 2 weeks of treatment, the HSD strategies would achieve better TCC control effects than the CD strategy. Some mechanisms might be developed by the biofilms to prevent the lose of TCC and the overproduction of TCC as well.

For all testing strategies, though there were only little effects on TCC at the first sample day, sudden TCC decreases at the starting point were noted. Meanwhile at this point sudden increases of biofilm coverage by HSD1 and HSD2 strategies while only slight change in cases of HSD3 and CD strategies (figure 4.24) were also observed before. An assumption was that the initial acute doses (a high concentration of chlorine, e.g. HSD1 and HSD2 strategies) could decompose the heavy biofilm matrix (small TCC values) but make the cells-mat thinner with wider distribution (large biofilm coverage); the initial mild doses (a low concentration and long duration, e.g. HSD3 and CD strategies) could not only decompose the biofilm matrix but also inhibit the extent of biofilm coverage.

4.5.3 Biofilm structure

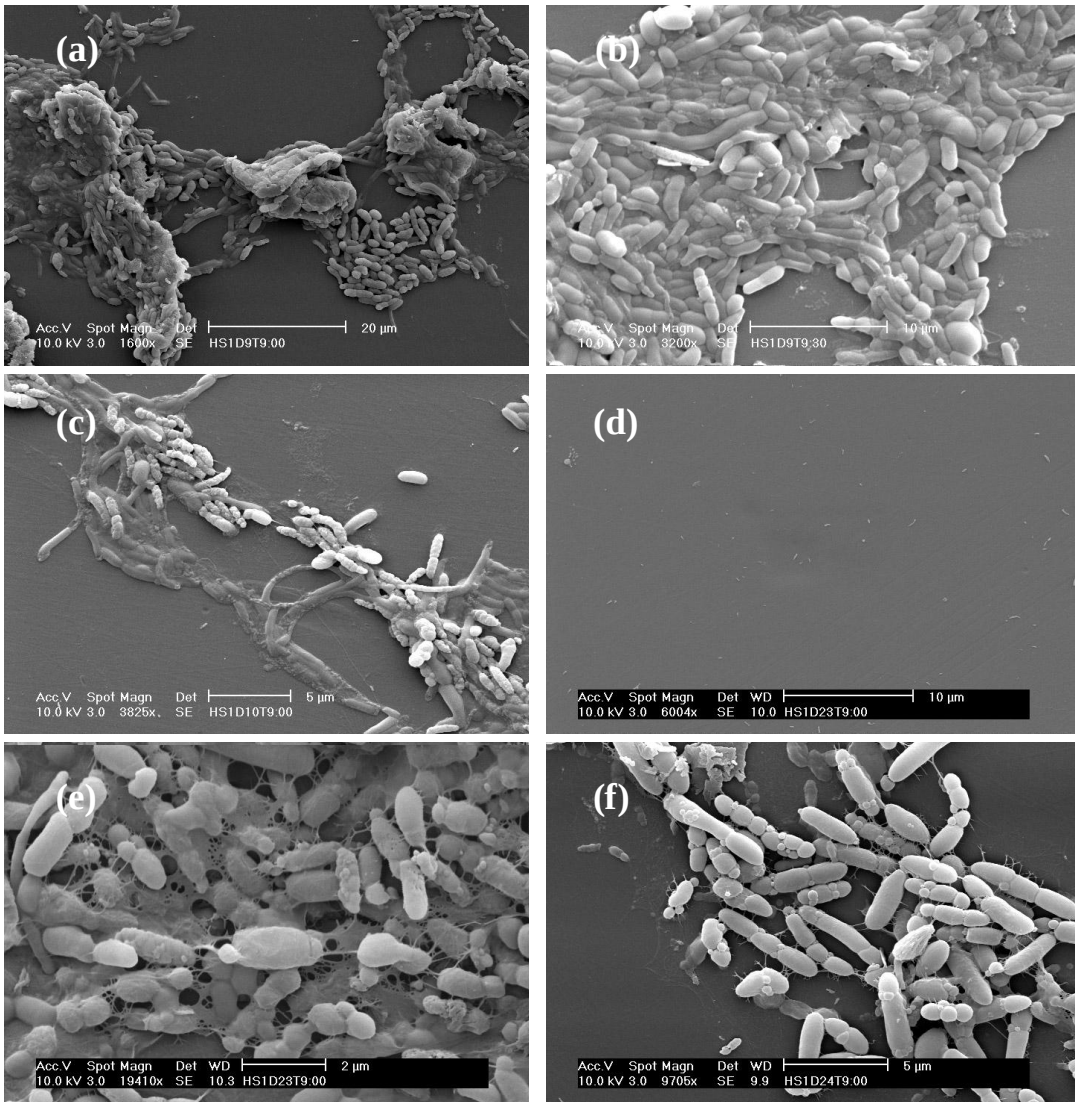


Figure 4.25 Biofilm structure treated with HSD1 strategy

Figure 4.25 showed the biofilms structures under HSD1 strategy. Figures 4.25 (a), (b) and (c) were taken from the first sampling day. On the 9th day intact biofilms before strategy was applied seemed to be health and prosperous like the ones growing in control reactors (a). After the first 30 minutes treatment with a dosage of 48mg/l, the biofilms structure was neglectfully effected (b). At this time, some cells might have been hurt or already died; however, the exterior morphology was unchanged. The influence on structure could be easily observed in (c), where the

biofilms had been treated after a whole day (one period) from beginning of the first HSD. Some cells lysed and only some residuals could be found. For the survivors, the cell wall became rough and heavy, most likely combined with EPS, as the resistance system probably had been triggered. Figures 4.25 (d), (e), and (f) were taken from the 3rd sampling day, when the biofilms had been treated with the HSD of free chlorine for about 2 weeks. There were only few survival biofilm bacteria existing (d). The biofilm matrix structure had changed a lot ((e) and (f)) compared with not only the intact ones, but also the counterparts with continuous dose strategies. The matrix seemed to be a net instead of a mat. The significant difference of the biofilms structure indicated the HSD strategies could facilitate alternative biofilm control mechanisms against the continuous dose strategies.

4.5.4 Summary

On summary, the HSD2 and HSD3 strategies could achieve better disinfection results than HSD1 and CD strategies, based on the considerations of highest HPC decreased and the lowest recovery thereafter. The population recovery phenomenon indicated that an appropriate dosing sequence would be required for HSD strategy, especially in the idling period when the agent was not available. For biofilm removal, all the HSD strategies presented better TCC control abilities than the CD strategy, while the HSD1, HSD3 and CD strategies achieved similar biofilm coverage control and all of them were better than HSD 2 strategy. The SEM showed the biofilm structure, especially for the biofilm matrix, was significantly affected via HSD strategies.

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the results presented and discussed herein, conclusions are drawn as followings.

1. From the biofilms survey in a local drinking water system, it was noted high HPC values (3~4 log CFU/cm^2) were found at some sites which indicated the possibility of the biofilms growth. The consumed chlorine, but not residual chlorine, was more correlated with biofilm bacteria population. The carbon source was most likely to be the controlling nutrition parameter for biofilm growth in this study.

2. A procedure for LIVE/DEAD assay of mono-layer *P. fluorescens* biofilms was recommended: 15-min of incubation period with the dye (50 μl/225 mm^2, SYTO 9 12 μM and PI 60 μM) followed by quick (in minutes) observation under fluorescence in moisture. Results also showed the kit was very sensitive to detect the viability of chlorine affected biofilms.

3. The free chlorine – silver ion combination showed best efficiency among three test agents (free chlorine, monochloramine, and free chlorine – silver ion combination). For biofilm disinfection, 4.51 and 3.84 log CFU/cm^2 reduction with a Cl_2 – Ag^+ concentration of 1.0 mg/l – 40 μg/l and 0.5 mg/l – 40 μg/l, respectively were achived. For biofilm removal abilities, 82.77%, 90.74%, and 75.62% biofilm coverage reduction with a Cl_2 – Ag^+ concentration of 2.0 mg/l

– 40 μg/l, 1.0 mg/l – 40 μg/l and 0.5 mg/l – 40 μg/l, respectively were obtained. Free chlorine was better for biofilm disinfection and biofilm removal than monochloramine at same dosage, however, the biofilm resistance factor of monochloramine was smaller than that of free chlorine.

4. For high slug dose strategies, the HSD 3 strategy (dosing 2 mg/l free chlorine for 6 hours and idling for 6 hours) was able to control the biofilms population within <1.11 ~ 2.01 log(CFU/cm^2), the suspended bacteria population below 1.00 log(CFU/ml), the biofilm coverage within 1.52 ~ 2.70%, and the TCC within 1.87 ~3.22 μg glucose/cm^2 after 2-week treatment, respectively. It was better than other HSD strategies and equivalent continuous dosing strategy based on both biofilm disinfection and biofilm removal abilities. It is important to design a HSD schedule in practical operation to achieve high biofilm control performance without compromising with bacteria recovery in the idling phase. The significant difference of the biofilms structure observed from SEM indicated the HSD strategies could facilitate alternative biofilm control mechanism against the continuous dosing strategy.

5.2 Recommendations

Due to time and manpower limitation, this study is only a primary study on biofilm control. Therefore, the following recommendations are made for further studies.

1. Annular reactor is good for biofilm development and analysis. However, if mono-species biofilms is anticipated, a hermetic environment should be provided to locate the equipments.

2. Less health effect biotic control strategy is the target of drinking water disinfection. Different less harmful agents (like enzyme) and efficient alternative operations (like slug dosing) could be the candidates for further study.

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