TU Delft

Assessment of Using Low-frequency Ultrasound Device for Domestic Drinking Water Disinfection

CIE5050-09 Additional Graduation Thesis

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Abstract:

Microorganisms may cause problems in drinking water distribution system (DWDS). It is hard to control and always forms the biofilm, which can result in the deterioration of drinking water quality. At household's tap, due to the favorable conditions, which incurs an easier formation of biofilm. Therefore, the idea of secondary disinfection at households to control the drinking water quality has come up. Ultrasound disinfection provides an easy-operated way for point-of-use disinfection, which can be influenced by the local hydraulic conditions.

Previous studies have monitored the spatial and temporal variations in the microbial community in DWDS but most of cases were based on long-term effect. In this study, a simulated household piping system was sampled intensively over short time scales. Several parameters on the dynamics of microbiological processes such as tATP, cATP, TOC and total cell concentration of drinking water samples were examined at four sampling points under various flow rate at different disinfection time.

The results highlighted that ultrasound disinfection can restrain cell activity but the effect was also influenced by flow rate. Both FCM and ATP data described the microbiological dynamics in the drinking water samples. The cell concentrations decreased when the flow rate was lower. Moreover, operation time of the ultrasonic device showed significant influence on the disinfection efficiency. At the same sampling point, when the system was operated at a constant flow rate, the cell activity decreased with the increase of the operation time of ultrasonic device. Considering the time that a cell travel through the system subjected to ultrasonic effects, the so called exposure time, it is observed that the cell activity decreased with the increase of time that the cell experienced ultrasound. As a result, the cell at the furthest sampling point under the most significant flow rate with longest ultrasound disinfection time showed the lowest activity.

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1. Introduction

The WHO drinking water guideline states: “Water entering the distribution system must be microbiologically safe and ideally should also be biologically stable” (WHO, 2006). Water treatment systems usually have source water protection and multiple barriers for pathogens to ensure that microbiological safety requirements are met (WHO, 2006). The words ‘Biological stability’ is a term that is not accurately defined. But it can be regarded as that the composition and the concentration of the microbial community stay nearly the same before and after running through the drinking water distribution system (DWDS) [1].

The microorganism causes many problems in drinking water distribution systems (DWDS). The primary formation it exists in DWDS is biofilm at the pipe wall [2]. Even after the disinfection process, microorganism regrowth is still one of the most critical factors that impact the water quality, especially between the DWDS and the households[3]. Because it changes the taste and the odor of the drinking water and even corrodes the pipes [4]. Moreover, the leaching of growth-promoting organic compounds in polymer-based pipes can also be found.

To solve the problem mentioned above, a variety of chemical and physical techniques are routinely used, however some cause disinfection residual. For example, monochloramine is the typical disinfection residual that can always be found in the DWDS in many countries [1]. Moreover, chemical treatment destroys the microorganisms on the surface of these clusters but leaves the innermost intact [5]. Due to the carcinogenicity and mutagenicity of chemical disinfection methods, more and more countries in Europe phase it out and turn to control the growth-supporting nutrients in the water to control the stability of water [6].

The Netherlands especially pays more effort on maintaining a high-quality distribution system. Chlorine is wiped out and thus reduces many disinfection by-products (DBPs). Apart from this, many approaches are used to achieve the goal, such as making the distribution system with biologically stable material, producing biologically stable drinking water, improving the distribution system to prevent stagnation [7]. However, no matter which disinfection method is used, the bacteria usually regenerate during processing and distribution, and the concentration in the range of mL-1 of 104-105 cells in which the different microorganisms multiply is attenuated is normal [8] [9]. Apart from that, at household connections, the deterioration of drinking water quality is hard to control since the unpredictable resident time and mixing of new drinking water with contaminated water from the household. Therefore, the idea of secondary sterilization at households’ tap to control the drinking water quality has come up.

Ultrasound processing, as a promising and no-residual water disinfection method, can disinfect bacteria suspensions through the cavitation caused by the changes in the pressure created by the ultrasonic waves [10]. Also, it can deagglomerate bacterial clusters or flocs [11] and at the same time, neither cause microbial resistance as chemical treatment such as chlorination nor be

limited when microorganisms are capable of photoreactivation (self-repair) as UV irradiation [11]. Therefore, ultrasound disinfection gives a hint about dealing with the drinking water between the distribution system and the household connection. Recent studies show that ultrasonic waves can prevent the fouling of heat exchangers and pipes at a certain level. Apart from these, the ultrasonic equipment is with relatively small volume, easy to equip, and uncomplicated to operate. Hence, adapt ultrasound disinfection equipment to household connection to control biofilm formation and reducing the contamination at the user's tap is available.

In this study, ultrasound disinfection equipment was investigated and applied on a set of copper piping system to imitate the part of the DWDS that is in the user's house. The long-term goal of the study is to figure out the optimal condition of ultrasound disinfection in microbial growth control in the domestic drinking water system. A series of research around this theme will be conducted, for example under the different temperatures and different segment time. At the first stage, the aim was to find out how the hydraulic condition and disinfection time affect the efficiency of ultrasound disinfection device and the formation of biofilm. Therefore, the different flow rates of process water were applied in, and the samples were taken under these various rates at different time. Several indicators of each sample, such as total cell number (TCC), total ATP (tATP), cellular ATP (cATP) and total organic carbon (TOC) were measured and compared.

2. Literature review

2.1 Microorganism regrowth in DWDS

Microbial regrowth in DWDS can cause hygienic, aesthetic and technical problems, such as changing in the odor of the drinking water and corrosion of pipes [12]. The primary formation that microorganisms exist in DWDS is biofilm, which is a persistent microbial community. It is estimated that 99% of the total population of bacteria in the world can be found in the form of biofilm [13].

Studies show that the significant growth of biofilm not only happens on metal pipes but also on increasingly-used polymer-based pipes [12] [14]. At the same time, the biofilm growth is affected by water characteristics and operational conditions of DWDS. In drinking water, the accumulation of organic molecules at surfaces creates a relatively nutrient-rich environment. The organic content plays as fuel to feed the growing microorganisms. Biofilm growth is also affected by the presence of inorganic nutrients in the water, for example, phosphorus, which is a significant element of ATP, DNA and RNA. Apart from the nutrient in water, temperature fluctuations can change the cell-to-surface attachment and then the activity of biofilm [14]. However, there is still a lack of an effective method to control biofilm growth [15].

In most European countries, drinking water quality is not monitored at household taps since the

limitation of accessing to the private home. However, at household pipes, the unpredictable resident time and higher temperatures both deteriorate the influence of disinfection and lead to bacteria growth [16]. Besides, it is already known that longer residence time (stagnation) of water contributes to microbial growth in pipes, but the exact impact of stagnation on the microbial quality is still not clear. Moreover, the abundance, structure as well as the composition of bacteria in drinking water are also affected by the hydraulic conditions [17].

2.2 Ultrasound disinfection mechanism

The reason that ultrasound power can generate chemical and physical effects lies in the cavitation phenomenon [18]. Cavitation appears due to the oscillation pressure field (ultrasonic) or fluctuating pressure in the shear layer of water jets (hydrodynamic), which cause pre-existing microscopic bubble nuclei. The bubble nuclei grow explosively and then collapses violently, results in fabulous effects on disinfection since it produces a lot of spots, free radicals, and turbulence that related to liquid circulation by using the energy provided by sound [19].

Within the bubble, hydroxyl and other radical attacks in the gas phase and results in pyrolysis in the gas phase as well as ion reactions. At or near bubble surface in liquid, hydroxyl and other radical attacks in the liquid phase. In bulk liquid solution, hydroxyl and some other radicals attack in the liquid phase. Apart from above, resonant vibration can result in violent bubble collapse, causing temperatures and pressures. Then the bacteria flocs can be deagglomerated by mechanical shear stress during this stage. When increasing the ultrasound dose, the cavitation can destroy cell walls [20].

2.3 Factors that affect microbial growth in DWDS

2.3.1 Adenosine Triphosphate (ATP)

Adenosine triphosphate (ATP) has been suggested as an indicator of biological stability analysis and microorganism regrowth in the distribution system because it is an energy-rich metabolic compound that is generated in all active organisms [1]. A strong relationship between total cell concentration and ATP has been observed in drinking water samples [21]. In this experiment, two kinds of ATP were measured, which were total ATP and cellular ATP. The total ATP (tATP) analysis measures ATP from both living and dead cells. Cellular ATP (cATP) represents ATP from living microorganisms and therefore is a direct indication of the living facilitates inventory management and process optimization.

The reaction that catalyzed by luciferase is:

$$\text{Luciferase} + \text{luciferin} + \text{ATP} \rightarrow \text{luciferase-luciferyl-AMP} + \text{PPi}$$

$$\text{luciferase-luciferyl-AMP} + O_2 \rightarrow \text{luciferase} + \text{Oxyluciferin} + \text{AMP} + CO_2 + h\nu$$

After the reaction, a yellow-green light can be observed, which at 560nm has its peak; the intensity of the light is proportional to the concentration of ATP [22].

The measurement method applied in this experiment is based on the bioluminescence, that is to measure the amount of light produced in the luciferin-luciferase assay [21]. A nucleotide-release buffer, as well as an ATP-activated light-producing substrate and enzyme, were added to expose the ATP in the water sample, then the intensity of the light emitted during the enzymatic reaction is measured. That is a representation of the amount of ATP that present in the water sample [21] [23].

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2.3.2 Total cell concentration (TCC)

In order to better understand the microbial growth as well as microbial survival during drinking water distribution, total cell counting is measured by flow cytometry (FCM). In general, the total bacterial cell concentration is not a design or operative parameters that would be taken into consideration, since the methods to quantify the cell number has not been comprehensive, which induce the difficulty to use the existing data to build up the regulation. At the same time, the study that provides valuable information about the processes and the dynamics of short-term changes is deficient. But FCM has its benefit that it has fast analysis speed, cultivation-independent, and can be combined with various fluorescent dyes so that the bacterial viabilities and activities can be inquired [24, 25]. Recently, online FCM with completely automatic measurement process (automatically collected, stained, incubated and analyzed at routine intervals) has applied a lot [26].

2.3.3 Total organic carbon (TOC)

Total organic carbon (TOC) is a non-specific test, which means TOC will not determine which particular compounds are present (most samples are complex mixtures which contain thousands of different organic carbon compounds). Instead, TOC will inform the user of the sum of all organic carbon within those compounds

3. Material and method:

In order to investigate the efficiency of ultrasound disinfection in microbial growth control in household water piping system at the normal temperature, a lab-scale experimental set-up has already been built. The water samples were taken from this set-up and sent to test some parameters, such as cell number, TOC and ATP. Because those parameters indicate bacteria activities and demonstrate the extent to which ultrasound disinfection technology can reduce microbial populations.

3.1 Description of water piping system:

The experimental set-up of the water piping system is shown in Figure 1. The piping system is made of copper with an internal diameter of 19 mm. The reason to choose copper pipe is that this material is the most common-used one to transport domestic drinking water. Several coupons are combined together. They are the smaller piping segments with two valves and a piece of pipelines. Those coupons are used for taking biofilm sampling as further studies. A flow meter is installed at the beginning of the system. The ultrasound disinfection equipment is installed in the middle of the system. Water enters in the system from the lower part of the set-up. After turning a loop, the direction of water flows through the ultrasound equipment is upward before it turns another circle to the outlet. We chose four sampling points which were located at the inlet, outlet, before and after the ultrasound equipment, respectively.

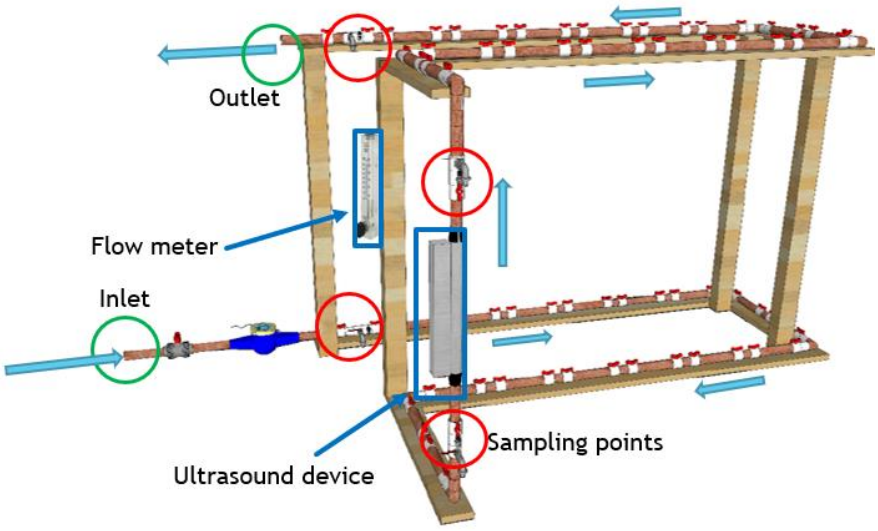

Figure 1. The experimental set-up of the water piping system

3.2 Equipment with setup

3.2.1 Ultrasound disinfection device

The ultrasound disinfection equipment is provided by H2O Technics B.V. with the type of Zinn. The device is a collaboration with Dunea™ and currently used in water supply system to fight *Legionella*. Inside the device, several disks stack together and shake from left to right violently to produce the ultrasound energy.

In this experiment, the ultrasound power can achieve 110 dB,40-45 Wt and the working frequency of the device is 40 kHz, it is a low-frequency ultrasound equipment (high-frequency is 30-100 MHz) [27].

Figure 2. Ultrasound disinfection device provided by H2O TECHNICS

3.2.2 Rotameter and pressure meter

The diameter of the PVC rotameter is 32mm and the rated capacity is from 100 L/h to 1000 L/h. It measures the volumetric flow rate by changing the flow area of the fluid to keep the differential pressure above and below the rotor is constant. The flow rate is controlled by the valve. As the hydraulic condition is an important factor that affects the microbial growth in DWDS, a direct way is to test the efficiency of ultrasound disinfection at a different flow rate.

3.2.3 Flow cytometer

More and more method for the analysis of the microbiological quality of drinking water has been provided by Flow Cytometer. The flow cytometer is used to detect physical and chemical characteristics of a population of cells or particles. In this study, at first two flow rates, online test

mode was used, this test mode needs no fluorescence staining of the microbial cell. The online flow cytometer we used is Sigrist BactoSense. To test the cell concentration of last two flow rates, BD Accuri ™ C6 Flow Cytometer was used. And we try to demonstrate that the efficiency of ultrasound disinfection equipment can be reflected through describing the total cell number.

3.3 Experiment

3.3.1 Sampling

In this experiment, process water was taken from four sampling points located at the inlet of the setting up, before and after ultrasound equipment and outlet of the setting up, respectively. The process water is the drinking water that stores in the bunker. So, the microbiology inside the water is more active than drinking water. The sampling points are shown in Figure 3.

During the experiment, the flow rate in the piping system was changed from 1.7 L/min to 6.8 L/min, by increasing 1.7 L/min for each time, this arrangement is based on the velocity of water (0.1- 0.4 m/S). Each time chose a flow rate and flushing the piping under that rate. After flushing and stabilization for approximate 30 minutes, when the temperature became stable at all sampling points, and the pipe was filled with flowing water, the ultrasound device could start to work.

Then duplicate water samples of 400 mL were taken from four different sampling points with an interval time of 30 minutes from 0 minute to 90 minutes. Then all the samples were sent to do the measurement at once. Samples were stored in clean bottles and put in 4℃ fridge if it didn't finish the test on time. All the bottles and caps were autoclaved before use.

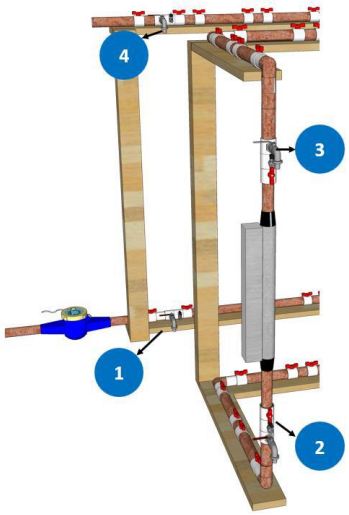

Figure 3. Sampling point diagram

3.4 Measurement

3.4.1 Adenosine Triphosphate (ATP)

Total ATP was determined using the UltraLyse 30[21] (LuminUltra Technologies Ltd, Canada) reagent and a luminometer (QuenchGone21™ wastewater Test Kit, LuminUltra Technologies Ltd, Canada). cATP was determined using the UltraLyse 7 (LuminUltra Technologies Ltd, Canada) reagent and the same luminometer.

To measure tATP, the procedures are as following: extraction, dilution, and assay. In extraction, 1mL of water sample was added into a 2mL UltraLyse 30[21] tube and inverted to mix. After waiting 1 minute for incubation, poured the contents of 2mL UltraLyse 30[21] into a new 8mL UltraLute/Resin (Dilution) Tube. Then 100µL of UltraLyse 30[21] liquid was taken into a new test tube, and 300µL of Luminase[W] was added in. Finally, the test tube was immediately inserted into the luminometer for assay.

To measure cATP, the procedures are as following: Measure the sample volume, filtration the sample, and extract ATP from the filter and dilute, then assay. First 60 mL water sample was measured and filtrated with 0.45 µm filter, then the ATP was extracted from the filter by adding 1mL UltraLyse 7 solution to the syringe and pushing the reagent into a 9 mL Ultralute Tube. Then 100µL Ultralute Tube liquid was taken into a new test tube and 100 µL Luminase[W] was added in. Finally, the test tube was immediately inserted into the luminometer for assay.

3.4.2 Total cell concentration (TCC)

The testing is a fully automated fluorescent staining online monitoring. The testing machines were from SIGRIST Process-Photometer, named BactoSense and BD Accuri ™ C6 Flow Cytometer. The BastcoSense was used to test the first two flow rates (1.7 L/min and 3.4 L/min). The measuring principle is the same as flow cytometry but without manually stain. It takes about 25 minutes to test one sample. BD Accuri ™ C6 Flow Cytometer was used to test the last two flow rates (1.7 L/min and 3.4 L/min). The samples for BD Accuri TM C6 Flow Cytometer needed SYBR green and propidium iodide stains for TCC and viability tests.

Two parallel water samples were taken from each sampling point at different ultrasound disinfection time, due to the tightening time and quick growing speed of microbiology in the water, only one sample at each sampling point at a different time was examined by using BastcoSense. The results contain TCC, HNAP, LNAP and HNAC data.

The samples for BD Accuri TM C6 Flow Cytometer need to be prepared before testing. Briefly,

samples were pre-heated to 35 °C for 5 min, stained with 10 µL mL−1 SYBR Green I (Molecular Probes, Eugene, OR, USA) and then incubated in the dark at 35 °C for 10 min. An Accuri C6 flow cytometer equipped with a 50 mW laser was used for the analysis.

Figure 4. BactoSense (left) and BD Accuri ™ C6 Flow Cytometer (right)

3.4.3 Total organic carbon (TOC)

TOC is the overall organic matter content in the water sample, it is an indicator of the level of natural organic matter (NOM) in the water and a general measured data. The primary source of NOM is the decomposition of plant and animal matter [28]. In this experiment, the TOC of every sample was measured. The measurement was based on 'Oxidative combustion-infrared analysis' method, which is a widely-used TOC measurement method.

The sample preparation is as following: Before the analysis, samples should be filtered using a 0.45 µm syringe filter. Prepare TOC standard solution by adding 1 mL of stock standard solution (1000 PPM) into a 100mL volumetric flask and dilute to 100 ml with ultra-pure water. Take 30 mL standard solution into TOC free vial and mark as a new standard solution. Take 30mL old standard solution that prepared last time into TOC free vial and mark as an old standard solution. Prepare three blanks by adding 30 mL ultra-pure water in the TOC free vial. Then filtrate 30 mL water sample into TOC free vial. After that, 1.6 mL of 2M hydrochloric acid is added into all the vials include old/new standard sample and water samples. All the vials are closed with the aluminium dish and cup. And then shake up the vials.

Afterwards, start up the computer and TOC analyzer. The TOC analyzer draws the water sample through a needle and oxidizes organic into carbon dioxide. The carbon dioxide is quantified by measuring conductivity. Once the test is completed, the analyzer will report the concentration of TOC.

Figure 5. Shimadzu Total Organic Carbon Analyzer (TOC-VCPH)

4. Results and discussion

4.1 ATP results and discussion

4.1.1 Total ATP

The total ATP value at four sampling points under the different flow rate are shown as following:

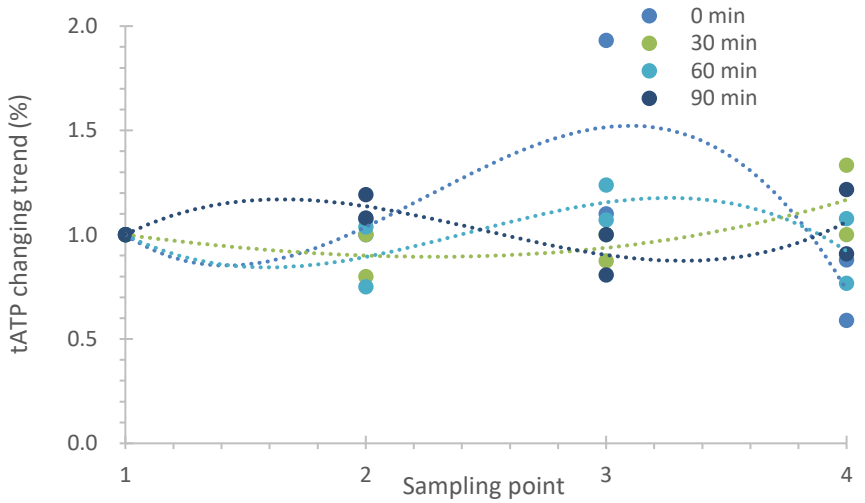

Figure 6. Total ATP at four sampling points when flow rate is 3.4 L/min

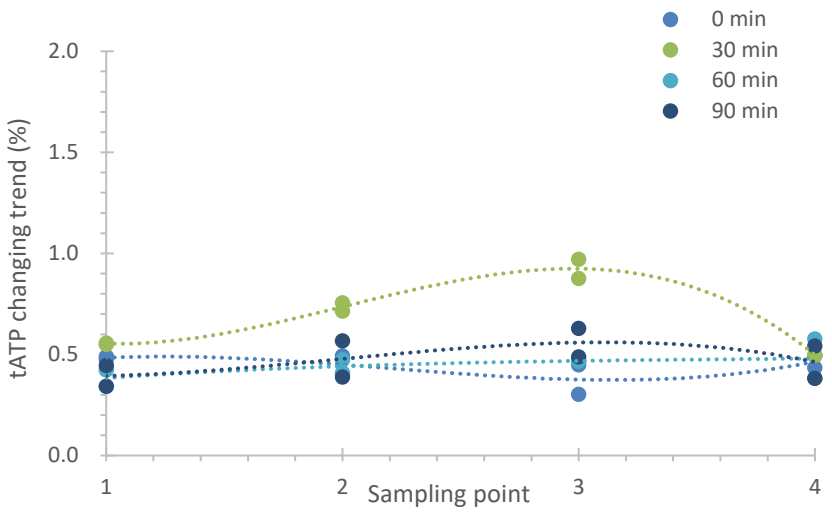

Figure 7. Total ATP at four sampling points when flow rate is 5.1 L/min

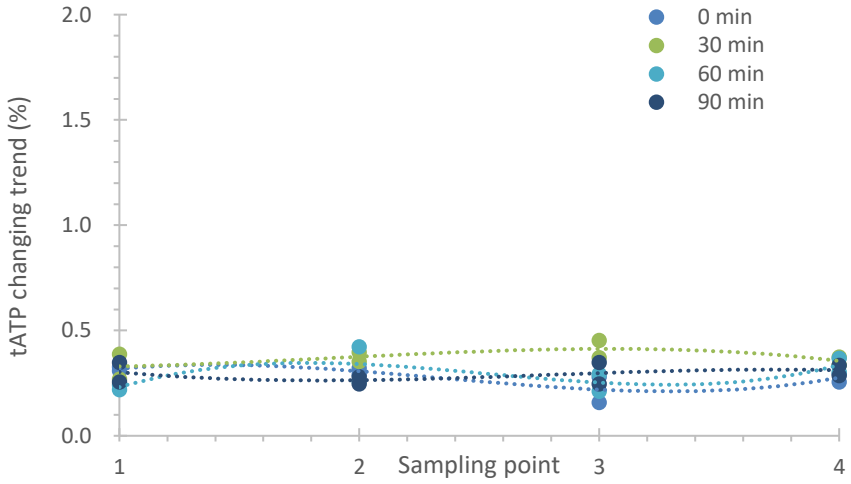

Figure 8. Total ATP at four sampling points when flow rate is 6.8 L/min

tATP at four different flow rate 1.7 L/min, 3.4 L/min, 5.1 L/min and 6.8 L/min were measured. At the same flow rate, when the ultrasound disinfection time was the same, the total ATP value at four different sampling points had no remarkable difference. However, with the increasing the flow rate, the total ATP value decreases from 550 pg ATP /mL to 150 pg ATP /mL at nearly all sampling points.

Since the ultrasound effect is a kind of energy that will continuously work on cell, the effect lasts for a while. There was another time that should be taken into consideration was the time from the water flows in the pipe until it flows out; this is the disinfection time that a cell truly experienced. At the same sampling point, with the higher flow rate, the shorter disinfection time was acted on water. This caused cell received fewer incentives, and then the released ATP became less. The low bacterial activity will result in the lower attachment strength of the cells to the piping materials

as the matrix exopolymer synthesis may have also been inhibited, lowered or delayed [27]. If we compare the data of four sampling points at the same time under the same flow rate, it was evident that the tATP at four sampling points remained almost the same level during the whole experiment. Except for an outlier in the 3.4 L/min experiment.

There are also some uncertainties. The experiments were conducted on different days from lower to higher flow rate. So, when the high flow rate experiment was carried out, there could already have some microbiology attached to the pipe wall. The system is small-scaled so the pipe length maybe not enough to observe a relationship with the travelled distance from inlet point and the location of the ultrasound device. The microorganism amount in the process water was various depend on the provided water by the water company. The water samples that were taken from the system at a particular time could not represent the instantaneous water the passed the sampling point as the water run too fast to be sampled. During the tATP test, the Luminase was sensitive. The luciferase enzyme activity decreased all the time, sometimes even it reached the bottom line of the test; it was still at low activity condition, thus affect the testing result. It is essential to do the luminance check every time before testing. Moreover, 1 mL water sample was diluted ten times, but only 300 µL of the diluted solution was sent to assay, this action also added some error.

4.1.2 cATP

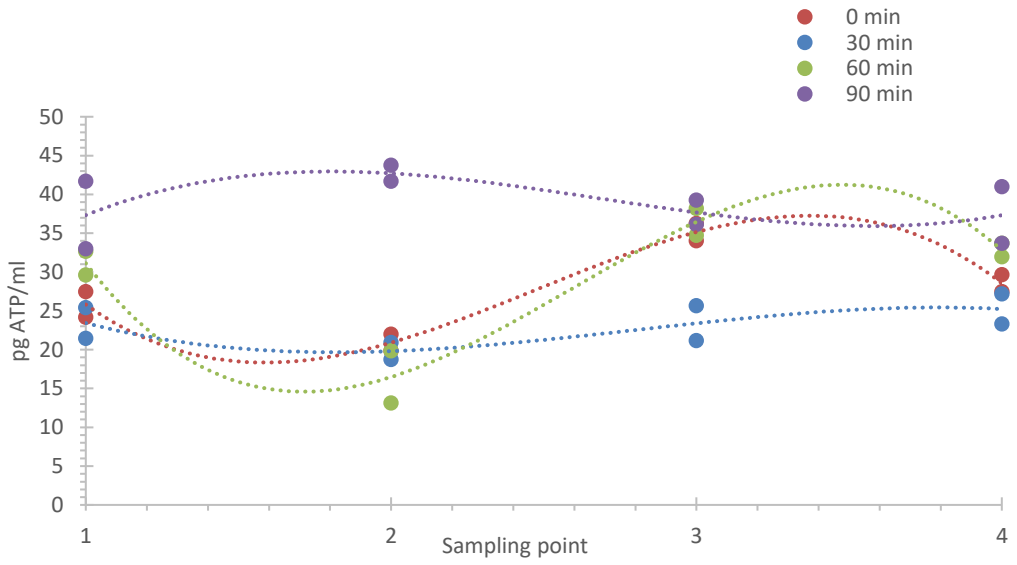

Figure 9. Cellular ATP at four sampling points when the flow rate is 1.7 L/min

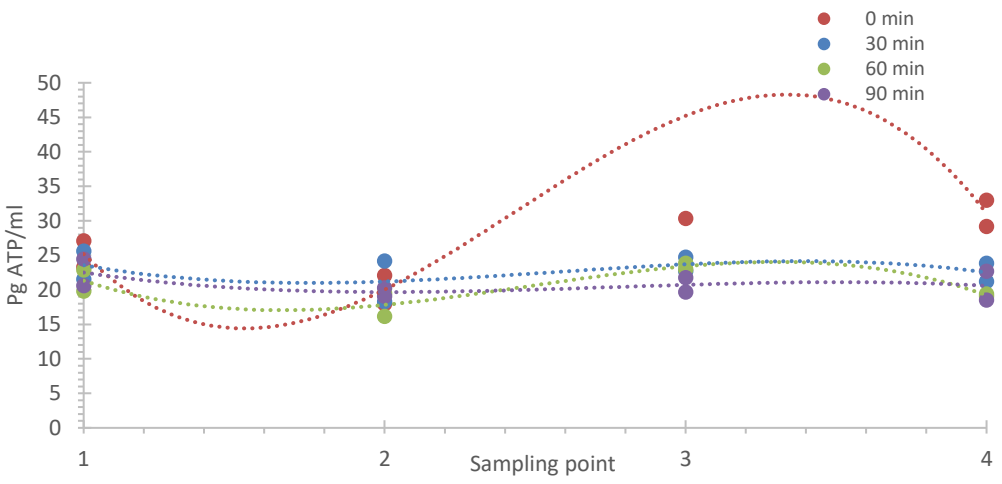

Figure 10. Cellular ATP at four sampling points when the flow rate is 3.4 L/min

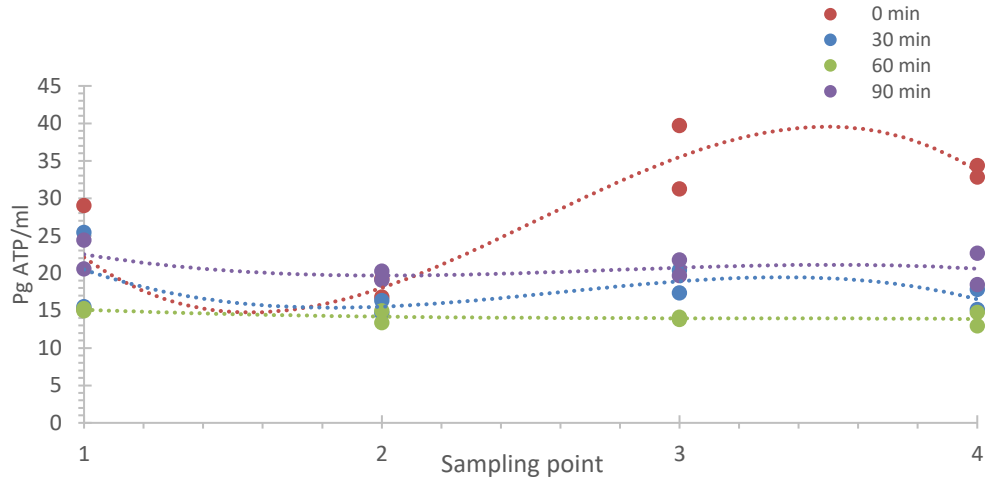

Figure 11. Cellular ATP at four sampling points when the flow rate is 5.1 L/min

For cATP results, the water samples at the same sampling point present good repeatability. Except for one outlier at the third sampling point when the flow rate was 3.4 L/min. cATP shows the same trend as tATP, the results of four sampling points at different ultrasound disinfection time present the same decreasing trend with increasing flow rate. Apart from this, the ratio of cATP against tATP at the same sampling points under the same flow rate over time is decreasing. This result was different with tAPT that rarely changed, reflects that the longer the ultrasound disinfection time, the better the disinfection effect it was.

If the cell wall is unbroken, the fluorescence dye can combine with some particular identifiable substance on the cell wall, so that reach the purpose of zrecognizing and count the cell [22]. Otherwise, if the ultrasound broke the cell wall and killed the cell, there would be some cellular ATP released from inside to the outside of the cell. From the result, it is easy to distinguish that the ratio of cATP and tATP stays nearly the same at four sampling points. It may be due to that

the cell lost some of its biological activity and cATP was not strong enough to cross the cell wall to release outside. So, we included this part of cATP but didn't take the cell where store this part of cATP into account as the total cell. Loss of biological activity has a great restricted effect on subsequent cell growth [29]. The cells will present lower attachment strength to the sonicated materials as the matrix exopolymer synthesis may have also been inhibited, lowered or delayed. And cells will not grow as fast and as much as before. On account of longer travelling time of the dropping water in the piping system, this part of water received more incentive from ultrasound, conduced to less cell activity, or even the broken structure. The water was mixed with up-flow water and was sampled, result in reducing cell concentration.

4.2 Total cell concentration (TCC) results and discussion

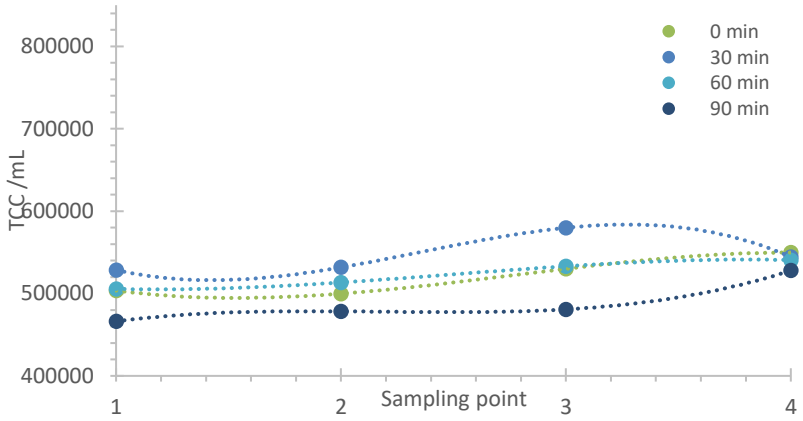

Figure 12. Total cell concentration at four sampling points when flow rate is 1.7 L/min

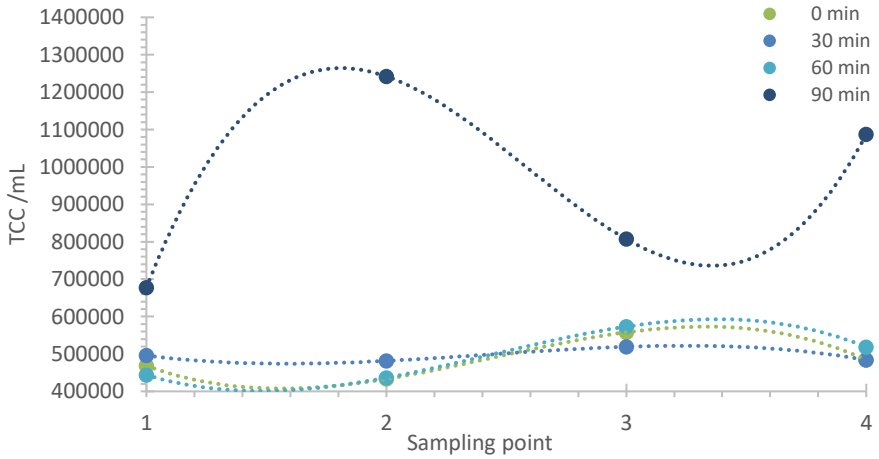

Figure 13. Total cell concentration at four sampling points when the flow rate is 3.4 L/min

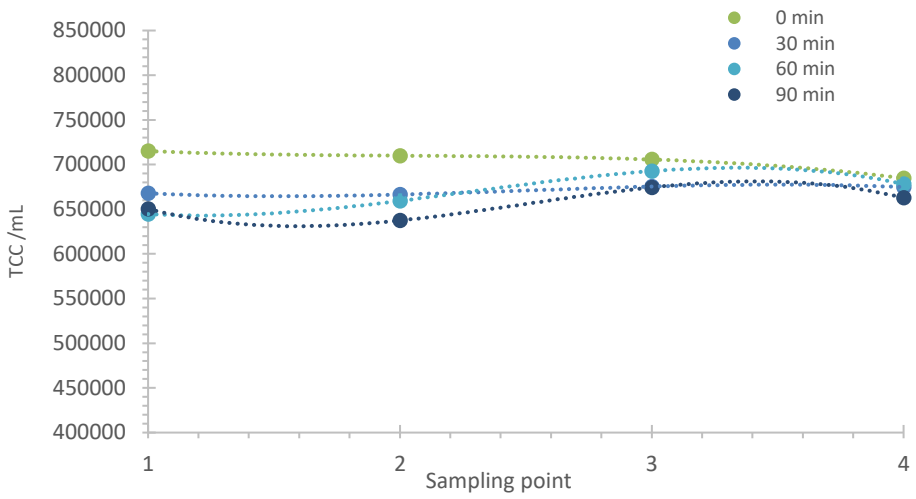

Figure 14. Total cell concentration at four sampling points when the flow rate is 5.1 L/min

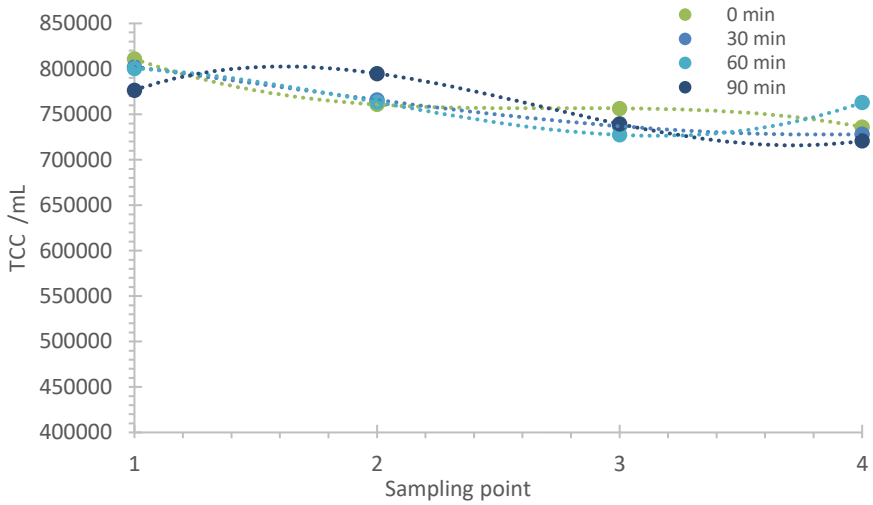

Figure 15. Total cell concentration at four sampling points when the flow rate is 6.8 L/min

Experiment results of total cell concentration (TCC) under four flow rates at four sampling points express that at the same sampling point. The increasing flow rate leads to a trend of higher cell number. When the flow rate was 3.4 L/min, the zero-time TCC at four sampling points was the lowest among the four flow rates. Thus, the TCC at different ultrasonic disinfection time at 3.4 L/min also implied almost the lowest value among the four flow rates. The inlet water quality is a factor that induced this phenomenon, and it is hard to say that the water quality is always consistent. Moreover, the experimental sequence is from a low flow rate to a high flow rate. When performing high flow rate experiments, some microorganisms may have grown on the pipe wall, although the pipe was drained after each experiment.

The most of data of HNAP (High Nucleic Acid Percentage) varies from 57% to 62%, but at 90 minutes when the flow rate was 3.4 L/h, the data of four sampling points showed a noticeable jump as the samples were not tested in time, even they were stored in the fridge. This situation appeared in the last four samples when the flow rate was 5.1 L/min as well.

In order to get rid of the interference of the process of water quality, all the data was compared with the first sampling point and zero time, respectively. Simply use the data at one sampling point to divide the data in the first sampling point at the corresponding time, and the data in the same sampling point at zero time to get the ratio, separately. The significant test was done to compare if there were changes in the same sampling point at different ultrasound times, and indifferent sampling points at the same ultrasound disinfection time.

Results showed that at the different flow rate, in the same sampling point but different ultrasound disinfection time, all the P-value was larger than 0.05. That means that there is no significant difference. These results represent that the length of the ultrasound disinfection time would not affect the disinfection effect at each sampling point.

The results at different disinfection time but different sampling point were alike above. Which indicated that no matter how far the sampling point is away from the ultrasound device, the effect on the cell in the water is the same. So, how extensive is the range of the ultrasound device is hard to define. Also, it would be difficult to draw a conclusion that ultrasound disinfection time affects that microorganism cell numbers in the drinking water piping system.

Also, the increase or decrease trend of all data was traced by using the following value to divide the previous one. However, they each other were irregular to follow. There was no continuously dropping down or going up that can prove the ultrasound disinfection destroyed the cell.

Combine ATP data with TCC data it can be found that even though tATP showed a decreasing trend, the overall total cell concentration was with a small variation and revealed that disinfection process did not damage the cell but reduced the cell activity. In previous, ATP and cell counts have been proposed as alternative parameters for HPCs to determine microbial regrowth in drinking water distribution system [29] [30]. The advantages of using ATP and cell counts over HPCs are that ATP and cell counts can be determined fast and are better indicators for active biomass (ATP) or total cell counts than HPCs, as only a small percentage of bacterial cells in drinking water can be cultivated by HPCs [21].

4.3 TOC results and discussion

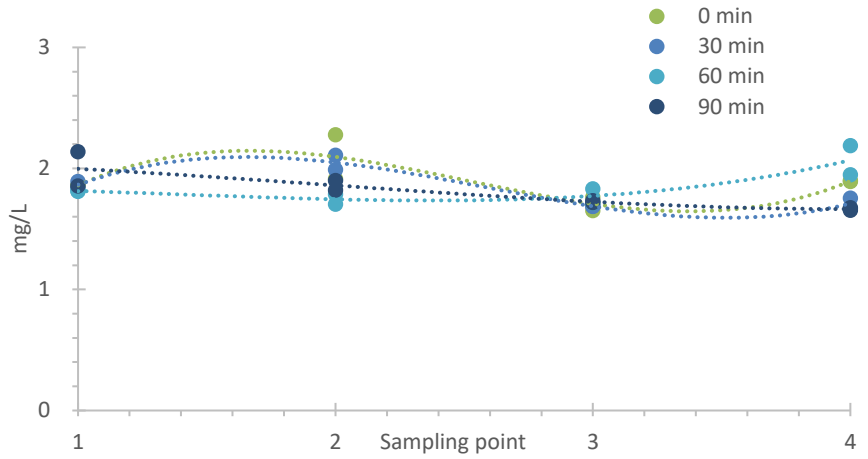

Figure 16. TOC values at four sampling points when the flow rate is 1.7 L/min

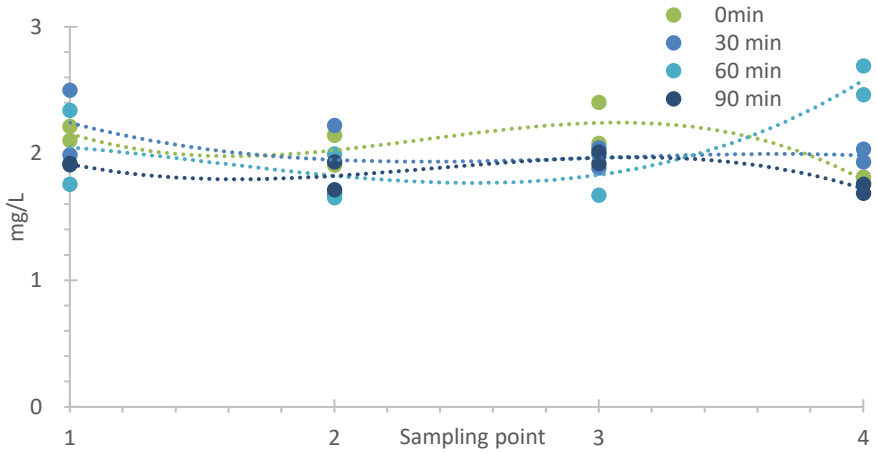

Figure 17. TOC values at four sampling points when the flow rate is 3.4 L/min

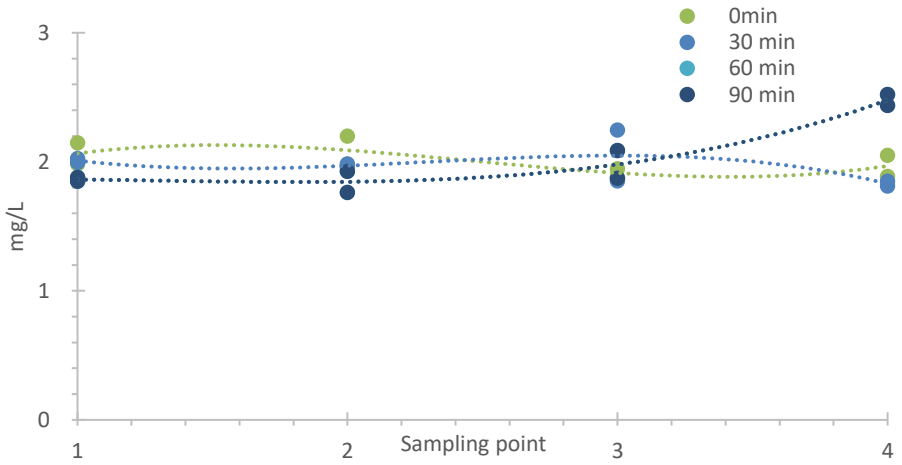

Figure 18. TOC values at four sampling points when the flow rate is 5.1 L/min

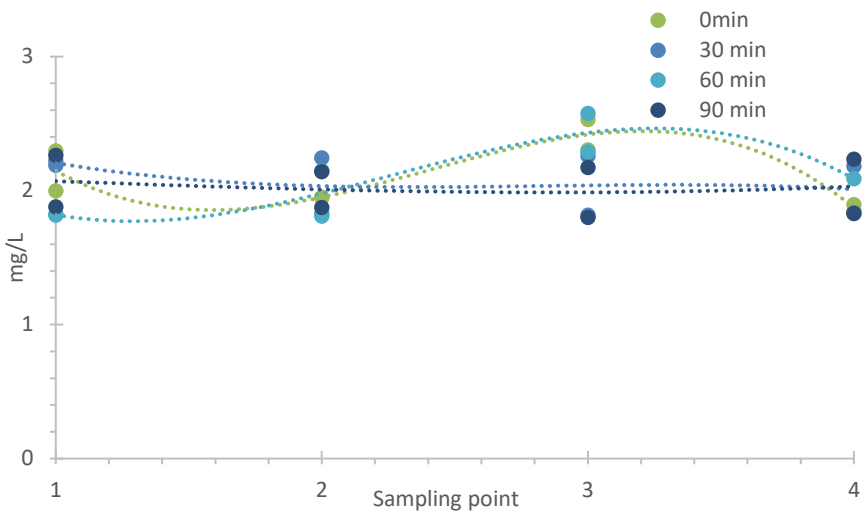

Figure 19. TOC values at four sampling points when the flow rate is 6.8 L/min

The detailed data are in an appendix. TOC concentrations were slightly but consistently stable at all sampling point. Thus, we can't conclude that ultrasound disinfection has a significant effect on TOC in flowing water. At the same time, the initial concentration of TOC was not that high.

5. Conclusion

Base on the results, ultrasound disinfection technology has little effect on drinking water itself. It can hardly influence the quality of the water. At the same time, the research aims to focus more on the long-term influence for the piping system that low-frequency ultrasound reduces the activity of the microbiology, thus reducing the growth of its cell, this action can effectively inhibit the further growth of biofilm.

- The ultrasound disinfection could restrain cell activity; at the same time, the effect was also influenced by the flow rate. The higher the flow rate was, the lower the cell concentration was.

- Moreover, the disinfection time dramatically affects the disinfection effect. At the same sampling point, when the flow rate was fixed, the longer time the device was on, the lower the cell activity. Ninety minutes of disinfection time made the lowest activity of the cell.

- Another time that describes the ultrasound device applied to a cell is counted from the time it enters the system to the time it exits the system. Based on that, at different sampling points but under the same flow rate, the longer time the cell experienced ultrasound, the lower activity it has. So, the cell at last sampling point under the largest flow rate with longest ultrasound disinfection time showed the most moderate activity.

- It is useful to adapt small volume of ultrasound disinfection equipment on household piping system, so the

6. Recommendation

The experimental set-up:

- Current experiment set-up does not have a control system to compare. All the experimental was compared with the data at zero time at the first sampling point or one by one before and after. A control pipe can be added parallel with the existing set-up to get rid of the influence of the changing process water.
- A more extended piping system may be better to see the ultrasound disinfection effect, according to the current experimental results, most of the data have a rare difference between four sampling points. A reason lies in that could be the pipe is too short to widen the gap.
- The coupons are connected by two different material (copper pipe and plastic valve), water leakage occurs all the time. It would be better to change the valve material to brass. This action is costly and will affect the biofilm formation rate.

Experiment and test:

- Choose normal flow cytometer that needs manually stained instead of the online flow cytometer that automatically stained, since the cells grow so fast that the later one is too slow to get one result. Plus, online flow cytometer does not provide information about cell viability.
- TOC test has little reference value.
- Future works should contribute to a better understanding of ultrasound mechanistic effects on bacterial cells and optimizing the industrial application. For example continuous disinfection and discontinuous disinfection, different low-frequency ultrasound, etc.

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Appendix

Abbreviation

TCC [/mL] Total cell count = The total number of bacteria detected inside the TCC gate. It is an addition of HNAC and LNAC: TCC = HNAC + LNAC

HNAP [/mL]: High Nucleic Acid Percentage = The percentage of HNA cells relative to TCC: HNAP = HNAC/TCC

LNAC [/mL]: Low Nucleic Acid Count = The number of LNA cells inside the TCC gate but below the HNA/LNA boundary.

HNAC [/mL]: High Nucleic Acid Count = The number of HNA cells inside the TCC gate and above the HNA/LNA boundary.

ATP original experimental data

tATP

Table 1. tATP at 3.4 L/min

RUL_tATP(pg ATP/ml)	Flow rate:3.4L/min=204 L/h			
Sampling point	0 min/tot	30 min/tot	60 min/tot	90 min/tot
1	464.78873	557.43243	520.27027	469.62233
1	516.43192	501.68919	501.68919	451.55993
2	499.21753	445.94595	390.20270	559.93432
2	516.43192	501.68919	520.27027	487.68473
3	964.00626	390.20270	483.10811	451.55993
3	568.07512	501.68919	557.43243	487.68473
4	568.07512	520.27027	520.27027	549.11433
4	499.21753	501.68919	427.36486	442.83414

Table 2. tATP at 5.1 L/min

RUL_tATP(pg ATP/ml)	Flow rate:5.1L/min=306 L/h			
Sampling point	0 min/tot	30 min/tot	60 min/tot	90 min/tot
1	227.83761	310.34483	178.30424	469.62233
1	246.06462	275.86207	212.59352	451.55993

2	246.06462	318.96552	185.16209	559.93432
2	209.61060	379.31034	212.59352	487.68473
3	291.63215	379.31034	226.30923	451.55993
3	255.17813	439.65517	260.59850	487.68473
4	247.08819	258.62069	198.87781	549.11433
4	247.08819	250.00000	246.88279	442.83414

Table 3. tATP at 6.8 L/min

RUL_tATP(pg ATP/ml)	Flow rate:6.8L/min=408 L/h			
Sampling point	0 min	30 min	60 min	90 min
1	152.39295	150.68493	125.98425	120.37037
1	159.31990	195.00403	110.23622	157.40741
2	145.46599	177.27639	165.35433	157.40741
2	166.24685	177.27639	133.85827	120.37037
3	152.39295	177.27639	141.73228	157.40741
3	159.31990	186.14021	118.11024	120.37037
4	145.46599	194.61538	157.48031	157.40741
4	147.92176	169.23077	157.40741	148.14815

cATP

Table 4. cATP at 1.7 L/min

RUL_cell ATP(pg ATP/ml)	Flow rate:1.7L/min=102L/h			
Sampling point	0 min	30 min	60 min	90 min
1	27.45632	25.41411	32.67683	41.66667
1	24.16610	21.46585	29.61647	32.98611
2	19.85478	20.89857	19.84303	43.75000
2	21.96506	18.72022	13.12997	41.66667
3	34.03676	21.14817	34.72222	36.11111
3	36.23780	25.64103	38.19444	39.23611
4	29.63467	23.28114	31.94444	40.97222
4	27.47901	27.18403	33.68056	33.68056

Table 5. cATP at 3.4 L/min

RUL_cATP(pg ATP/ml)	flow rate:3.4L/min=204L/h			
Sampling point	0 min	30 min	60 min	90 min
1	27.09069	25.61837	19.80500	24.44842

1	23.26266	21.49588	22.85192	20.57245
2	17.96231	18.25677	19.50030	19.08169
2	22.08481	24.14605	16.14869	20.27430
3	60.07067	22.67373	23.85212	21.76506
3	30.32980	24.73498	22.95766	19.67800
4	29.15194	21.20141	19.08169	22.65951
4	32.97998	23.85159	19.37984	18.48539

Table 6. cATP at 5.1 L/min

RUL_cATP(pg ATP/ml)	flow rate:5.1 L/min=306 L/h			
Sampling point	0 min/cell	30 min/cell	60 min/cell	90 min/cell
1	29.06162	25.44351	15.27431	24.44842
1	15.17274	15.52288	14.96259	20.57245
2	19.25770	16.33987	13.40399	19.08169
2	16.80672	14.70588	14.96259	20.27430
3	31.27918	17.39029	13.81962	21.76506
3	39.74238	20.42484	14.13134	19.67800
4	32.83066	15.17274	14.75478	22.65951
4	34.40151	17.85714	12.98836	18.48539

TCC

Table 7. Total cell concentration at 1.7 L/min (/mL)

Flow rate=1.7 L/min	0 min	30 min	60 min	90 min
1	503630	528584	505516	466155
2	499766	531728	513402	478345
3	529985	579724	533077	480747
4	549694	544388	540540	527861

Table 8. Total cell concentration at 3.4 L/min (/mL)

Flow rate=3.4 L/min	0 min	30 min	60 min	90 min
1	468290	496039	443754	676921
2	433255	481503	436447	1242131
3	557891	519128	572939	807173
4	483861	483616	518118	1087009

Table 9. Total cell concentration at 5.1 L/min (/mL)

Flow rate=5.1 L/min	0 min	30 min	60 min	90 min

1	715000	667800	644660	650280
2	709920	666440	659360	637420
3	705640	675420	692660	674460
4	684740	674660	678220	662700

Table 10. Total cell concentration at 6.8 L/min (/mL)

Flow rate=6.8 L/min	0 min	30 min	60 min	90 min
1	810940	802180	800360	776500
2	760780	765800	763340	794860
3	756520	736980	727380	739360
4	736040	728200	763000	720800

TOC

Table 11. Total organic carbon at 1.7 L/min (mg/L)

TOC/(mg/L)	Flow rate:1.7 L/min=102 L/h			
Sampling point	0 min	30 min	60 min	90 min
1	1,861	1,836	1,811	2,138
1	1,861	1,893	1,816	1,856
2	1,913	2,108	1,705	1,820
2	2,278	1,990	1,785	1,900
3	1,763	1,686	1,715	1,732
3	1,652	1,688	1,831	1,717
4	1,891	1,754	1,948	1,654
4	1,900	1,666	2,188	1,675

Table 12. Total organic carbon at 3.4 L/min (mg/L)

TOC/(mg/L)	Flow rate:3.4 L/min=204 L/h			
Sampling point	0 min	30 min	60 min	90 min
1	2,102	2,499	2,340	1,922
1	2,215	1,989	1,758	1,912
2	2,143	2,222	1,652	1,934
2	1,908	1,679	1,998	1,713
3	2,081	2,046	1,993	2,012
3	2,403	1,885	1,671	1,922
4	1,767	2,035	2,692	1,757
4	1,815	1,934	2,464	1,687

Table 13. Total organic carbon at 5.1 L/min (mg/L)

TOC/(mg/L)	Flow rate:5.1 L/min=306 L/h			
Sampling point	0 min	30 min	60 min	90 min
1	2,146	1,995	1,914	1,878
1	1,983	2,024	1,959	1,849
2	2,198	1,984	2,137	1,927
2	1,981	1,955	2,215	1,763
3	1,880	2,246	2,029	2,088
3	1,947	1,851	1,974	1,871
4	2,050	1,811	1,931	2,436
4	1,887	1,849	1,805	2,521

Table 14. Total organic carbon at 6.8 L/min (mg/L)

TOC/(mg/L)	Flow rate:6.8 L/min=408 L/h			
Sampling point	0 min	30 min	60 min	90 min
1	2,298	2,222	2,210	2,263
1	1,998	2,189	1,824	1,879
2	1,948	2,244	1,816	2,143
2	1,952	1,824	1,809	1,874
3	2,304	2,259	2,149	2,174
3	2,532	1,818	2,289	1,800
4	1,896	2,183	2,576	2,235
4	1,849	1,836	2,089	1,830