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DEVELOPMENT OF A NOVEL LOW FREQUENCY ULTRASONIC STERILIZATION SYSTEM

NOWA METODA STERYLIZACJI ULTRADŹWIĘKAMI NISKIEJ CZĘSTOTLIWOŚCI

Autorzy opracowali nowatorski systemu sterylizacji/dezynfekcji ultradźwiękami o niskiej częstotliwości (ok. 20 kHz) i przedstawili sposób jego aplikacji na istniejących basenach i SPA. Oscylator został dołączony do powierzchni wibrującej jako element w postaci okrągłego stożka i dlatego przemieszczająca się fala jest generowana jako fala kulista. Fala kulista odbija się od powierzchni komory sterylizacyjnej co skutkuje wytworzeniem wtórnej fali kulistej od powierzchni. Moc generowana dzięki efektowi kawitacji, falę pierwotną i przyspieszenie wibracyjne została zrównoważona. Z tych przyczyn istnieje możliwość stabilnej sterylizacji zawracanej wody drogą kontroli częstotliwości +/- 1KHz. Bakterie Legionella pneumophila subsp. pheumophilila ATCC 33152, typowe bakterie tzw. higieniczne w ilości 10000 cfu/100 ml zostały użyte w badaniach. (Cfu to jednostka tworząca kolonię (colony-forming unit) – jednostka określająca liczbę mikroorganizmów lub komórek w materiale badanym: dopisek tłumacza). Po 1 sekundowej ekspozycji na proces niskoczęstotliwościowy (kolonie umieszczono w 150 ml butli szklanej) nie zaobserwowano obecności żywych kolonii. Badania powtórzono, na tej samej kulturze bakterii i czasie ekspozycji, w nierdzewnej komorze wypełnionej 15L wody gorącej źródlanej wody o pH=9.2 z liczebnością kolonii bakterii równej 11000 cfu/100 ml. Również po tym eksperymencie nie zaobserwowano obecności żywych kolonii bakterii. Następnie szczepy o 140 cfu/ml Escherichia coli JCM1649, 210 cfu/ml Enterobacter faecalis JCM5803, i 190 cfu/ml Staphylococcus aureus NBRC100910 były kolejno eksponowane przez 5 min na proces niskoczęstotliwościowy. I w tych przypadkach, po ekspozycji, nie zaobserwowano obecności żywych kolonii.

Aparatura ultradźwiękowa o parametrach 150 mm x 150 mm x 494 mm, 250 W/200 V, została zainstalowana po systemie filtracyjnym na rurze ciągu recyrkulacji wody w SPA. Nie wykryto obecności kultur Legionella lub coli po pierwszych 9 dniach kontroli. Miesięczne inspekcje, ukierunkowane na te kultury bakterii, przez 7 lat i 8 miesięcy nie wykryły obecności bakterii. Po zastosowaniu systemu sterylizacji ultradźwiękami o niskiej częstotliwości, autorzy skutecznie zredukowali dawkę chloru, używaną do dezynfekcji.

We developed a novel low frequency (around 20 KHz) ultrasonic sterilization apparatus and its operation method for actual pool and spa is represented. Oscillator is attached to vibration surface as circular cone shape and, therefore, traveling wave is generated as spherical wave. The spherical wave reflects at the surface of sterilization chamber, resulting in secondary spherical wave from the surface. The power generated by cavitation, straight wave and vibration acceleration are equalized. By these reasons, it is possible to sterilize recirculating water stably by controlling frequency +/- 1 KHz. 10000 cfu/100 ml of Legionella pneumophila subsp. pheumophilila ATCC 33152 was used as a typical hygienic bacterium and no cfu was detected after 1 sec low frequency treatment using 150 ml glass bottel. The strain was added to 15 L of pH 9.2 of a hot spring water in a stainless chamber to become 11000 cfu/100 ml and treated at the same condition, resulting in no detection of the strain. 140 cfu/ml of Escherichia coli JCM1649, 210 cfu/ml of Enterobacter faecalis JCM5803, and 190 cfu/ml of Staphylococcus aureus NBRC100910 were respectively irradiated for 5 min. No colony detection were shown in all cases. The ultrasonic apparatus, 150 mm x 150 mm x 494 mm, 250 W/200 V, was installed after the filtration facility at the pipe line of circulation of a spa. No detection of Legionella and coli form group for initial 9 days were recorded. Monthly inspection of these bacteria for 7 years and 8 months at this time represent no detection of these bacteria. We can successfully reduce the dosage of chlorine after introducing the low frequency ultrasonic sterilization apparatus.

1. Introduction

Legionella genus is one of important criteria for maintaining legal water quality of pool and spa, especially facilities operated continuously for 24 hr with circulation of water. Conventional circulation method of water in pool and spa facilities use filtration process for purification of water before circulation. In many cases, transparency of filtered water is sufficient but bacteria, such as coli form bacteria and Legionella spp. still remain. Therefore, chlorination is forced to use for sterilization of filtered water for recirculation and also to satisfy Japanese guideline for public bath. Nevertheless, hygienic bacteria are found in some facilities after chlorination due to original water quality used for pool and spa and unknown reasons. Ozonation, UV radiation, and other sterilization methods are additionally considered for sanitation of bacteria but relatively high initial and maintenance costs inhibit their application to actual facilities. To solve the problem, we developed a novel low frequency ultrasonic (around 20 KHz) sterilization apparatus and its operation method for actual facilities. In this report, we will describe the detail of the apparatus and results for sanitation of typical hygienic bacteria by laboratory experiments and actual spa.

2. Materials and Methods

2.1 Possible Theory of Sterilization by Low Frequency Ultrasonic Wave

Destruction of bacterial cells by low frequency ultrasonic wave is conventionally used for extraction of cell components in the fields of microbiology, molecular biology, and etc. Sterilization of bacteria by ultrasonic wave means physical destruction of cell. Whenever bacteria are irradiated by ultrasonic wave of certain frequency, membrane of cells is destroyed at a moment by disruption of bubbles in water. The image of theory of sterilization by ultrasonic wave is shown in Figure 1.

2.2 Characteristics of Oscillator and Generator

Figures 2 and 3 show newly developed oscillator and generator, respectively. Oscillator is attached to vibration surface as circular cone shape and, therefore, traveling wave is generated as spherical wave. The spherical wave reflects at the surface of sterilization chamber, resulting in secondary spherical wave production from the surface. The power generated by cavitation, straight wave and vibration acceleration are equalized (Figure 2).

Figure 3 represents scheme of newly developed low frequency ultrasonic sterilization apparatus. The apparatus is composed of circular cone shaped oscillator, reaction chamber, and controller. It is possible to select strength in proportion to temperature and flow rate. Besides, stable power output is performed.

2.3 Laboratory Test 1 (Small Scale)

We used *Legionella pneumophila subs. pneumophilila* ATCC 33152 as typical hygienic strain for pool and spa. 10,000 cfu/100 ml of the precultured strain solution were aseptically added to sterilized 150 mL glass bottle. The bottle was set in a water chamber and ultrasonic treatment was performed at 1, 3, 5, and 10 seconds respectively. After irradiation, all of solution were respectively centrifuged and remaining pellet were cultivated for 5 days at 37 0C using GVPC agar medium. Generated colonies in each day were counted and represented as cfu/ml. All experiments were performed at least three times and average value is described.

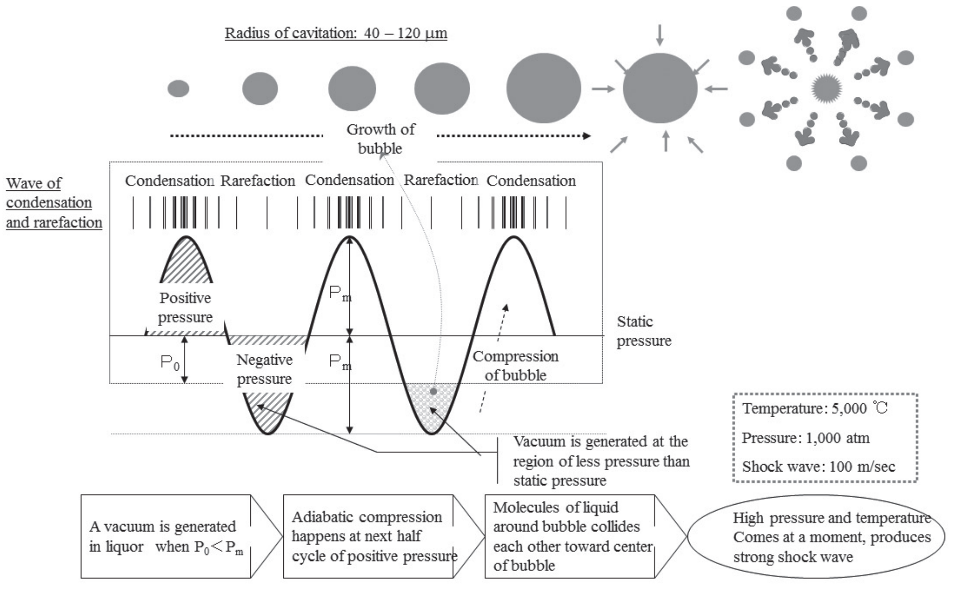

Fig. 1. A proposed theory of sterilization by low frequency ultrasonic wave
Rys. 1. Proponowana teoria sterylizacji za pomocą fal ultrasonicznych o niskiej częstotliwości

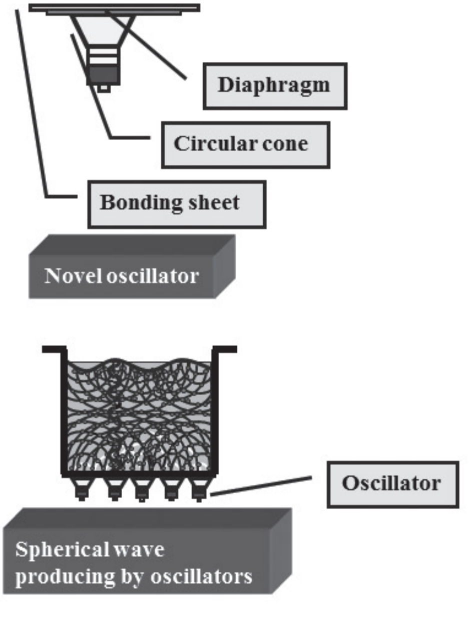

Fig. 2. Newly developed circular cone shaped oscillator
Rys. 2. Nowo rozlinięty oscylator stożkowy

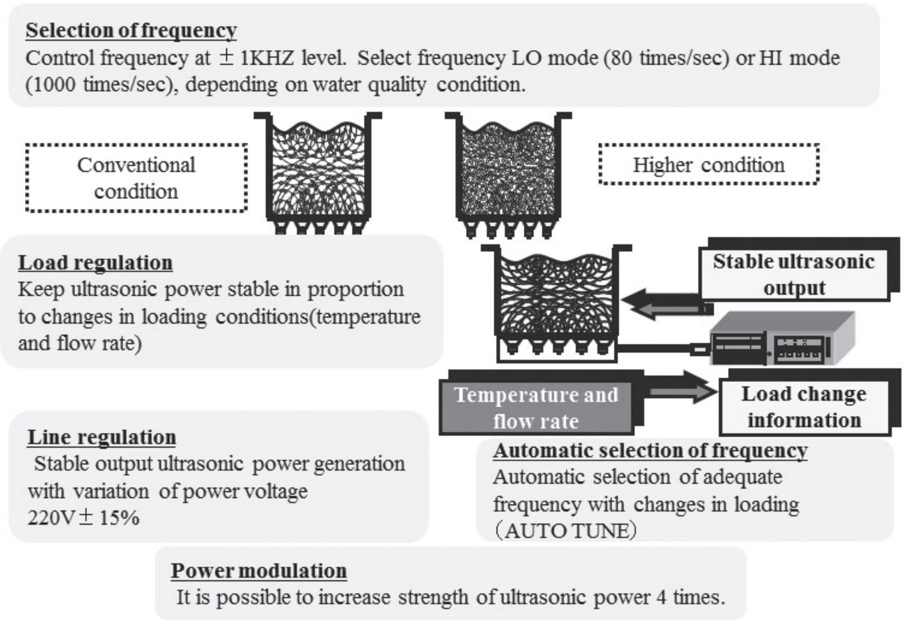

Fig. 3. Novel low frequency ultrasonic sterilization apparatus
Rys. 3. Aparat do sterylizacji ultrasonicznej o niskiej częstotliwości

2.4 Laboratory Test 2 (Large Scale)

Legionella pneumophila subs. pneumophilila ATCC 33152, *Escherichia coli JCM1649, Enterobacter faecalis* JCM5803, and *Staphylococcus aureus* NBRC100910 were respectively used as typical hygienic bacteria.

For *counting of Legionella* strain, same method described in previous session is used. Standard Methods Agar Deeps was used for counting of *E. coli, Ent. faecalis, and St. aureus* and cultivated at 37^0C for 48 hrs. *Legionella pneumophila subs. pneumophilila* ATCC 33152 was added to 15 L stainless chamber of 0.9 % NaCl solution and hot spring water collected at Meiho Onsen, Gifu, Japan to become 11,000 cfu/100 ml, respectively. Irradiation of ultrasonic wave was 1 second. The pH and ORP values of respective water before and after treatment were also monitored.

E. coli, Ent. faecalis, and St. aureus were respectively added to become 1.4 x 10^2 CFU/ml, 2.1 x 10^2 CFU/ml, and 1.9 x 10^2 CFU/ml in 3 % sodium chloride solution and irradiated for 5 minutes.

2.5 Installation of Ultrasonic Sterilization System to a Spa and its Operation

We installed a novel low frequency ultrasonic sterilization apparatus at a Spa of Meguminoyu, Kagamigahara, Gifu, Japan. The volume of the bath tub was 28 m^3 and whole water is filtered and circulated 8 times in 16 hrs operation in a day. The outline of the system is shown in Figure 4 and the method for installation of oscillator to pipe-line is illustrated in Figure 5. Besides, actual scene is shown in Figure 6.

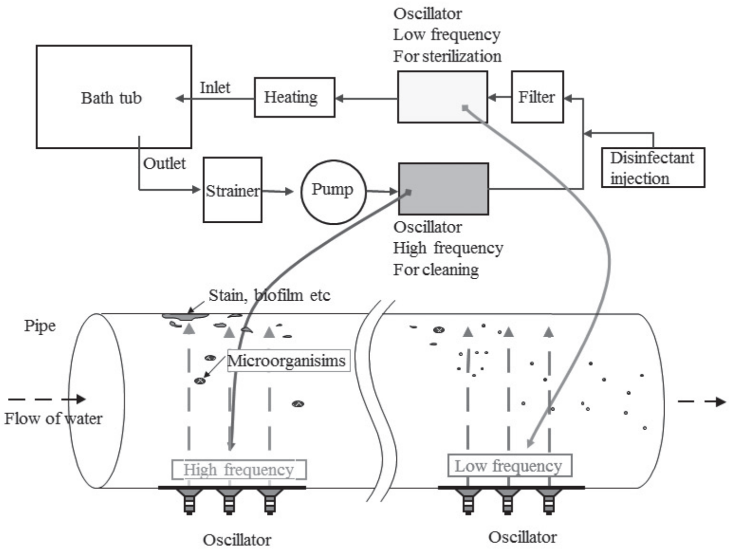

Fig. 4. Instalation of ultrasonic sterilization system in pipe of a Spa
Rys. 4 Instalacja systemu sterylizacji w rurociągu prowadzącym do Spa

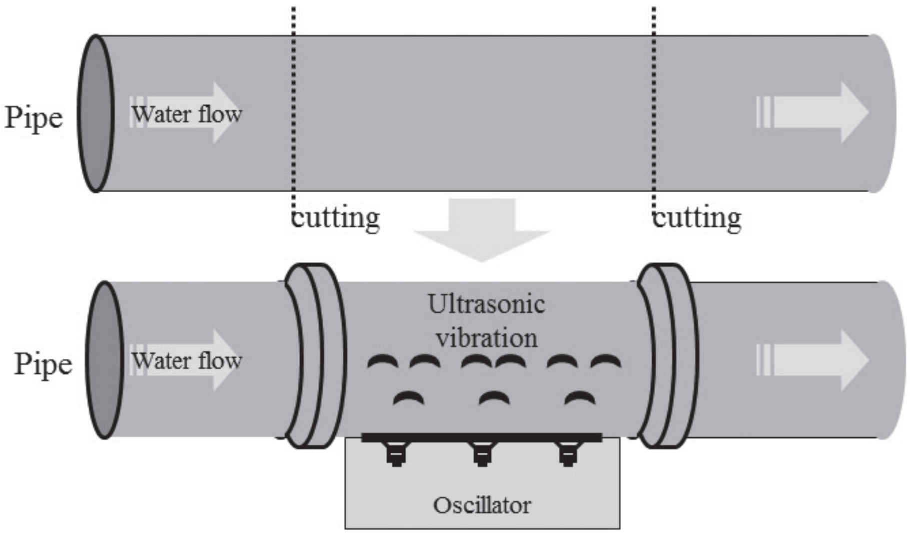

Fig. 5. Fixing of an oscillator at a pipe line
Rys. 5. Naprawa oscylatora w rurociągu

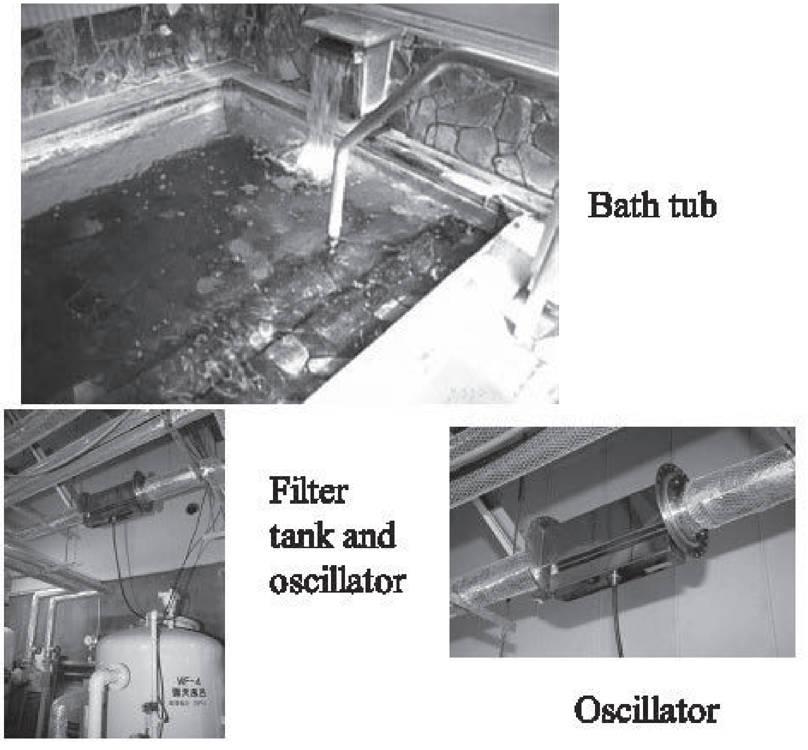

Fig. 6. Scene of actual spa operating ultrasonic sterilization
Rys. 6. Przedstawienie aktualnego działania ultrasonicznej sterylizacji w spa

The size of flange part is 180 mm in diameter and 15 mm length. Oscillator part is 150 mm in diameter and 494 mm length, and 3 mm thickness. Materials used is stainless steel SUS316L and power is 100 W/200 V at conventional and 250 W/200V at maximum condition.

Test period was 9 days from 13th November 2006 to 21st November 2006. Before the experiment, water quality of bath tub was maintained by circulation and chlorine addition. At the first day of experiment, water was discarded and chlorine addition was stopped. Then, fill the bath tub with fresh water and spa powder was added to feel more comfortable for client using the bath, and ultrasonic treatment has started. The irradiation was performed at 1 hr interval while Spa open (approximately from 8:00 to 24:00) and spa powder is supplemented once a day. Water for analysis was collected aseptically once a day by 9th day for monitoring of *Legionella* and coli form bacteria levels, and consumption of $KMnO_4$ and turbidity as indicators of organics. Coli form bacteria was counted after 48 hrs incubation at 37 using desoxychlorate agar and consumption of $KMnO_4$ and turbidity were measured according to the Standard Methods.

3. Results and Discussion

3.1 Laboratory Test 1 (small scale)

No colony was detected after 1 sec low frequency treatment. We treated up to 10 seconds and could not find any colony on each plate. Initial level of *Legionella* cells were 10,000 cfu/100 ml and the results represent that low frequency ultrasonic treatment is very effective to sterilize *Legionella* cells with in short second.

3.2 Laboratory Test 2 (large scale)

We studied the effect of water quality on sterilization of Legionella cells. The results is shown in Table 1. In both cases of authentic physiological solution of sodium chloride and actual spring water, Legionella could not detect at all after 1 sec irradiation of low frequency ultrasonic wave. Initial pH value 9.2 of spa water did not change after treatment, ORP as well. Other typical hygienic bacteria, E. coli, Ent. *faecalis*, and *St. aureus* were respectively irradiated. 1.4 x 10^2 CFU/ml of E. coli, 2.1 x 10^2 CFU/ml of *Ent. faecalis*, and 1.9 x 10^2 CFU/ml of *St. aureus* were respectively irradiated for 5 min and no colony were found after treatment (Table 2)

Table 1. Effect of low frequency ultrasonic irradiation on cell number of Legionella and water quality
Tabela 1. Efekt ultrasonicznego naświetlenia niskiej częstotliwości

	Legionella, cfu/100 ml		pH		ORP, mv	
	Before IR	After IR	Before IR	After IR	Before IR	After IR
Physiological solution	1.1×10^4	ND	–	–	–	–
Spa water	1.1×10^4	ND	9.2	9.2	60	60

All samples were irradiated for 1 second. IR is the abbreviation of irradiation.
ND means not detected.

Table 2. Sterilization of Escherichi coli, Enterobacter faecalis, and Staphylococcus aurerus by low frequency ultrasonic wave
Tabela 2. Sterylizacja Eshcerichi coli, Enterobacter faecalis i Stapholococcus aurerus przy pomocy fal niskiej częstotliwości

	coli form bacteria, cfu/ml	
	Before IR	After IR
3 % NaCl	1.4×10[3]	ND

	, cfu/ml	
	Before IR	After IR
3 % NaCl	2.1×10[2]	ND

	Staphylococcus aureus, cfu/ml	
	Before IR	After IR
3 % NaCl	1.9×10[2]	ND

All samples were irradiated for 5 minutes. IR is the abbreviation of irradiation. ND means not detected.

3.3 Operation of Low Frequency Ultrasonic Sterilization System at a Spa

The ultrasonic apparatus, 150 mm x 150 mm x 494 mm, 250 W/200 V, was installed after the filtration facility at the pipe line of circulation of a spa. The results are shown in Table 3. No detection of Legionella and coli form group for initial 9 days were recorded. Consumptions of $KMnO_4$ were started at 1.0 mg/l level and increased gradually up to around 4.0 mg/l, then, bath tub water was discarded and fresh water was filled to the bath as conventional operation. While, values of turbidity are stable around 0.1. In any cases, Legionella, coliform bacteria, consumption of $KMnO_4$, and turbidity are respectively below the water quality standards of spa, Japan. Monthly inspection of these bacteria for 8 years and 86 months at this time represents no detection of these bacteria at all.

Table 3. Long term irradiationn of low frequency ultrasonic wave to actual spa water.
Tabela 3. Naświetlanie wód spa ultrasonicznymi falami niskiej częstotliwości

Period	*Legionella* cfu/100ml	coli form bacteria cfu/100 ml	Consumption of $KMnO_4$ mg/l	Turbidity
1st	0	0	6.0	Bellow 0.1
Water exchanged and stopped to add chlorine				
2nd	0	0	1.0	Bellow 0.1
3rd	0	0	1.4	Bellow 0.1
4th	0	0	2.2	0.1
5th	0	0	2.0	0.1
6th	0	0	3.8	0.1
7th	0	0	4.0	0.1
8th	0	0	3.6	0.1
Water exchanged				
9th	0	0	1.0	Bellow 0.1
Legal standards	Bellow 10	Bellow 1	Bellow 25	Bellow 5

4. Conclusions

Hot spring is the one of important and comfortable facilities for Japanese for relaxing and maintaining physiological and psychological activity normal. For keeping hygienic condition, Japanese government recommends to use chlorine for sanitation of hot spring and public bath where water in bath tub is circulating. In this manual, 0.2 - 0.4 mg/l of the residual chlorine concentration should be kept for 2 hrs during daily operation period (Ministry of Health, Labor and Welfare, Japan, 2001) [1]. Many people smell chlorine in this concentration and feel uncomfortable while staying in these facilities. How to reduce the dosage of chlorine and keep the sanitary condition at lower residual chlorine level is wide concern.

For this purpose, we focused on the characteristics of ultrasonic wave and found that low frequency ultrasonic wave (around 20 kHz) has activity to destroy bacterial cells in liquid in short time. We developed a novel low frequency (around 20 kHz) ultrasonic sterilization apparatus and tested its ability using mainly Legionella and other hygienic bacteria by both laboratory tests and actual spa. We found that newly developed apparatus is very useful to kill bacteria in a very short time. Comparing to chlorine disinfectants, low frequency ultrasonic wave is more effective to sterilize Legionella. It takes 5 minutes to sterilize the strain at 0.5 mg/l of residual chlorine level and 30 – 60 minutes to reduce 99 % of the strain at 0.1 mg/l of residual chlorine (Tokyo Metropolitan Institute of Public Health, 2014) [2] but in case of low frequency wave, only one second is necessary for sterilize 104 cfu/100 ml of the strain. Chlorine disinfectants reduce activity proportional to increase in pH value. For example, effective HClO level at pH 7.0 is 76 % but the level drops to 24 % at pH 8.0. Therefore, more costly dose of chlorine is required to keep residual chloride concentration at 0.2 – 0.4 mg/l. On the other, low frequency ultrasonic sterilization is not reduced sterilization activity at pH 9.2. Shimizu et al (2014) [3] has reported the effect of low frequency ultrasonic treatment to reduce the level of a plant pathogen, Pythium sp.

Finally, we can conclude that low frequency ultrasonic sterilization is effective to kill bacteria in a moment and decrease the dosage of chlorine at spa and pool.

References

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3) Shimizu, S. et al (2014) J-GLOBAL 14A0730185 (in Japanese)