ภาควิชาจุลชีววิทยา
คณะแพทยศาสตร์ศิริราชพยาบาล
มหาวิทยาลัยมหิดล
2 ถนนพรานนก บางกอกน้อย
กรุงเทพฯ 10700

Department of Microbiology
Faculty of Medicine Siriraj Hospital
Mahidol University
2 Prannok Road, Bangkoknoi
Bangkok 10700, THAILAND

Tel. (662) 0-2411-3106, 0-2419-7053-57, 0-2419-7061-63, 0-2419-7067-69
Fax. (662) 0-2411-3106, 0-2411-0263, 0-2418-4148

Assessment of WATERLIFE system in killing *Mycobacterium bovis* BCG Pasteur, *E. coli*, *Staphylococcus aureus*, *Bacillus* sp. and *Candida albicans* in water

Angkana Chaiprasert, Therdsak Prammananan, Srisuda Pannanusorn, Prapaporn Srilohasin, Nida Chairatana

Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

INTRODUCTION

The WATERLIFE procedure bases on the principle of natural oxidation. Because of charging the oxygen molecules, which are located in the air, positively and negatively charged oxygen ions are produced. These ions neutralize their charge with oxidizable partners (organic and inorganic substances like virus and bacteria) in the water and eliminated them.

The blower fan sucks the air which should be treated and passes it on directly to the ionization unit. The ionized oxygen ions are led directly to the emission sheet where they react with germs existing in the water and in the air and eliminate them.

In the year 2001 Christian A. (Institut für Lufthygiene, Berlin) tested WATERLIFE for decontamination of a gram-negative bacterium, *Pseudomonas diminuta,* and found that the system could successfully eradicate the bacterium of recirculation water in circulation air humidifiers.

In 2003 Horn S. from Department of water hygiene and environmental microbiology, Ruhrgebiets, Gelsenkirchen, Germany evaluated WATERLIFE system to kill *Legionella pneumophila* in water. He tested by inoculating *Legionella pneumophila* ATCC 3152 into 400 litres water tank to obtain a final concentration of $1.4x10^5$ cfu/ml and incubated at 36 ºC for 8 days. He took the first sampling for identification of the existing bacterial concentration and set the WATERLIFE system into operation and took water samples at 1h, 2h, 3h, 22h, 26h, 28.5h, 48h, 72h and 96h for the first test. The result showed

no detectable colonies after one hour of treatment. The second test, he used the same condition for inoculation and incubation. The water sample was taken at time zero for determination of cfu. After WATERLIFE system is operated the water samples were assessed at 0.25h, 0.5h, 0.75h, 1h, 1.5h, 2h, 4h, 6h, 24h and 96h. There was no colonies detected at 2h and more than 2h operation.

These above data revealed that the WATERLIFE system is effective for killing gram-negative bacteria contaminated in water at 10^5 cfu/ml in not more than 2 hours.

OBJECTIVE OF THIS STUDY

To assess the activity of WATERLIFE system produced by LUWATEC GmbH Weissenfels, Germany in killing *Mycobacterium bovis* BCG Pasteur , *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* sp. and *Candida albicans* in water.

MATERIALS AND METHODS

The WATERLIFE system (LUWATEC GmbH Weissenfels, Germany) as shown in Figure 1 was used in all experiments.

ESCHERICHIA COLI, STAPHYLOCOCCUS AUREUS AND *BACILLUS* SP.

These bacteria were grown in LB broth at 37degree Celcius for 16 hr for preparation of the inoculum. Approximately 10 to the fifth cell/ml of 20, 30 and 200 liter water (for *E. coli*, *S. aureus*, and *Bacillus* sp.) were used for each experiment. At time zero, 5, 15, 30, 60, 90, 120 min after turn on the WATERLIFE system, 1 ml of treated water was taken out for dilution (from 10 to the minus 1 to the minus 3) and spreading on LB agar plate for colony counting in triplicate set. At 180 and 240 min after the WATERLIFE treatment, 10 ml of the water were taken out and concentrate 10 time before spreading on LB agar plate for colony counting. All the tested plates were incubated at 37 degree Celcius for 1-3 days, the number of colonies grown on each plate were count with naked eyes.

CANDIDA ALBICANS

The yeast was grown on Sabouraud dextrose agar (SDA) at room temperature for 2-3 days then subcultured to Sabouraud broth and incubated at 37 degree Celcius overnight for preparation of inoculum. Ten to the fifth cell/ml of *C. albicans* of 200 liter water were also used for this experiment. After turning on the WATERLIFE system at time zero, 5, 15 30, 60, 90, 120, 180 and 240 min, the water samples were taken out as previously described for bacteria, diluted or concentrated for appropriate concentration and spread on SDA for colonies counting. All plates were incubated at the same condition as bacteria and at each time point the plates were done in triplicate.

MYCOBACTERIUM BOVIS BCG (PASTEUR)

The preparation of *M.bovis* BCG inoculum was done in Mycobacterium Growth Indicator Tube (MGIT) by performing standard growth curve of cell concentrations range from 10 to the sixth to 10 to the first cell/ml in duplicate sets. The 10 ml of 1 MacFarland cell-suspension were inoculated in 200 ml Middle-Brook 7H9 (M7H9) broth plus 10%OADC supplement in 500 ml Erlenmeyer Flask, and incubated at 37 degree Celcius for 3.3 days. These 200 ml culture of *M. bovis* BCG were used as inoculum in 27 liter water in 55 liter-plastic tank stand in the safety cabinet class 2 (Gelman B2000, Australia) during the test with WATERLIFE system. At time zero, 5, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min the water samples were taken out for spreading on the M7H10 agar plates for colonies counting (in triplicate sets) and also 0.5 ml each were inoculated in a MGIT (in duplicate) at each time point to detect viability of *M. bovis* BCG in MGIT 960 system. The tested plates and the MGITs were incubated at 37 degree Celcius for 1 month for assessment of the bacterial growth.

Results

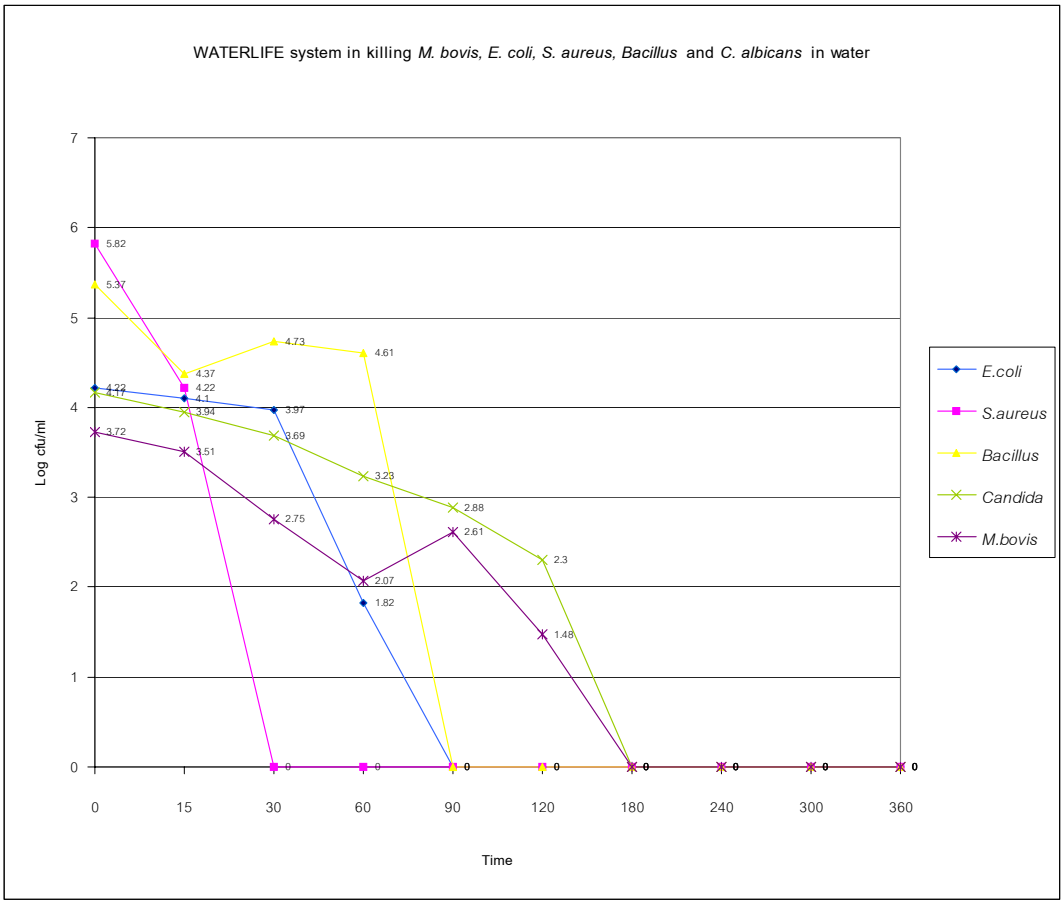
Number of colonies of bacteria, yeast and mycobacterium are shown in Table 1. In 5 species tested, *S. aureus* is the most sensitive to the ionizing oxygen whereas *C. albicans* and *M. bovis* BCG are the most tolerant. The corelation of the log number of viable cells to the exposured time to ionizing oxygen from WATERLIFE system are shown in Figure 2.

Species	Number of Colonies cfu/ml at different time point (X from tripicate sets)									
	0	15	30	60	90	120	180	240	300	360
Escherichia coli	16667	12667	9433.3	66.667	0	0	0	0	0	0
Staphylococcus aureus	660000	16667	0	0	0	0	0	0	0	0
Bacillus sp.	236667	23333	54000	40667	0	0	0	0	0	-
Candida albicans	14667	8767	4966.7	1700	766.7	200	0	0	0	0
Mycobacterium bovis BCG pasteur	5266.67	3233.3	565	116.67	403.33	30	0	0	0	0

Table 1 Number of colonies of *E. coli, S. aureus, Bacillus* sp., *C. albicans*, and *M. bovis* BCG grown on appropriate media after treatment with WATERLIFE system at different time points (number in the table indicate minute).

Figure 1 The WATERLIFE system from LUWATEC (Weissenfels, Germany)

Figure 2 Sterility curves of *E.coli, S. aureus, Bacillus* sp., *C. albicans*, *and M. bovis* BCG after treatment with the WATERLIFE system about 6 hours (360 minutes).



CONCLUSIONS

From our experiments, it reveals that the WATERLIFE system can destroy both gram negative bacteria like *E.coli* and gram positive bacteria such as *S. aureus* and *Bacillus* sp., the yeast *C. albicans* and the higher bacteria *M. bovis* BCG. *Staphylococcus aureus* is the most sensitive to this system and is killed after 30 minutes of water-treatment. *Bacillus* and *E. coli* are moderately sensitive to this system. Cell concentrations of 10 to the fifth of these bacteria in water are killed after 90 minutes treatment with WATERLIFE system. The yeast *C. albicans* and higher bacteria like *M. bovis* BCG are the most tolerate tested organisms. They are killed after 120 minutes of treatment (see table 1 and figure 1). Determination of mycobacterial growth using MGIT 960 system also reveals concordant results with

colony count on M7H10 agar plates. The tubes show no increasing of growth unit (GU) after WATERLIFE treatment for 180 minutes or more.

In addition to above mentioned results, the WATERLIFE system also revealed that the production of bio-film in the tested water-tank could be destroyed and prevented. With this principle of using ionizing oxygen molecules, some other bacteria contaminated in water and environment such as *Pseudomonas aeruginosa, Comamonas acidovorans, Stenotrophomonas maltophilia, Bacillus amyloliquefaciens and Sphingobacterium thalpophilum* were also destroyed. A systematic approach for testing with these bacteria are further recommended.

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