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Evaluation of USR Technology on the Destruction of HPC Organisms

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Abstract: The primary aim of this study was to investigate the effect of ultrasonic reactor (USR) at different sonication times on HPC. Heterotrophs are broadly defined as microorganisms that require organic carbon for growth. A variety of simple culture-based tests, which are intended to recover a wide range of microorganisms from water, are collectively referred to as heterotrophic plate count or HPC. USR is able to inactivate bacteria through a number of physical, mechanical and chemical effects arising from acoustic cavitation. Results showed that a significant increase in percent kill for HPC bacteria with increasing duration of sonication in 42 kHz after 90 min sonication.

Key words: Ultrasonic reactor (USR), sonication, Heterotrophic Plate Count (HPC), acoustic cavitation

INTRODUCTION

General description: Heterotrophs are those microorganisms that cannot synthesize all their required nutrients and therefore rely on external sources of organic and inorganic material for nutrition. Most bacteria, including many of the bacteria associated with drinking water systems, are heterotrophs (Bartram *et al*., 2003).

Use of HPC in water management: HPC testing has a long history of use in water microbiology. At the end of the 19th century, HPC tests were employed as indicators of the proper functioning of processes (and of sand filtration in particular) and thereby as indirect indicators of water safety. Use as a safety indicator declined with the adoption of specific fecal indicator bacteria during the 20th century. HPC measurements nevertheless continue to figure in water regulations or guidelines in many countries. HPC measurements nevertheless continue to figure in water regulations or guidelines in many countries. HPC measurements are used (Eaton *et al*., 1998; Federal-Provincial-Territorial Committee on Drinking Water in Canada, 2004).

To indicate the effectiveness of water treatment processes, thus as an indirect indication of pathogen removal; As a measure of numbers of regrowth organisms that may or may not have sanitary significance and as a measure of possible interference with coliform measurements in lactose-based culture methods. This application is of declining value as lactose-based culture media are being replaced by alternative methods that are lactose-free.

Health effects: Unlike other indicators, such as Escherichia coli or total coliforms, low concentrations of HPC organisms will still be present after drinking water treatment. In general, water utilities can achieve heterotrophic bacteria concentrations of 10 colony-forming units (cfu) per milliliter or less in finished water. Within a distribution system, increases in the density of HPC organisms are usually the result of bacterial regrowth. The density reached can be influenced by the bacterial quality of the finished water entering the system, temperature, residence time (i.e., stagnation), presence or absence of a disinfectant residual, construction materials, surface-to-volume ratio, flow conditions, the availability of nutrients for growth and in chloraminated systems, the chlorine/ammonia ratio and the activity of nitrifying bacteria. As mentioned, the heterotrophic population in potable water may include a broad range of genera, including some opportunistic bacterial pathogens. In numerous studies, heterotrophic bacteria isolated from water have been shown to possess very few virulence factors and are therefore of no human health consequence. At a recent expert meeting dealing with

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HPC in drinking water management, it was also concluded that HPC in drinking water are not a health concern to the general public (Bartram *et al*., 2003; Eaton *et al*., 1998; Federal-Provincial-Territorial Committee on Drinking Water in Canada, 2004).

History of USR: The wider uses of ultrasonic in environmental remediation have been reviewed recently. Many of these studies have been on a small batch scale. The destruction of microorganisms by power ultrasound has been of considerable interest since the 1920s when the work of Harvey and Loomis was first published. Their work examined the reduction in light emission from a seawater suspension of rod shaped Bacillus fishery caused by sonication at 375 kHz under temperature-controlled conditions. They showed that heating appeared to injure the bacterial colonies but that ultrasonic appeared to have a greater effect. In the 1960s research concentrated on understanding the mechanism of ultrasonic interaction with microbial cells (Hughes and Nyborg, 1975).

Cavitations phenomenon and associated shear disruption, localized heating and free radical formation were found to be contributory causes. By 1975, it was shown that brief exposure to ultrasonic caused a thinning of cell walls attributed to the freeing of the cytoplasm membrane from the cell wall (Hughes and Nyborg, 1975).

USR disinfection: The basis for ultrasonic applications is that acoustic cavitation can effect a number of mechanical, acoustic, optical, chemical and biological changes in liquid. Earlier studies had shown that all biological effects are due to acoustic energy absorption by biological cell (Joyce *et al*., 2003).

Cavitation can be in general defined as the phenomena of the formation, growth and subsequent collapse of microbubbles or cavities occurring in milliseconds releasing large magnitudes of energy (Fig. 1).

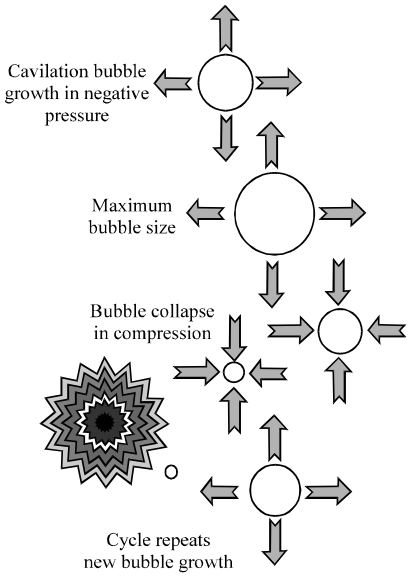

Fig. 1: Cavitation and implosion process

The local effects of the cavitation phenomena can be given as generation of very high temperatures (of the order of 1000-5000 K) and pressures (100-50,000 bar) as well as release of free radicals due to pyrolysis of water (Joyce *et al*., 2003; Mason *et al*., 2003). Also, cavitation bubbles produce enough energy to mechanically weaken or disrupt bacteria or biological cells via a number of processes (Mason *et al*., 2003; Parag *et al*., 2003):

- Forces due to surface resonance of the bacterial cell are induced by cavitation. Pressures and pressure gradients resulting from the collapse of gas bubbles which enter the bacterial solution on or near the bacterial cell wall. Bacterial cell damage results from mechanical fatigue, over a period of time, which depends on frequency (Fig. 2).

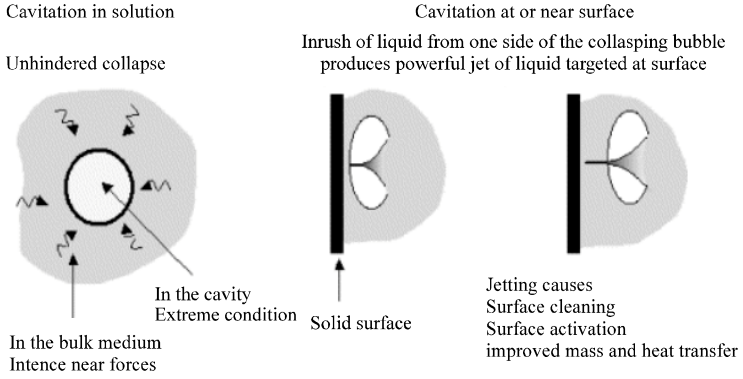

Fig. 2: Effects of cavitation in solution and near to a surface

- Shear forces induced by microstreaming occurs within bacterial cells.
- Chemical attack due to the formation of radicals (H. and OH.) during cavitation in the aqueous medium. These radicals attack the chemical structure of the bacterial cell wall and weaken the cell wall to the point of disintegration.
- Amongst the final products of this sonochemical degradation of water is hydrogen peroxide (H_2O_2), which is a strong bactericide.

MATERIALS AND METHODS

Disinfection apparatus: Disinfection was carried out with an ultrasonic processor equipped with a power supply and operating at 42 kHz. The fixed power has been applied throughout the experiments. The temperature of the sonicated solutions was maintained at 20°C. Ultrasonication was applied to samples with the following characteristics (Table 1).

Methods: All experiments were performed at a constant ultrasonic frequency of 42 kHz; in a cell filled with capacity 1.5 Lit bath. 500 mL HPC suspension was placed in the reaction vessel and sonicated with an ultrasonic cleaning bath (Bransonic Cleaning bath), one operating at 42 kHz (Fig. 3). The bacterial suspensions were sonicated using different sonication times. Samples were taken after 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85 and 90 min and analyzed. Two different methods were used to estimate the effect of USR on HPC (WHO/SDE/WSH, 2002; WHO, 1996).

Pour plate method: The pour plate method involves adding a small volume of sample (0.1-2.0 mL) to melted agar (44-46°C) and then pouring the mixture into plates. The plates are then incubated for the required time. Plates can be stored at 35°C for 48 h or at 20-28°C for 5-7 days. Using the pour plate method, colonies are generally small and compact and therefore easier to count. On the other hand, because the colonies are submerged, they are often slower growing and difficult to transfer (if necessary). Also, because the sample is being added to agar between 44 and 46°C, this could result in heat shock to the bacteria.

Spread plate method: The spread plate method has the advantage of using solidified agar, eliminating the possibility of heat shock. The sample is spread on the surface of the agar (0.1-0.5 mL) and the plates are incubated as required. Some media give better results under specific conditions. The resultant colonies can be easily transferred and their colony morphology can be distinguished. Since the sample applied has to be absorbed into the agar surface, only a small sample volume can be used.

Table 1: Characteristics of USR

Parameters	Characteristics
Input	220-230 V
Output	70 W
Power	155 W
Frequency	42 kHz
Capacity	1.5 Lit

Fig. 3: Laboratory US reactor for irradiation treatment

RESULTS

The results indicate that sonication of a 500 mL suspension of HPC at 42 kHz produces a significant effect. The disinfection time for the analyses of polluted water fungi were 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85 and 90 min (Table 2).

The results using ultrasound bath for the HPC inactivation of water show that the highest and lowest HPC reduction after disinfection within 90 min by USR was 99.82 and 95.17% (Fig. 4-6).

In this study results showed that a significant increase in percent kill for HPC with increasing duration of exposure in 42 kHz. Ultrasound bath, applied under such conditions, leads to greater efficiency in lyses of HPC cell.

Table 2: Percent kill average of HPC organisms

Disinfection time (min)	Percent kill average (%)
20	95.17
25	96.69
30	97.31
35	98.36
40	98.45
45	98.54
50	98.58
55	98.64
60	98.73
65	98.84
70	98.88
75	98.89
80	99.63
85	99.71
90	99.82

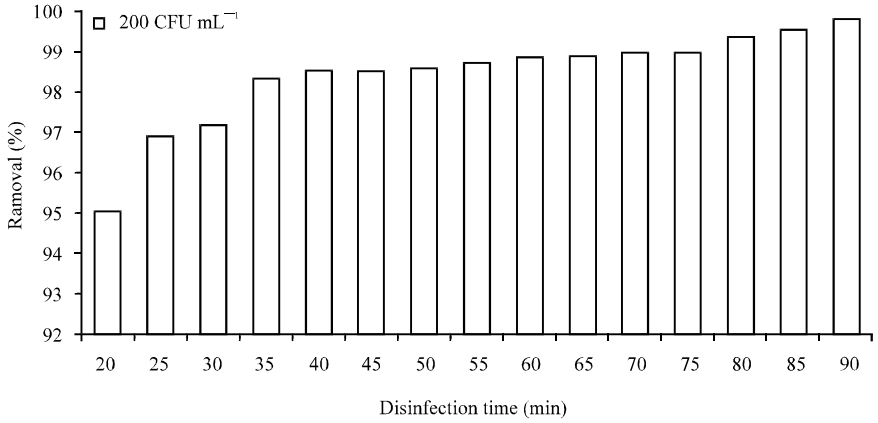

Fig. 4: Removal percentage of HPC organisms by USR vs. Disinfection time for 200 cfu mL^{-1}

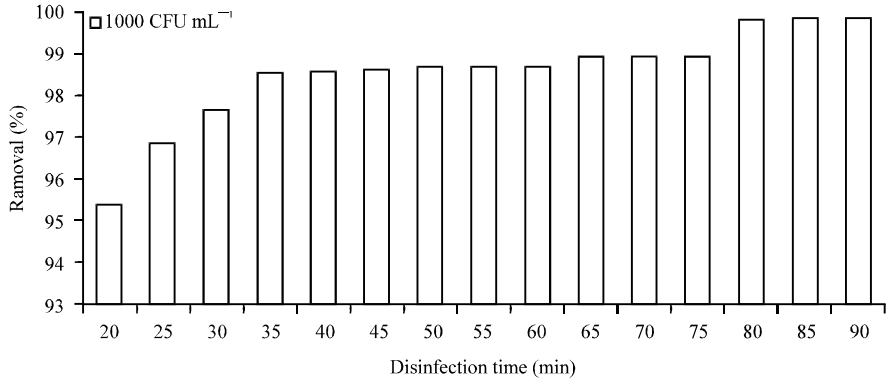

Fig. 5: Removal percentage of HPC organisms by USR vs. Disinfection time for 1000 cfu mL^{-1}

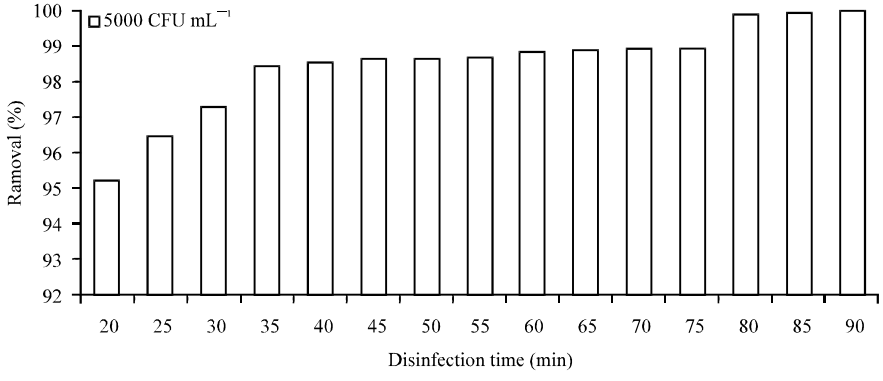

Fig. 6: Removal percentage of HPC organisms by USR vs. Disinfection time for 5000 cfu mL^{-1}

DISCUSSION

Ultrasonic (lower frequency) in low volumes of bacterial suspension results in a continuous reduction in bacterial cell numbers. Also, Ultrasonic (lower frequency) in large volumes results in an initial rise in cell numbers suggesting declumping of the bacteria but this initial rise then falls as the declumping finishes and the kill rate becomes more important.

This study showed that it is possible to decrease the number of HPC bacteria present in the water and that the process depends on exposure time, type of organism,

frequency and intensity of the ultrasonic. Also, cavitation intensity and hence the efficacy of sonochemical processes depend strongly on the equipment as well as the liquid phase physicochemical properties

The results using ultrasonic bath for the bacterial inactivation of water show that disinfection with ultrasonic is suitable for water treatment. But, in order to effectiveness of treatment, would need to be applied in combination with another treatment processes.

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