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Sonolysis of Indicator Microorganisms of Water Quality

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Abstract: The aim of this study was to investigate the sonolysis of water microorganisms. Ultrasound waves as a disinfection technology at a frequency 42 kHz was used to inactivation of indicator microorganisms of water like the *Escherichia coli* (*E. coli*), *Clostridium perfringense* (Cp) and Heterotrophic Plate Counts (HPC). In ultrasound technology, the phenomena of cavitation which involves formation, growth and violent collapse of vapor bubbles in a liquid media is known to generate a high intensity pressure which affects the microorganisms viability. Therefore, ultrasound waves display a strong influence on the rate of microorganisms inactivation in aqueous suspension. Results showed a significant increase in sonolysis for indicators with increasing duration of sonication time in the 42 kHz. Sonolysis in 42 kHz is capable to some degree of inactivation indicator microorganisms. On the other hand, more than 99% of microorganisms was inactivated within 90 min.

Key words: Sonolysis, water drinking, ultrasound wave, disinfection technology, frequency, inactivation, indicator microorganisms, *Escherichia coli*, *Clostridium perfringense*, heterotrophic plate counts, cavitation, intensity, sonication time

INTRODUCTION

Water is very important for life. It covers 71% of the earth's surface and makes up 65% of our bodies. Everyone wants clean water to drink, for recreation and just to enjoy looking at. If water becomes polluted, its loses its value to us economically and aesthetically and can become a threat to our health[1,2]. Water-related diseases are a human tragedy, killing millions of people each year, preventing millions more from leading healthy lives and undermining development efforts[3,4]. About 2.3 billion people in the world suffer from diseases that are linked to water[5]. Some 60% of all infant mortality is linked to infectious and parasitic diseases, most of them water-related. In some countries water-related diseases make up a high proportion of all illnesses among both adults and children. To keep our used water from spoiling our water resources, we have to remove the pollutants before the water gets back into the environment. Therefore, it is of the utmost importance to produce through treatment processes a final potable water which is microbiologically safe[6,7].

***Escherichia coli*:** The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination. Sewage may contain many types of disease-causing organisms. Water can be contaminated in a variety of ways. Main sources of *E. coli* are municipal sewage discharges or runoff from failing septic systems, animal feed operations, farms and faeces deposited in woodlands from warm blooded animals. In urban areas, the *E. coli* from the excrement of warm blooded animals (such as pets in a park or on the street) may be washed into creeks, rivers, streams, lakes, or groundwater during rainfalls or snow melts. The contamination in water is often highest immediately following a storm, because of the runoff. In addition, infected bathers can unknowingly contaminate water, or contamination can occur from boaters discharging wastes directly into the water. When these waters are used as sources of drinking water and the water is not treated or inadequately treated, *E. coli* may end up in drinking water[8,9].

Heterotrophic Plate Counts (HPC): General description HPC measurement detects a wide spectrum of heterotrophic microorganisms, including bacteria and fungi, based on the ability of the organisms to grow on rich growth media, without inhibitory or selective agents. The spectrum of organisms detected by HPC testing includes organisms sensitive to disinfection processes, such as coliform bacteria; organisms resistant to disinfection, such as spore formers and organisms that rapidly proliferate in treated water in the absence of residual disinfectants been developed, there is no single universal HPC measurement. Heterotrophic microorganisms include both members of the natural (typically nonhazardous) microbial flora of water environments and organisms present in a range of pollution sources. They occur in large numbers in raw water sources. HPC organisms can grow in water and on surfaces in contact with water as biofilms[10,11].

***Clostridium perfringens*:** General description *Clostridium* sp. are Gram-positive, anaerobic, sulfite-reducing bacilli. They produce spores that are exceptionally resistant to unfavourable conditions in water environments, including UV irradiation, temperature and pH extremes and disinfection processes, such as chlorination. The characteristic species of the genus, *C. perfringens*, is a member of the normal intestinal.ora of 13-35% of humans and other warmblooded animals. Other species are not exclusively of faecal origin. Like *E. coli* and *C. perfringens* does not multiply in most water environments and is a highly specific indicator of faecal pollution. The presence of *C. perfringens* in drinking water can be an index of intermittent faecal contamination[12,13].

Disinfection technique: Common disinfection methods used in water treatment include chlorination, ozonation and ultraviolet irradiation. The purpose of water purification is to change the aquatic environment so that it does not support detrimental microbiological life forms. The most important and final process used in the destruction of micro-organisms is disinfection. Several studies have shown that the efficiency of disinfection technique is dependant on the concentration of suspended solids. Because suspended solids can protect bacteria from being destroyed by disinfectants. For example, the efficiency of ultraviolet irradiation is affected by high concentrations of suspended matter. Also, chlorine is traditionally used for disinfection. With the use of chlorine a possibility exists that by-products may form, which are potentially toxic and carcinogenic. Other alternative disinfection method is physical unit and advanced oxidation processes. These processes involve the generation of highly reactive free radical intermediates[14,15].

Ultrasound waves technology: In the water treatment, the destruction or transformation of organic pollutants and the removal of biological contamination are the prime objectives of fundamental and applied investigations involving ultrasound. The use of ultrasound in the water industry are now of considerable interest. The one way to inactivate microorganisms is with ultrasound waves. When liquids are exposed to these vibrations, both physical and chemical changes occur as a result of a physical phenomenon, known as cavitation. Cavitation is the formation, expansion and implosion of microscopic gas bubbles in liquid as the molecules in the liquid absorb ultrasound energy. Compression and rarefaction waves rapidly move through the liquid media. If the waves are sufficiently intense they will break the attractive forces in the existing molecules and create gas bubbles. As additional ultrasound energy enters the liquid, the gas bubbles grow until they reach a critical size. On reaching a critical size, the gas bubbles implode or collapse. The energy that exists within the cavity and in the immediate vicinity of the gas bubbles just before collapse causes both physical and chemical effects in the liquid. Physical effects result when cavitation is intense enough to rupture cell membranes, free particulates from solid surfaces and destroy particles and organisms through particulate collisions or by forcing them apart[16-18].

MATERIALS AND METHODS

Ultrasound batch reactor: Ultrasound was applied to samples using a laboratory cleaning bath with the following characteristics:

Input: 220-230 V 155 W, Output: 70 W 42 kHz

Microbiological experiments: Ultrasound reactor has been used for microbiological experiments. Ultrasound device used was Bransonic which is operated at 42 kHz. Electrical power 70 W and input power 155 W are applied. Experiments involved sonicating of indicators and observing the effectiveness of ultrasound frequency upon their growths. Before ultrasound, the concentration of bacteria in water was adjusted. Samples were added to the ultrasound bath in which sonication could be performed. The samples were sonicated in periods of 1, 15, 30, 45, 60, 75 and 90 min. For each trial namely, each sample was exposed to all of the duration. The number of trials per the mentioned exposure levels was variable. Finally, required samples for analyses were taken after 1, 15, 30, 45, 60, 75 and 90 min and all the analyses were performed according to the procedures outlined in standard methods[19]. In this study, dependent variable was removal with ultrasound frequency and independent were intensity and exposure time.

RESULTS AND DISCUSSION

The major objective was to study the sonolysis of indicator microorganisms of Water quality. This study show that a significant of bacterial counts was only possible when long sonication time (up to 90 min) and

Table 1: The effect of ultrasound waves time on removal of indicators of water quality

Ultrasound waves time (min)	Removal efficiency *Escherichia coli*	*Clostridium perfringens*	Heterotrophic plate counts
1	0.00	0.00	0.00
15	72.50	79.10	70.52
30	84.00	89.46	89.97
45	98.50	99.76	98.59
60	99.23	99.81	98.64
75	99.70	99.84	99.00
90	99.88	99.88	99.94

frequency (42 kHz) were applied. The results in Table 1 indicate that sonication of 500 mL suspension of indicators at 42 kHz produces a significant effect. On the other hand, maximum reduction for *E. coli*, *Clostridium perfringens* and Heterotrophic plate counts were 99.88, 99.88 and 99.94, respectively. The majority of these reduction found to occur in the 90 min.

Table 1 presents the ultrasound time versus the removal efficiency. The results show that the ultrasound waves is suitable for water treatment and disinfection.

Ultrasound as an exclusive disinfection method will only be possible if new full scale reactors show significantly better efficiency than lab scale device. Ultrasound reactors are small devices that easily can be any place on a treatment plant. In order to definitely damage cell walls higher ultrasound energy input is necessary. Sonolysis of microorganisms most likely results from a combination of physical and chemical mechanisms which occur during acoustic cavitation. The possible mechanisms by which cells are rendered inviable during ultrasound waves includes free-radical attack, including hydroxyl radical attack and physical disruption of cell membranes.

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