OPERATIONAL AND KINETIC PARAMETERS OF ULTRASONIC DISINFECTION

by

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ABSTRACT

Ultrasonic pressure waves are powerful means of destroying organic matter and pathogenic organisms in polluted water bodies. The potential is due to “cavitation phenomenon”, which consists of the formation, growth and implosive collapse of acoustic cavities that are made of microbubbles filled with gases and vapors of the surrounding liquid. When these bubbles grow to sufficiently large sizes, they implode violently in a “catastrophic collapse”, releasing extremely high temperatures and pressures and creating “local hot spots” in the surrounding. At such, molecules of gases entrapped in the microbubbles are thermally fragmented into atomic or radical species. When the surrounding liquid is water, gaseous bubbles are filled with molecules of water vapor, which undergo pyrolysis during cavity collapse to fragment into hydrogen and hydroxyl radicals. The destruction of organic matter and bacterial cells in sonicated water is due both to the chemical reactivity of the radicals and to very unique mechanical effects of cavity collapse.

The purpose of this study was to investigate the operational parameters of ultrasonic disinfection and to develop a kinetic model to describe the rate of bacterial kill under ultrasonic irradiation. The method involved the use of three different frequencies, 20 kHz, 300 kHz, 520 kHz to inactivate pathogenic organisms in water represented by *Escherichia* C*oli*. Bacterial count tests were periodically performed throughout the sonication period to determine the quantity of surviving cells in the reactor effluents. Effects of the operation mode (continuous vs pulse), the buffer concentration, initial *E.coli* density, and solid particle addition were investigated to select optimum conditions.

It was found that maximum rate of kill was accomplished at 20 kHz within 20 min. The degree of cell inactivation could be enhanced by the addition of sand or talc with effective diameters of 53 μm. There was no significant difference between continuous and pulse mode of operations, to be attributed to the relatively long pause period applied. The rate of inactivation was described by a modified form of Chick’s law, showing that ultrasound mimicked the role of chemical biocides in water disinfection systems.

ÖZET

Sesüstü dalgalar, kirli sularda bulunan organik maddelerin ve hastalık yapıcı organizmaların yok edilmesinde etkili bir yoldur. Sesüstü dalgaların etkinliği suda oluşturduğu ‘’kavitasyonlar’’ sayesindedir. Kavitasyon, içinde bulunduğu sıvı bünyesindeki buhar ve gazlarla dolarak oluşan mikro-kabarcıkların, daha da gelişip, meydana getirdiği şiddetli ‘’içsel patlaması’’ olarak tanımlanır. Bu kabarcıklar, yeterli büyüklüğe ulaştığında, etkili bir biçimde patlayarak içinde bulunduğu ortama çok yüksek ısı ve basınç yayar ve sıvı içinde ‘’lokal enerji merkezleri’’ yaratır. Bu durumda, kabarcıklar içine hapsolan gazlar parçalanır ve radikaller oluşur. Sesüstü dalgaların uygulandığı sıvı su ise, kabarcıklar su buharı molekülleri ile dolar ve kavitasyon sırasında su buharı moleküllerinin hidrogen ve hidroksil radikallerine ayrılır. Organiklerin ve bakteri hücrelerinin parçalanması radikallerle meydana gelen kimyasal reaksiyonlar ve kavitasyona özgü mekanik etkilerin sonucudur.

Bu çalışmanın amacı, sesüstü dalgalarla dezenfeksiyonun operasyonel parametrelerinin araştırılması ve bakteri ölüm hızını ifade eden kinetik bir modelin geliştirilmesidir. Hastalık yapıcı bakterilerin işaretcisi olan *Escherichia* C*oli* hücrelerinin yok edilmesinde üç ayrı frekans: 20 kHz, 300 kHz ve 520 kHz denenmiştir. Tüm çalışmalarda, periyodik olarak reaktörlerden alınan numunelerde yaşamını sürdürebilen hücreler sayılmıştır. Sistem verimin en iyi olduğu şartların belirlenmesi amacıyla, sürekli ve kesikli uygulamaların, başlangıç hücre ve tampon çözelti konsantrasyonlarının, ve sisteme katı parçalarının eklenmesinin etkileri araştırılmıştır.

Sonuç olarak, ölüm hızının 20 kHz frekans ile çalışan sistemde en yüksek olduğu belirlenmiştir. Hücre ölümü kum ve 53 μm’den küçük talk parçacıkları ile artırılmıştır. Sesüstü dalgaların sürekli ve kesikli uygulamaları arasında belirgin bir fark gözlenmemiştir. Buna, kesikli uygulama sırasında sistemin kapalı olduğu sürenin uzunluğunun sebep olduğu düşünülmüştür. Hücre ölüm hızının, Chick’s kanunun modifiye formu ile tanımlanabilmesi, ses ötesi dalgaların, kimyasal dezenfektanlara benzer bir rol oynadığını göstermektedir.

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LIST OF ABBREVIATIONS AND SYMBOLS

Symbols/Abbreviations	Explanation	Unit
a	Empirical Constant	
b	Empirical Constant	
CFU	Colony Forming Units	
C_P	Specific Heat at Constant Pressure	$J.g/^0C$
C_{solid}	Concentration of Solids	g/L
d	Particle size	µm
DBPs	Disinfection By Products	
DI water	Deionized Water	
DO	Dissolved Oxygen	
E	Efficiency	#/J
E.coli	*Escherichia Coli*	
EC	Electrochemical	
Eq.	Equation	
h	Hour	
J	Joules	
k	Rate Coefficient	$min^{-(m+1)}$
kHz	kilohertz	
M	Molar	moles/L
m	Empirical Constant	
MHz	Megahertz	
min	Minutes	
M_L	Mass of Liquid	g
mL	Milliliter	
MLSB	Membrane Laurly Sulphate Broth	
n	Empirical Constant	
N	Number of colonies	#
N_{20}	Concentration of colonies at 20 min	#/mL
nm	Nanometer	

Symbols/Abbreviations	Explanation	Unit
N_0	Initial Number of Colonies	#/mL
P	Power	W
P_d	Power Deposited	W
R^2	Correlation coefficient	
T	Temperature	^0C
t	Time	min
$t_{99.9\%}$	Time required to achieve 99.9% kill	min
UV	Ultraviolet	
V	Volume	mL
W	Watt	
ΔN	Change in Colony Concentration	#/mL
Δt	Change in Time	min
μm	Micrometer	
#	Number	
%	Per cent	
•OH	Hydroxyl Radical	
^0C	Degrees Centigrade	
^0F	Degrees Fahrenheit	

1. INTRODUCTION

Water is an essential component of this planet and plays an essential role in supporting all life. When contaminated, however, it can transmit a wide variety of diseases and illnesses to populations. Therefore it is of the utmost importance to produce through treatment processes final potable water which is both microbiologically and chemically safe and, at the same time is aesthetically acceptable to the population (Phull et al., 1997). Potable and wastewaters have highly complex compositions which include variety of potential contaminants. It therefore follows that a variety of treatment processes will be necessary to deal with this range of contamination, including physical, chemical and biological processes.

Disinfection can be considered as one of the most important treatment processes in terms of protection of the public health from waterborne microbial diseases. So, it has become a challenging aspect of water treatment because of rapid elevation of health standards and the growing concern for pollution-free water resources. The most commonly practiced methods are those that involve chemical (e.g., chlorination and ozonation) and physical (e.g., heat treatment and / or ultraviolet light at 254 nm) processes. Chlorination, as the most common and cost-effective of all, has been noted in recent years for its adverse health effects originated by residual chlorine, which reacts with natural organic matter to form carcinogenic by products (Ince and Belen, 2001).

The major problems associated with chlorination process can be summarized as: (i) microorganisms (especially bacteria) are capable of producing strains that are tolerant to normal chlorine treatment levels. This can be overcome by using higher chlorine levels than those normally used; however, this can lead to formation of unpleasant flavors and odors due to the formation of chlorophenols and other halocarbons. (ii) certain species of microorganisms produce colonies and spores that agglomerate in spherical or large clusters. Chlorination of such clusters may destroy microorganisms on the surface leaving the innermost intact.

(iii) fine particles such as clays are normally removed by flocculation using some chemicals such as aluminum sulphate. The flocs can entrap bacteria and their spores protecting them from chlorination. The vast majority of particles are removed, but one or two may pass through the system unaffected by the final disinfection stage (Phull et al., 1997).

Increasingly severe regulations on trihalomethanes (THMs) favor the change to other disinfectants, particularly ozone. Ozonation is a viable alternative because of its non-residual effect, however, more researches is required to lower its operational costs and to protect the water from re-infection in the distribution system. UV disinfection is another alternative. Several studies have shown that the efficiency of UV disinfection methods is highly dependent on the suspended solids concentration of the sample, due to the fact that they protect the organisms, decrease the penetration capacity of UV, thus increasing the required UV dose (Blume and Neis, 2004).

Ultrasound is a promising tool of disinfection and decontamination of water bodies. The well-known effectiveness of power ultrasound (20-100 kHz) for its surface cleaning action has been successfully utilized in some patented systems applied in institutional and medical facilities for disinfecting non-disposable implements and accessories (Mason, 1999). Bacterial removal by these systems involves their dislocation from adhered surfaces and crevices, which are rather difficult to reach by conventional cleaning methods. Recent studies with aqueous systems have shown the biocidal effects of ultrasound on bacteria, viruses and fungi in water via cavitation phenomenon associated with mechanical effects: shear forces and dispersion; and chemical effects: localized heating and free highly reactive radical formation (Ince and Belen, 2001).

In the following sections basic theories of ultrasonic technologies, associated chemical reactions, important system parameters and factors affecting the performance (e.g., frequency, solvent characteristics, power, and addition of solid particles) will be discussed. Furthermore, applications of ultrasonic technologies for disinfection purposes and previous studies will be presented.

The objectives of this study were (i) to compare the efficiencies of ultrasonic systems with different frequencies, powers and configurations; (ii) to investigate the effects of initial cell concentration; mode of irradiation (continuous versus pulse), and the addition of solid particles with different particle sizes on the performances of the systems.

2. THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1. Microbial Contamination of Drinking Water

Drinking water is a major source of microbial pathogens in developing regions due to rapid and uncontrolled urbanization. Poor water quality, sanitation and hygiene account for some 1.7 million deaths a year world-wide, mainly through infectious diarrhea. Nine out of ten deaths are in children and virtually all of the deaths are in developing countries. Major enteric pathogens in these children include: rotavirus, *Campylobacter jejuni*, enterotoxigenic *Escherichia coli* (*E.coli*), *Shigella* spp. and *Vibrio cholerae* O1, and possibly enteropathogenic *E.coli*, *Aeromonas* spp. *V.cholerae* O139, enterotoxigenic *Bacteroides fragilis*, *Clastridium difficile* and *Cryptosporidium parvum*. All except the latter are easily controlled by chlorination of water, but recontamination of treated water is a huge problem (Ashbolt, 2004).

2.1.1. *Escherichia coli* (*E.coli*)

Escherichia coli (*E.coli*) is a bacterium commonly used as an indicator of water quality for freshwaters. Natural habitat of *E.coli* is the intestinal tract of warm-blooded animals, and although typically non-pathogenic, its presence in water indicates fecal contamination and the potential for waterborne disease.

Routine monitoring of enteropathogens, which can cause serious diseases such as cholera, typhoid, salmonellosis, and dysentery, is unreliable since these organisms are difficult to detect. Instead, an indicator organism, such as *E.coli*, is used to determine fecal contamination. The presence of *E.coli*, a normally non-pathogenic intestinal organism of warm-blooded animals, is easy to test for and is relatively more abundant than the enteropathogens thus leaving a safety margin for the detection of disease-causing organisms.

E.coli is considered a more specific indicator of fecal contamination than fecal coliforms since the more general test for fecal coliforms also detects thermotolerant non-fecal coliform bacteria (Francy et al., 1993). The *E.coli* test is recommended by the United States Environmental Protection Agency (USEPA) which confirms presumptive fecal coliforms by testing for the lack of an enzyme that is selective for the *E.coli* organism. This test separates *E.coli* from non-fecal thermotolerant coliforms. It should be noted that *E.coli* may not be an appropriate indicator for protozoan and viral diseases caused by such organisms as *Cryptosporidium*, *Giardia*, and the hepatitis virus due to their lower numbers in water and lower infectious doses.

The presence of *E.coli* in surface waters is often attributed to fecal contamination from agricultural and urban/residential areas. However, variation in *E.coli* concentrations from site to site and the contribution of human vs. agricultural sources are not readily understood. In addition, *E.coli* concentrations at a particular site may vary depending on the baseline bacteria level already in the river, inputs from other sources, dilution with precipitation events, and die-off or multiplication of the organism within the river water and sediments. The concentration of *E.coli* in surface water depends for the most part on the runoff from various sources of contamination and is thus related to the land use and hydrology of the contributing watersheds.

Sediments may affect the survival and often act as a reservoir of *E.coli* in streams. Sedimentation and adsorption, which offer protection from bacteriophages and microbial toxicants, can lead to higher concentrations of *E. coli* in sediments than in the overlying water column (Burton et al., 1987). Thus, the sediment often acts as a reservoir for *E.coli* in the stream. In addition, fecal bacteria may persist in stream sediments and contribute to concentrations in overlying waters for months after initial contamination (Sherer et al., 1992).

2.2. Methods of Disinfection

2.2.1. Chemical

Disinfection is usually a chemical unit operation in the water treatment process and objective is the inactivation of pathogenic microorganisms in order to minimize the risk of waterborne illnesses. When the municipal drinking water is provided from especially surface water bodies, disinfection operation must be included in the purification treatment process. Disinfection of domestic effluents is also mandatory before their utilization in crop irrigation, for recharging groundwater through soil infiltration, or before their disposal in soil or into large water bodies.

Disinfection methods were primarily developed for drinking water and they were used later for disinfection of domestic effluents. Methods can be divided into three main categories: chemical, physical and photochemical. The criteria for choosing the most suitable method depend on water quality parameters, water utilization or disposal methods, ecological concerns and economic factors.

Chemical disinfection methods are based on the oxidation potential of chemicals which can oxidize and damage the cell wall of microorganisms and eventually cause lethal damage. The most commonly used chemicals are chlorine (Cl_2), hypochlorites (ClO^-), chloramines (RNHCl), chlorine dioxide (ClO_2), bromine (Br_2), and ozone (O_3). If only the standard oxidation potential of these chemicals is considered, their efficacy decreases in the following order:

$$O_3 >> Cl_2 > Br_2 > ClO_2 > ClO^- > RNHCl$$

Selection of the disinfectant not only depends on the oxidation potential or germicidal efficacy, but also requires the consideration of parameters like suspended solids, oxidizable organic and inorganic matter, dissolved oxygen, pH, temperature, etc. Water characteristics also determine the dose of the chemical agent (Acher et al., 1997).

Engelbrecht et al. (1977), determined the chlorine resistance of poliovirus type 1 Mahoney strain, *E. coli*, *S. typhimurium*, and mixed culture of wastewater yeasts and acid-fast bacilli in a completely mixed batch reactor. The inactivation response was determined for free chlorine residual of 0.5 mg/L. The order of resistance was as follows: acid-fast organisms > yeasts > polio virus > *S. typhimurium* > *E.coli*. Farooq and Akhlaque (1983) conducted ozone disinfection studies using five cultures of organisms. Their resistance to ozone observed in the following order: *M. fortium* (acid-fast organism) > polio virus type 1 Mahoney strain > *C. parapsilosis* (yeast) > *E. coli* > *S. typhimurium*. Comparison of order of resistance of the five different organisms with residual chlorine and ozone shows that it changes with the type of disinfectant, implying different mechanism of disinfection takes place with different disinfectants. It is believed that chlorine selectively oxidizes essential cellular functional units such as enzymes, coenzymes, and H-carriers of both pathogens and other organisms, where as ozone acts as a general protoplasmic oxidant.

Disinfection of *E.coli* by ozonation in natural water and wastewater presents a complexity because ozone will also react with dissolved, colloidal and particulate matter, and these reactions might interfere with some of the reactions responsible for *E.coli* inactivation. Design of disinfection systems thus might require the simultaneous consideration of all reactions affecting the concentration of dissolved ozone and ultimately inactivation process. Hunt and Marinas (1999) investigated the apparent decomposition of dissolved ozone in the presence of humic acid and *E.coli* cells. They concluded that the rate for the ozone inactivation of *E.coli* in the presence of humic acid was slower than that in the absence of natural organic matter. The transmission electron micrographs revealed that noticeable changes in the cells did not take place until most of the cells present in the sample were not viable and subsequent exposure to ozone resulted in structural changes, membrane deterioration, and ultimately lysis of inactivated cells.

Disinfection of water supplies is of paramount importance for the prevention of waterborne diseases but may pose chemical threat to human health due to disinfection residues and their by-products (DBPs). DBPs are formed when the disinfectants react with natural organic matter and/or inorganic substances present in the water. More than 250 different types of DBPs have already been identified. Major classes of DBPs are Trihalomethanes (THM), Haloacetic acids (HAA), Haloacetonitrile (HAN), and inorganic compounds (Sadiq and Rodriguez, 2004).

For chlorination, generally chlorine gas is bubbled into water and hydrolysis to HCl and HOCl takes place. The HOCl undergoes subsequent reactions resulting in formation of THMs (Sadiq et al., 2002).

Ozone is becoming a popular disinfectant due to its effectiveness for killing harmful microorganisms and also because it does not produce significant concentrations of THMs or other chlorinated disinfection by-products. However, the increased use of ozonation for disinfection purposes has led to reevaluation of the chemistry involved in the ozonation of water that contain natural organic matter and bromide. The major organic DBPs resulting from ozonation of surface and ground water have been identified as low molecular weight aliphatic organics, particularly aldehydes, carboxylic acids and ketones. Additionally, ozonation of bromide –containing water can cause by-products which are potential carcinogens (Huang et al., 2005). Furthermore, ozonation of drinking water transforms the natural organic matter into a more biodegradable form, which can cause significant bacterial regrowth in distribution systems (van der Kooij et al., 1989)

Yang et al. (1997) have conducted an ecologic epidemiological study to examine whether chlorinated drinking water was associated with cancer risks. The results of this study suggest a positive association between consumption of chlorinated drinking water and rectum, lung, bladder, and kidney cancer and increased mortality rates.

Tokmak et al. (2004) investigated the occurrence of THMs in water supply system of Ankara, Turkey and carried out risk estimations indicating that each year

1 of 5 million individuals in Ankara could get cancer from the daily intake of water, mainly because of exposure to chloroform among the four THMs through oral digestion.

The manufacture of chlorine and its derivatives, storage, transport and use pose a continuous threat to operators and environment. Ozone is also threatening the health of operators and the environment even at low concentrations.

2.2.2. Physical

Physical methods are based on mechanical separation of organisms from water by filtration or synthetic membranes. These methods are generally used in combination with other methods in order to increase their efficiency.

Ultrafiltration or microfiltration membranes constitute an effective barrier against the development of bacteria, viruses and other microorganisms taken into consideration in the disinfection process. They are capable of clarification and disinfection of water within one operation. Konieczny (1998) investigated the ultrafiltration of well and surface water using polymeric membranes made of polyacrylonite and polysulfone; and stated that with respect to E.*coli*, full disinfection of water was observed, and mesophilic bacteria are also removed to a high degree; further, it was observed that water flux was declined in time due to clogging.

2.2.3. Photochemical

Photochemical disinfection methods were developed because of the potential hazards associated with chemical disinfection methods, as environmentally friendly techniques which make use of natural sunlight and artificial UV radiation.

2.2.4. Solar/UV, UV-IR and Photocatalytic

Sunlight exposure is considered to be the most important cause of ‘’natural’’ disinfection in waste stabilization ponds. Colley et al. (1999) examined the influence of the DO, pH, particulate and dissolved constituents on sunlight inactivation of faecal microorganism. They concluded that the sunlight is the main factor causing ‘’natural’’ disinfection, observed damages on DNA and cell membrane, and inactivation strongly depended on DO, light absorbing constituents in water, independent of pH for enterococci but higher with pH>8.5 for *E.coli*. The sunlight was either used as global irradiation or concentrated by mirrors and reflectors.

McLoughlin et al. (2004) studied the solar disinfection of contaminated water and compared three different small- scale reactors: Pyrex tubing and aluminium reflectors of compound parabolic, parabolic and V-grooves. Results indicated that compound parabolic reflector promoted a more successful inactivation of *E.coli* as compared to other two. Kehoe et al. (2001) stated that agitation, turbidity decrease the inactivation rates of *E.coli* covering the surface of the reactor with aluminium foil increases the efficiency, and inactivation kinetics was independent of the volume of water treated.

An increasing awareness of the disadvantages of chemical disinfectants has resulted in selection of ultraviolet (UV) radiation as a promising alternative. In 1988, nearly 300 operating wastewater treatment plants were using UV disinfection. The number of utilities using UV disinfection has increased considerably and has expected to increase significantly over next decade (Taghipour, 2004).

The inactivation of bacteria by UV radiation results primarily from the absorption of the radiation by the DNA of the microorganisms and resultant dimerization of thymine bases in the DNA, which affects the normal DNA replication. This photodimerization process has also been observed in the RNA of the viruses (Harris et al., 1987). The germicidal effect of UV radiation is greatest in

the far-ultraviolet (190-300 nm) wavelength range. Practical UV disinfection systems have traditionally used low-pressure mercury lamps; pulsed xenon arc flashlamps is also a viable alternative (Wang et al., 2005)

Exposure of UV damage cells to higher wavelength light (300-500 nm), primarily in the visible range, may often repair much of the damage to the DNA (Harris et al.,1987) investigated the sensitivity of bacteria and virus to UV radiation and examined the influence of photoreactivation on the sensitivity of the bacteria. *E.coli* and *S.faecalis* were selected for this study. The viruses in general, were found to be more resistant to UV radiation. Potential photoreactivations of 3.4 and 2.4 logs were observed for *E.coli* and *S.faecalis*, respectively. This may have important implication in such a situation where UV disinfected secondary effluent is discharged into receiving environments like lakes and rivers. Sunlight penetrating these water bodies may reactivate a significant fraction of the bacteria.

One of the factors affecting the performance of the UV disinfection is the quality of water. Suspended particles in water can increase microbial survival by shielding organisms from UV irradiation. Loge et al. (1999) concluded that UV can not penetrate particles by transmission through solid material. The synergetic use of UV with other forms of particle – penetrating irradiation, like ionizing radiation (electron beams and gamma irradiation) in disinfection process is a potential option for addressing this issue. Taghipour (2004) studied the impacts of UV irradiation, gamma irradiation and the combination of both on *E.coli* inactivation and concluded that particle-associated microorganisms, which are protected from UV, can be inactivated by ionizing radiation at a rate similar to that for free microorganism inactivation.

Photocatalytic removal of bacteria by Titanium dioxide (TiO_2) is another alternative disinfection method. When TiO_2 particles are illuminated with near UV radiation, electron hole pairs are generated within the metal oxide semiconductor. The valance band hole has a very positive reduction potential and is capable of oxidizing water, or hydroxide ions, to form hydroxyl radicals which are known to be powerful, indiscriminate oxidizing agents (Dunlop et al., 2002). Choet al. (2004)

demonstrated that there is an excellent linear correlation between steady state concentration of hydroxyl radicals, $[\bullet OH]_{SS}$ and the rate of *E.coli* inactivation in UV/TiO_2 process. Rincon and Pulgarin (2004) pointed out the efficiency of photocatalytic disinfection of E.*coli* was positively influenced by addition of H_2O_2 and evaluate the effects of addition of organic and inorganic matter.

2.2.5. Electrochemical

Electric fields and currents have been shown to be capable of disinfecting drinking water and reducing the number of bacteria and yeast in food. Several mechanisms have been proposed to account for the lethality of electrochemical exposure, including oxidative stress and cell death due to electrochemically generated oxidants, irreversible permeabilization of cell membranes by the applied electric field, and electrochemical oxidation of vital cellular constituents during exposure to electric current or induced electric fields. Chemical oxidants are generated when electric current is applied to aqueous suspensions of microbes with immersed electrodes. Electrolysis generates variety of oxidants in the presence of oxygen, including hydrogen peroxide and ozone, as well as free chlorine and chlorine dioxide when chloride ions are present in the solution. Current research indicates that antimicrobial agents and electric current act synergistically to inactivate microbes (Drees et al., 2003).

Diao et al. (2004) carried out experiments to investigate the mechanism of electrochemical (EC) disinfection of artificial wastewater contaminated by *E.coli*. Comparative disinfection tests with chlorine, ozone and hydroxyl (·OH) radicals produced by Fenton reactions were also performed. It was demonstrated that EC process was highly effective for wastewater disinfection. Scanning electron microscopy showed different appearances of damage to in the surface morphology and structure of cells after different forms of disinfection. Substantial leakage of intercellular materials was found for the *E. coli* cells, which was also observed for the cells treated by Fenton reactions. However, such cell lysis was noticeable to a less extent in ozonation and hardly noticeable for chlorinated cells.

Kerwick et al. (2005) reports on a series of experiments evaluating the disinfection efficacy of an electrochemical disinfection technology against *E.coli* and bacteriophage *MS2*. They concluded that both organisms were inactivated by 4 logs in the absence of chlorine; and stated that electrochemical disinfection can be effective without generation of chlorine species.

2.2.6. Ultrasonic

Disinfection by ultrasound is a novel technique, the details of which are discussed in the following section.

2.3. Introduction to Ultrasound and Applications

Ultrasound is simply defined as any sound frequency above that to which human hear has no response (i.e., above 16 kHz). In practice, three ranges of frequencies are reported for three distinct uses of ultrasound (Ince et al., 2001):

(i) High frequency or diagnostic ultrasound (2-10 MHz),
(ii) Low frequency or conventional power ultrasound (20-100 kHz), and
(iii) Medium frequency" ultrasound (300-1000 kHz)

Ultrasound has many uses in different areas: at homes as burglar alarms, and jewelry cleaners; in medicine, to remove kidney stones without surgery, to treat cartilage injuries, and to image fetal development during pregnancy. In industry, ultrasound is important for emulsifying cosmetics and foods, welding plastics, cutting alloys, and large-scale cleaning. None of these applications, however, take advantage of the effects that ultrasound can have on chemical reactivity. The chemical applications of ultrasound, "sonochemistry", have become an exciting new field of research during the past decade (Suslick, 1994).

Benefits of the use of ultrasound in the water industry are now of considerable interest. Table 2.1 summarizes the uses of ultrasound in water treatment process.

Table 2.1. Potential uses of ultrasound in water treatment processes (Mason et al., 1993).

Potential Use/or Effect of Ultrasound	Application
Biotechnology	Enhancing enzyme activity
Coagulation and Flocculation	Enhancement of the process
Decontamination	Surface Cleaning Destruction of chemicals and biological contaminants, PCBs, chlorinated hydrocarbons, pesticides Treatment of leachate, destruction of r red list pollutants
Degassing	Removal of excess chlorine and ozone Methane removal
Dewatering	Improved efficiency of dewatering in digestion process
Disinfection	General disinfection of drinking water Destruction of specific organisms such as cryptosporidium and giardia Improve efficiency of bactericide and disinfectants Enhancing UV treatment Disinfection of pathogenic bacteria in sewage sludge
Dispersion	Deagglomeration of particles Particle size reduction Homogenization, mixing, emulsification Dispersion of chemicals Break up of bacterial clumps
Filtration	Improving efficiency of filters and membranes
Oxidation	Enhancing oxidation process by improving gas mixing

The action of ultrasonic waves in liquids can introduce or accelerate a wide variety of chemical reactions. Sonochemistry involves the introduction of very large amounts of energy in a very short period of time. The effects of ultrasound on

chemical transformations are not the results of any direct coupling of the sound field. The reason why ultrasound is bale to produce chemical effects is through the phenomenon of cavitation.

2.3.1. Cavitation Bubbles and their Role in Sonochemistry

Sound is nothing more than waves of compression and expansion passing through gases, liquids or solids. Like any sound wave, ultrasound is transmitted via waves, which alternately compresses and stretch the molecular spacing of the medium through which it passes (Figure 2.1). If a large negative pressure, i.e. sufficiently below the ambient, is applied to the liquid so that distance between the molecules exceeds the critical distance necessary to hold the liquid intact, the liquid will break down and voids will be created, and the ‘’cavitation bubbles’’ will form (Mason,1999).

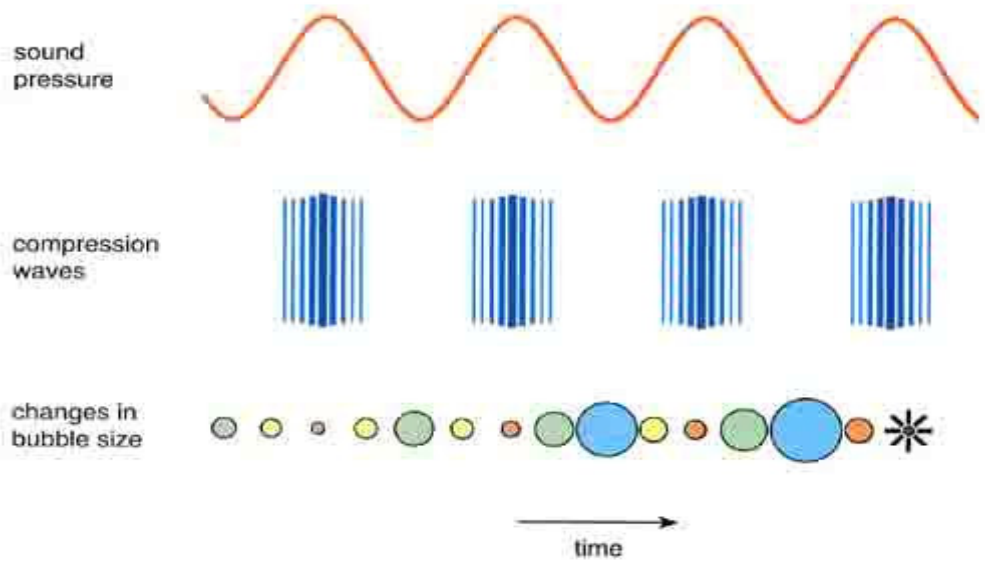

Figure 2.1. Development and collapse of cavitation bubbles (Suslick, 1994)

Formation of bubbles is a nucleation process. Nucleation of bubbles occurs at weak points in the liquid (Suslick, 1990). In practice cavitation can be produced at considerably lower applied acoustic pressures as compared to pure waters due to

the *weak spots* in the liquid, which lower its tensile strength. Weak spots include the presence of gas nuclei in the form of dissolved gas, minute suspended gases or tiny particles. When produced in a sound field at sufficiently high power the formation of cavitation bubbles will be initiated during the rarefaction cycle. These bubbles will grow over a few cycles taking in some vapor or gas from the medium. Some bubbles suffer sudden expansion to an unstable size and collapse violently, in a so called “catastrophic collapse” (Ince et al., 2001). Collapsing of cavity bubbles generates the energy for chemical and mechanical effects.

Compression of a gas generates heat. The compression of cavities when they implode in irradiated liquids is so rapid than little heat can escape from the cavity during collapse. The surrounding liquid, however, is still cold and will quickly quench the heated cavity. Thus, one generates a short-lived, localized hot spot in an otherwise cold liquid. Such a hot spot is the source of homogeneous sonochemistry; it has a temperature of roughly 5000 ^{0}C (9000 ^{0}F), a pressure of about 1000 atmospheres, a lifetime considerably less than a microsecond, and heating and cooling rates above 10 billion ^{0}C per second. (Suslick, 1994) Thus, each cavitation bubbles acts as a localized micro reactor generates instantaneous temperatures of several thousand degrees and pressures in excess of one thousand atmospheres (Mason, 1999).

The enormous local temperatures and pressures and extraordinary heating and cooling rates generated by cavitation collapse mean that ultrasound provides an unusual mechanism for generating high-energy chemistry. Like photochemistry, very large amounts of energy are introduced in a short period of time, but it is thermal, not electronic excitation (Suslick, 1990)

Cavitation can be classified as ‘’transient (inertial)’’ and ‘’stable (non-inertial)’’. Transient cavities generally exist for no more than a few acoustic cycles during which time they expand to at least double their initial radius before collapsing violently with in a few seconds. Stable cavities are those that oscillate often non-linearly, about some equilibrium size with a lifetime of tens of cycles (Mason, 1999).

2.3.2. Homogeneous and Heterogeneous Systems

The acoustic streaming strongly stirs the liquid or solution submitted to ultrasound. Therefore, when two non-miscible liquids are sonicated, they emulsify and the reaction of the molecules constituting these two liquid phases is favored by the increasing interface. When the system is solid-liquid system, the solid is disrupted or eroded by the jetting phenomenon. If the solid is a reactant or catalyst, rate increases are observed. In these cases, the sonochemistry that takes place is called ‘’heterogeneous’’. The great majority of the sonochemical reactions heaving real practical interest and potential industrial applications are heterogeneous reactions where the reactivity changes associated with the ultrasonic irradiation are due to physical effects of ultrasound. The major ultrasound frequencies used in heterogeneous sonochemistry are in the range of power ultrasound, 20 -100 kHz (Reisse, 1995).

Reisse (1995) stated that ‘’homogenous sonochemistry’’ involving media, pure liquid or solution are homogenous before the ultrasonic irradiation starts. That is, as soon as cavitation occurs, the media become in heterogeneous. However in sonochemistry it is normal to consider the original state of the system to which the ultrasound is applied (Mason, 1999).

In heterogeneous systems, when cavitation occurs in a liquid near a solid surface, the dynamics of cavity collapse changes dramatically. In pure liquids, the cavity remains spherical during collapse because its surroundings are uniform. Approaching to a solid boundary, however, cavity collapse is very asymmetric and generates high-speed jets of liquid. The potential energy of the expanded bubble is converted into kinetic energy of a liquid jet that moves through the interior of the bubble and penetrates the opposite bubble wall. Liquid jets drive into the surface with velocities of roughly 400 kilometers/hour. These jets hit the surface with tremendous force. This process can cause severe damage at the point of impact and can produce newly exposed, highly reactive surfaces (Suslick, 1994).

2.3.3. Origins of Sonochemical Activity

Homogeneous sonolysis is induced directly by the outcome of extreme conditions in collapsing micro bubbles (Reisse, 1995). Cavitation in any liquid system will result in the formation of radicals. This is because the cavity is unlikely to enclose a vacuum – it will almost certainly contain vapor from the liquid medium itself or from any volatile reagents which are dissolved in it. On collapse, these vapors will be subjected to extremely large increases in temperature and pressure resulting in molecular fragmentation and consequent generation of highly reactive species. Such radicals might then react either within the collapsing bubble or after migration into bulk liquid. In the case of water sonication gives rise to highly reactive •OH and •H radicals, which undergo a range of subsequent reactions. An important product from sonolysis of water is hydrogen peroxide. Together with the radical species, it provides a powerful bactericide and chemical oxidant (Mason et al., 1993).

In sonochemistry, there are three potential sites for chemical reactions: (i) reactions inside the cavitation bubble; (ii) reactions at or near the bubble/liquid interface (iii) reactions in the liquid immediately surrounding the bubble (Mason, 1999).

In order for a chemical to experience the extreme conditions generated inside the cavitation, it must enter the bubble, hence it should be volatile. In the sonication of water, small quantities of •OH and •H radicals are generated inside the bubble may either react in the gas phase or recombine at the cooler gas-liquid interface and/or in the solution bulk to produce hydrogen peroxide as represented in following reactions (Ince et al., 2001):

$$H_2O \rightarrow \bullet OH + \bullet H \quad (2.1)$$

$$\bullet OH + \bullet H \rightarrow H_2O \quad (2.2)$$

$$2\bullet OH \rightarrow H_2O \quad (2.3)$$

$$2\bullet OH \rightarrow H_2O_2 \quad (2.4)$$

$$2\bullet H \rightarrow H_2 \quad (2.5)$$

It is tempting to conclude from this that ultrasound has no effect on materials which cannot enter the bubble. This is not the case as has been shown in a range of reactions during which the very short-lived radicals produced within the bubble migrate to the interface and beyond to undergo reactions with nonvolatile species dissolved in the bulk medium The collapse of the bubble also produces very large shear forces in the surrounding liquid capable of breaking the chemical bonding in any polymeric materials dissolved in the fluid (Mason et al., 1993).

2.3.4. Parameters Affecting Efficiency of Sonochemical Reactions

2.3.4.1. Frequency. It is significant to select a correct frequency in order to have high degrees of ultrasonic treatment. The choice mainly depends on nature and physicochemical properties of target material and localization with respect to cavitation bubbles (Petrier and Francony, 1997). Hydrophobic chemicals with high vapor pressures and high volatility have a strong tendency to diffuse into gaseous phase; therefore, most effective site for their destruction is the interior of the bubble to undergo pyrolytic destruction or bubble-bulk liquid interface for their hydroxylation or thermal decomposition. Thus, volatile pollutants should be exposed to low frequency- power ultrasound where stable cavities with long life times are generated. On the other hand, hydrophilic compounds which are less volatile tend to remain in the bulk liquid during ultrasonic irradiation; the major reaction site is the liquid medium, where they may be effectively oxidized by radicals ejected during cavity collapse, which is more pronounced in high frequency systems having transient cavities with short life times (Ince et al., 2001).

Irradiation frequency has a significant effect on the diameter of the pulsating bubble, the duration of collapse, and the formation of radicals and hydrogen peroxide. Petrier and Farconny (1997) stated that the size of the pulsating bubble and the acoustic period decrease with increasing frequency. They pointed out that although more H_2O and O_2 are cleaved, at low frequencies radicals have time to combine inside the bubble since the duration of collapse is longer, whereas, with an increase of the frequency, acoustic periods are shorter, diameter of bubbles decreases, consequently, the cavitation intensity decreases and more radicals

could escape even though the fragmentation water and oxygen is lower. In an other study, Petrier (1992) compared the effectiveness of 20 and 514 kHz irradiation for the generation hydrogen peroxide in the water at the same power input and concluded that the rate of production 12 times faster in the system with higher frequency.

2.3.4.2. Power. A minimum intensity for sonication is required to reach cavitation threshold. If the energy is too low the cavitation threshold may not be reached and/or the yield may be very low (Mason and Cordemans, 1998). Above the cavitation threshold the only effect of power increase is to produce more bubbles, with each bubble having the same cavitational energy level. In general, an increase in intensity will provide an increase in the ultrasonic effect; however only up to the point where decoupling occurs, which is the build up of large numbers of cavitation bubbles reducing the transfer efficiency of power (Mason, 1999). Therefore, the power should be optimized in all cases.

2.3.4.3. Solvent Vapor Pressure. More volatile solvent will support cavitation at lower acoustic energy and produce vapor filled bubbles. However, the collapse of these bubbles is cushioned by vapor and therefore less energetic (Mason, 1999).

2.3.4.4. External (Applied) Pressure. Increasing the external pressure will mean that a greater rarefaction pressure is required to initiate the cavitation. More importantly, raising the external pressure will give rise to a larger intensity of cavitational collapse and consequently enhanced ultrasonic effect (Mason, 1999).

2.3.4.5. Temperature. As the temperature is increased, the vapor pressure of any liquid involved in the reaction is also increased. That is, the more vapor enters the bubble; the less violent is the collapse (Mason et al., 1993). The temperature increase causes decrease in solvent viscosity and surface tension.

2.3.4.6. Solvent Viscosity and Surface Tension. Viscosity is a measure of resistance to shear force it is more difficult to produce cavitation in viscous liquid.

Cavitation requires the generation of liquid-gas interface. Employing solvent of low surface tension would lead to a reduction in the cavitation threshold (Mason, 1999)

2.3.5. Enhancement of Cavitation

Scientists and engineers should consider two basic strategies for maximizing reaction efficiencies. The first strategy is the optimization of power and reactor configuration, including (i) selection of transducer (piezoelectric or magnetic material that converts electrical impulses to mechanical vibrations); generator (probe or plate-type); (ii) configuration and dimensioning of the reactor and (iii) optimization of the power efficiency (effective power density delivered to medium). The second strategy is for the enhancement of cavitation involves the addition of gases and solids (Ince et al., 2001)

2.3.5.1. Gas Entrainment. The first effect of cavitation in solution is degassing so that over the first few minutes of sonication cavities becomes less extensive. For this reason it is quite common to entrain a gas during sonication. A bubbled gas will generate large number of nucleation sites for cavitation and provide bubbles of uniform energy of collapse (Mason and Cordemans, 1998). The selection of the gas is very important, since the final temperature of collapse is closely related to a parameter called "polytrophic ratio, ''γ'', which is the ratio of specific heats of the ambient gases entrapped in the bubble (Ince et al., 2001). The energy developed on collapse of these gas –filled bubbles will be greatest for gases with the largest ratio of specific heats (Mason, 1999).

2.3.5.2. Addition of Solids. The addition of solids, such as glass beads, ceramic disks, SiO_2, TiO_2, Al_2O_3 and talc in to the sonication medium is another common method for the improvement of cavitation effects. The presence of such material is reported to be especially useful for micronization of species, and for the abrasion, activation and alternation of chemical properties of catalyst surfaces during ultrasonic irradiation of liquid media (Ince et al., 2001).

It is known that synergetic effect of a photocatalyst of TiO_2 fine particles with oxidation potential of the positive hole under UV irradiation has an ability to enhance the sonochemical reaction. Davydov et al. (2001) showed that the use of 20 kHz–ultrasound during photocatalysis (sonophotocatalytic systems) had a pronounced effect on the rate and the efficiency of salicylic acid destruction as compared to UV-light photocatalysis alone.

Mrowetz et al. (2003) also showed the similar effects at 20 kHz on the destruction of salicylic acid and the oxidation of formic acid. 20 kHz is suitable for obtaining the physical actions by ultrasound such as mixture of different media or dispersion of particulate in liquid phase to improve the photocatalytic events. Even with the use of inert particles such as alumina or silica under appropriate conditions of particle size and the additive, it is possible to improve sonochemical reactions, since the particle addition plays a role of cavitation nuclei to increase in site of chemical reactions (Tuziuti et al., 2004).

Keck et al. (2002), studied the influence of quartz particles (2-25 µm) on the chemical effects of ultrasound in aqueous system using high power ultrasound generator (68-1028 kHz, 100 W, reactor volume 500 mL). They showed that in pure water, regardless of the particle size, concentration and frequency affect the formation rate of hydrogen peroxide under Ar/O_2 (4:1). They observed maximum rate by using 206 kHz in the presence of 3-5 µm quartz particles (4-8 g/L); and stated that the yield of hydrogen peroxide formation and the degradation of organic compounds are higher under this condition.

2.3.5.3. Operational Mode. It is known that yields of sonochemical reactions increase markedly by applying pulsed ultrasound .The mechanism of enhancement comes from the behavior of residual cavitation nuclei during the inactive period of the pulse. Bubbles in a sound field, which are generated by acoustic cavitation, receive two kinds of force of acoustic origin: the primary Bjerknes force and the secondary Bjerknes force. The former determines the motion of each bubble in the sound field. Bubbles larger than the resonant size go to the nodes of sound pressure distribution in a standing wave field and they do

not contribute to the formation of a reaction field, whereas smaller bubbles which generate an extreme environment at the collapse of a bubble go to the pressure antinodes. The latter force acts between oscillating bubbles as an attractive force if they oscillate in phase, and this force makes clusters of tiny bubbles. Once bubble clusters are formed, ultrasound cannot reach inside a cluster because of impedance shielding.

A pulsing operation inhibits the formation of clusters during the inactive period of ultrasound and enhances chemical reactions. Fluid motion in a reactor cell also acts in a similar manner as a source of the suppression of cluster formation leading to higher efficiency of chemical reactions. But, if the time-averaged input power to the transducer is constant, a pulsing operation needs higher amplitude to compensate for the inactive period, and this may bring a quenching phenomenon at excessive amplitude, which reduces reaction yields (Mitomo and Hatanaka, 2002)

2.4. Application of Ultrasound for Disinfection Purposes: Literature Review

The destruction of microorganisms by ultrasound has been of considerable interest since 1920's when the work of Harvey and Loomis was first published. Their work examined the reduction in light emission from seawater suspension of rod shaped *Bacillus fishers* caused by sonication at 375 kHz under temperature controlled conditions. They showed that heating appeared to be injuring bacterial colonies but that ultrasound appeared to have a greater effect. In the 1960s, researches were concentrated on the understanding the mechanism of ultrasound interaction with microbial cells. By 1975, it was shown that brief exposure to ultrasound caused a thinning of cell walls attributed to the freeing of the cytoplasm membrane from the cell wall (Mason et al., 2003).

Dahi (1976) studied the physicochemical aspects of disinfection by means of ultrasound and ozone; and concluded that ultrasonic treatment intensifies the action of ozone with respect to both oxidation of chemicals and inactivation of microorganisms. It is also stated that ultrasonic treatment increases the ozone decomposition and the activity of free radicals in water, as well as the ozone transfer efficiency, and the aeration parameter, k_{La}.

In 1980, it was further shown that bacterial survival under ultrasonic effects exhibits an exponential behavior and that although the shear forces set up by cavitation bubbles are insufficient to rupture the cells unless by prolonged contact, they disengage the more delicate attachment sites of the DNA to membrane (Graham et al., 1980).

Scherba et al. (1991) exposed aqueous suspensions of specific bacteria, fungus and viruses to ultrasound at 26 kHz frequency and discovered that the relative percentage of bacteria killed increased with an increase in exposure time and increased ultrasonic intensity.

Mues et al. (1995) showed that zooplankton such as *Arthemia daphnia* or *Notolka* are inactivated by a disruption of their chitin carapace caused by

ultrasonically created microjets which are mechanical effects of ultrasound, generated by the asymmetrical collapse of cavitation bubbles, and having velocities up to 100 m/sec.

Phull et al. (1997) investigated the effect of ultrasound (38 kHz, 5 W/cm^2) up on destruction of *E.coli*. It was stated that the ultrasound could be used effectively for water disinfection and has several advantages. When used in conjunction with chlorine it significantly reduced the number of bacteria present in the water samples. They demonstrated that ultrasound could substantially improve the effect of biocide (chlorine) in disinfection. Neither chlorination alone nor sonication alone was able to completely destroy the bacteria present. It is significant to note that extending the time of chlorination and sonication from 5 min to 20 min seems to double the biocidal effect of individual techniques when sonication is combined with chlorination, however, the biocidal action is significantly improved. They claimed that ultrasound reduces the amount of chlorine required for disinfection; and size of particles (from 40 to 1μ in 8 min); increasing the power of ultrasound leads to greater efficiency; and high frequency ultrasound is more beneficial than low frequency at the same acoustic power, when the improvement on the biocidal action of chlorine considered.

Hua and Thompson (2000) investigated the impact of power intensity, dissolved gas and ultrasonic frequency on the ultrasonic inactivation of *E.coli*. They concluded that the inactivation of *E.coli* exhibits pseudo-first order behavior and depends moderately on total power and the power intensity at frequency of 20 kHz; the nature of dissolved gas does not strongly influence the magnitude of inactivation coefficient varied from 0.027-0.047 1/min; ultrasonic frequency within limits of 205-1017 kHz with same power displayed a strong influence on rate of inactivation and the most effective ultrasonic frequency was found to be 205 kHz.

Radel (2000) evaluated the viability of yeast cell suspensions as a function of treatment time during exposure to both standing and propagating wave fields with frequencies slightly above 2 MHz and change in yeast cell morphology caused by ultrasonic treatment were examined by transmission electron microscopy (TEM).

The electron microscopy of the cells which were sonicated by standing wave fields shows morphological changes. Microorganisms tend to be concentrated in the pressure nodal planes where the cavitation is absent in the case of standing waves, causing the microbial inactivation to seem to be weakened. The ultrasound seems to alter the integrity of cell vacuole, while cell nucleus and envelope are not affected. In treatment with propagating waves, cells display significant loss of viability.

Ultrasound technologies have a wide range of hospital and dental applications which include cleaning and disinfection of surgical and dental instruments. Jatzwauk et al. (2001) measured the germicidal efficacy of sonication, with or without chemical disinfectants, in an ultrasonic bath delivering a frequency of 35 kHz and an intensity of 0.66 W/cm^2. Cultures of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* were exposed to ultrasound and to an amine-based disinfectant in non-bactericidal concentrations. Ultrasonication for 60 min alone did not cause a significant killing of the bacteria and yeast. However, they were able to show that sonication can act as a powerful synergistic agent to increase the cidal efficacy of the disinfectant against *S. aureus* and *P. aeruginosa, C. albicans* was more resistant to the combination of ultrasound and chemical disinfection. The key role in the action of ultrasound in cleaning of instruments and perhaps in enhanced disinfection is played by cavitation phenomena.

Ince and Belen (2001), evaluated aqueous phase disinfection of *E.coli* with power ultrasound (20 kHz) and effects of solid catalysts on the process kinetics. They found out that disinfection by ultrasound is accelerated with solids in the order of activated carbon>ceramic>metallic zinc. The acceleration of disinfection was the result of the chain of events between increased cavitational nuclei and enhanced mechanical effects of ultrasound for attrition, milling and dispensing solid particles and activating their surfaces. The observed order of effectiveness was attributed to: (i) Surface (AC has specially manufactured surface properties) and (ii) Crystalline properties of the solids.

Blume et al. (2002), studied the effect of frequency, pressure, intensity, dissolved oxygen and residence time on the inactivation of plankton by ultrasound in drinking water. They revealed that ratio of survival is less in low frequency, that is, increase in frequency decreases the effectiveness; survival decreases with increasing, intensity, contact time, pressure, and oxygen concentration.

Joyce et al. (2003) investigated the effect of power at different powers and frequencies on *Bacillus subtilis*. The results showed a significant increase in per cent kill with increasing duration of exposure and intensity of ultrasound in the low-kHz range (20 and 38 kHz). Results obtained at two higher frequencies (512 and 850 kHz) indicated a significant increase in bacterial count suggesting declumping. In assessing the bacterial kill with time under different sonication regimes three types of behavior were characterized: (i)High power ultrasound (lower frequencies) in low volumes of bacterial suspension results in a continuous reduction in bacterial cell numbers i.e. the kill rate predominates. (ii) High power ultrasound (lower frequencies) in larger 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. (iii) Low intensity ultrasound (higher frequencies) gives an initial rise in cell numbers as a result of declumping. The kill rate is low and so there is no significant subsequent decrease in bacterial cell numbers. They concluded that high frequency alone was not particularly effective for disinfection.

Joyce et al. (2003) summarized the process that weaken or disturb biological cells as follows:

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

(b) Shear forces induced by microstreaming occurs in bacterial cells. High shear forces and liquid jets may damage the cell wall/membrane.

(c) Chemical attack due to the formation of radicals (•H and •OH) during cavitation in the aqueous medium (Fragmentation of water molecules during the collapse of cavity bubbles). These radicals attack the chemical structure of the bacterial cell wall and weaken the cell to the point of disintegration.

(d) High local temperatures and pressures disintegrate biological cells and/or denature any enzymes.

(e) Amongst the final products of this sonochemical degradation of water is hydrogen peroxide (H_2O_2, recombination of radicals, which is a strong bactericide.

Duckhouse et al. (2004) stated that ultrasound alone is capable of killing bacteria when sufficient power is applied but ultrasound at low powers can also be used to improve the effectiveness of biocides. They explored the effect of the timing of the ultrasonic treatment at 20 and 850 kHz on the biocidal efficiency of sodium hypochlorite solution towards suspensions of *E*.coli and noted remarkable frequency effect. At lower frequency, the improvement in biocidal activity is greatest when it is applied at the same time as the hypochlorite. When the ultrasound is used as pretreatment before the hypochlorite addition under silent condition, frequency of 850 kHz is better.

Jyoti and Pandit (2004) investigated the synergistic effect of ozone and cavitation for water disinfection. They revealed that synergetic processes, increases the ozone decomposition and reduces to half or one third the required concentration of ozone and stated that reduced amount of chemicals means reduced amount of toxic products associated with these biocides.

Furuta et al. (2004) investigated the ultrasonic inactivation of *E.coli* by high intensity ultrasonic waves from horn type sonicator (27.5 kHz) utilizing the ‘’squeeze- film effect’’, the film refers to the space between the end of the probe of the sonicator and the bottom of the reactor is designed to prevent the influence at the site of acoustic pressure and to provide enough cavitation. They observed that

inactivation showed pseudo first order behavior, the inactivation rate constant gradually increased with increasing amplitude of vibration face and showed rapid increase above 3 µm (p-p). In contrast, the H_2O_2 formation was not observed below 3 µm (p-p), indicating that ultrasonic shock wave might be more important than indirect effect of OH radicals formed by cavitation. The optimum thickness of squeeze film was determined as 2 mm for the *E.coli* inactivation; more that 99% of bacterial removal was achieved with in 180 sec sonication at the amplitude of 3 µm and 2 mm of thickness of squeeze film.

Blume and Neis (2004) evaluated the scientific and economic potential of ultrasound applications as a pre-treatment step in combination with UV to optimize the disinfection process. They were stated that efficiency of UV applications is limited for samples with high concentrations of suspended matter. Suspended solids can act as protection to bacteria and viruses; and large bio-particles, bigger than 50µm in diameter are hard to penetrate so that the required UV demand is raised drastically. In this study, although the concentration of suspended particles cannot be reduced by sonication, the particle size distribution was significantly changed. Almost no particles greater than 50 microns were left after sonication. It was found that disinfection efficiency was enhanced by 1.2 orders of magnitude (compared with the not pretreated samples by sonication). They observed that even if the specific energy consumption of ultrasound was higher than that of UV Lamps, the combination of these processes is much more economical.

Tsukamoto et al. (2004) investigated the *Saccharomyces cerevisiae* (yeast cells) by ultrasonic irradiation with a horn type sonicator emitting at 27.5 kHz. Inactivation of yeast cells showed pseudo-first order behavior, and the inactivation by ultrasonic irradiation was found to be decreasing with increasing initial yeast cell concentration.

Dadjour et al. (2005), conducted a study to investigate the kinetics of disinfection of *Escherichia coli*, in the presence of a TiO_2 photocatalyst, using an ultrasonic irradiation system. TiO_2 was found to significantly improve the disinfection process. A 98% reduction in the concentrations of viable cells was

obtained in the presence of TiO_2 during a 30 min period of irradiation, while only a 13% reduction was observed when an ordinary ultrasonic irradiation system was used. The rate of cell killing was also higher in the presence of TiO_2 compared with Al_2O_3. The rate of disinfection was proportional to the amount of TiO_2 in the concentration range examined. Cell concentrations were decreased by an order of 5 within 10 min of irradiation in the presence of 2.0 g/mLTiO_2. No significant effect of cell concentration on the cell-killing process was found in the range of 10^3 to 10^7 CFU/mL. The mechanism of cell killing was further investigated by examining the effects of OH radical scavengers, such as histidine and glutathione. The rate of disinfection was decreased in samples containing these radicals, indicating the importance of radicals in the process.

3. MATERIALS AND METHODOLOGY

3.1. Materials

The test bacteria, the reagents and the consumables are described in the following sections.

3.1.1. Test Bacteria

The experiments were carried out with a pure culture of *Escherichia Coli* (*E.coli*) which was obtained from Boğaziçi University – Department of Biochemistry in frost form in vials. The Figure 3.1 shows the Scanning Electron Microscopy photographs of E.*coli*.

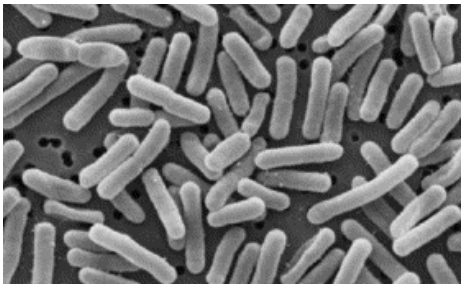

Figure 3.1. Scanning electron microscopy (SEM) photographs of *Escherichia Coli* cells (Diao et al., 2004).

3.1.2. Growth Medium

E.coli was grown in Oxoid® MM0615 - Membrane Laurly Sulphate Broth (MLSB) medium which consisted of 39.0 g/L Bacteriological Peptone; 6 g/L Yeast extract; 30 g/L Lactose; 0.2 g/L Phenol red; and 1.0 g/L Sodium laurly sulphate The pH and the temperature of the medium was 7.4±0.2 and 25 ^{0}C.

3.1.3. Petri Dishes, Pads, and Filter Papers

E.coli were filtered through Millipore® 0.45 µm, white 47 mm gridded sterile filters and incubated in disposable Millipore® Petri dishes with ready to use, sterile, white absorbent pads with a diameter of 47 mm.

3.1.4. Dilution and Filtration Water

Dilution water for the test solutions and filtration water were prepared by dissolving 13.6 g of Riedel-de Haën, Potassium dihydrogen phosphate, KH_2PO_4 in 1 L of ultra pure deionized water.

3.1.5. Solid Particles and Sieves

Sand particles were collected from Balıkesir-Ayvalık coastal region. Talc was Riedel-de Haën and in powder form. Particle sizes were determined by using USA Standard Testing Sieves with ASTM-11 Specifications; which were No: 18, 60 and 270 with opening sizes 1.00 mm, 250 µm, and 53 µm, respectively.

3.2. Apparatus and Equipment

1. Ultrasonic Reactor-1 (20 kHz, Bandalin Sonoplus HD2200, glass cell, V=80 mL)
2. Ultrasonic Reactor-2 (300 kHz, Undatim Ultrasonics, glass cell, V=100 mL)
3. Ultrasonic Reacto-3 (520 kHz, Undatim Ultrasonics, glass cell, V= 300 mL)
4. Unicam Heλios Alpha/Beta Double Beam Spectrophotometer
5. Autoclave
6. Shaking Water Bath (Julabo SW22)
7. Incubator (Gallenkamp)
8. Filtration Assembly (Millipore)

3.3. Methods

3.3.1. Preparation of the Growth Medium and Stock Culture

Growth medium of *E.coli* was prepared by dissolving 76.2 g MLSB medium in 1 L of deionized water in a glass bottle and sterilized in autoclave for 15 minutes at 121 ^{0}C and cooled before usage. Growth medium bottles were removed from autoclave immediately after sterilization in order to avoid the breakdown of lactose. This medium was used for both preparation of the stock culture and incubation of samples withdrawn throughout the experiments. A clean and healthy colony of *E. coli* was selected by a sterile loop from a mother dish and placed in 100 mL of growth medium and inoculated in a shaking incubator for 24 hr, at 37 ^{0}C. This solution was used as stock solution and prepared freshly.

3.3.2. Preparation of the Test Solutions

Test solutions of *E.coli* were prepared by appropriate dilutions of the stock solution using phosphate buffered ultra pure deionized water to prevent cell damage by osmotic pressure. The composition of dilution water as defined previously, was sterilized in an autoclave for 15 minutes at 121 ^{0}C before use.

3.3.3. Experimental Setup

Ultrasonic irradiation of bacterial suspensions was carried out by three different systems with different operational parameters, reactor volumes, and characteristics as defined below. The schematic views of the systems are presented in Figure 3.2. It is important to note that the reason for different input powers and solution volumes in each system is that the systems were operated at their previously optimized values.

3.3.3.1. System-1. This system consisted of a 20 kHz Bandalin Sonoplus HD2200 transducer, 180 W - horn type sonicator, and a glass cell with a water volume of 80 mL, equipped with a water cooling jacket to maintain constant liquid

temperature. The horn was submerged 1.5 cm into the test solution. The applied power was 59 W (Figure 3.2. a).

3.3.3.2. System-2. This system consisted of a plate type transducer connected to a power generator (Undatim Ultrasonics) operated at 25 W and emitting a frequency of 300 kHz; and a glass cell with a water volume of 100 mL equipped with a cooling water jacket (Figure 3.2.b).

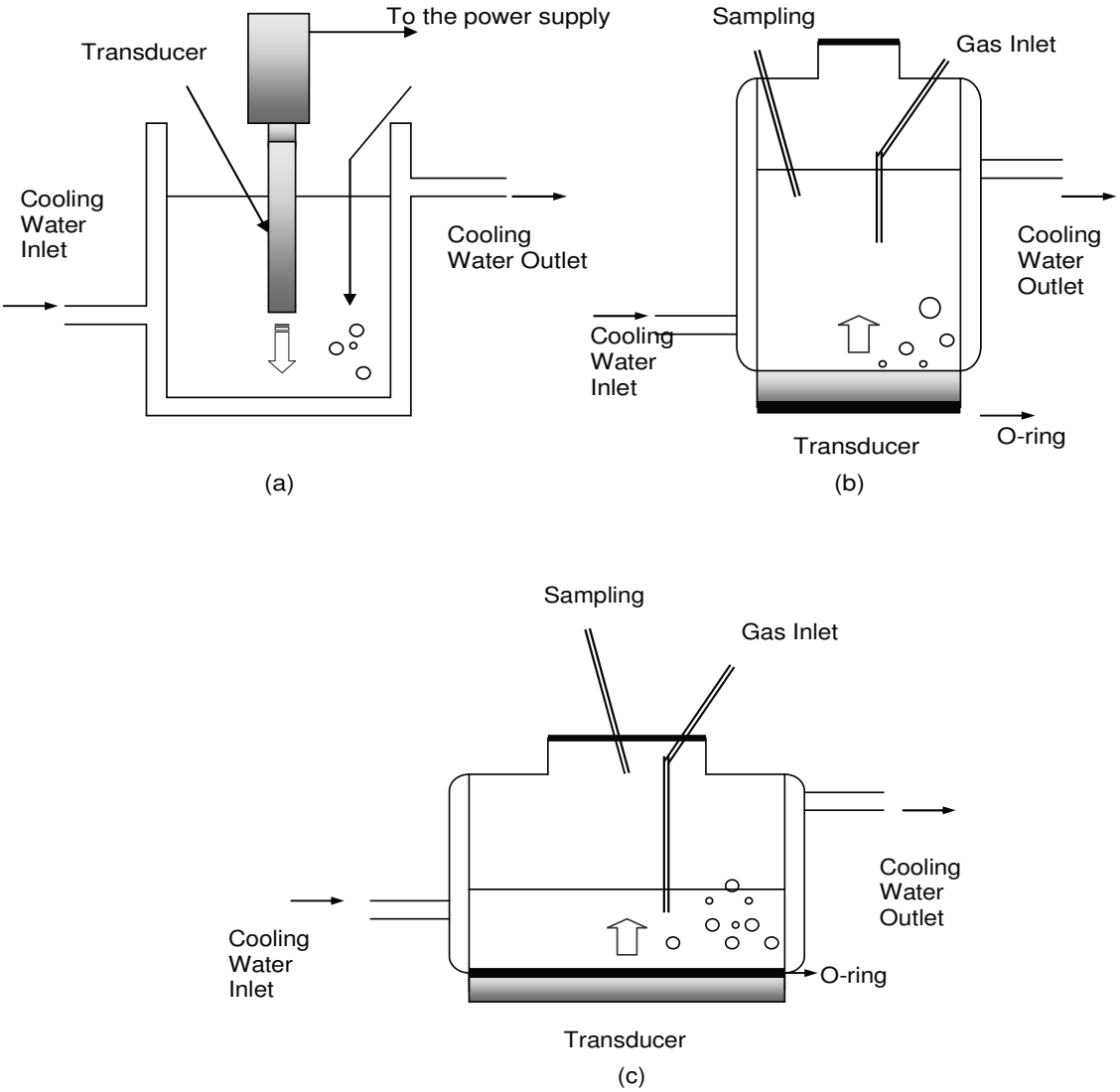

Figure 3.2. Schematic diagram of experimental systems: (a) System -1, (b) System- 2, (c) System- 3.

3.3.3.3. System-3. This system consisted of a plate type transducer, a power generator (Undatim Ultrasonics) operated at 100 W and emitting a frequency of 520 kHz, and a glass cell with a water volume of 300 mL equipped with a cooling water jacket. The applied power was 40 W (Figure 3.2.c).

3.3.4. Sonication Experiments

In all experiments, argon gas was bubbled mildly and continuously to enhance the cavitation events, to compensate for degassing effects, and to improve the degree of mixing in the test solution. The effect of buffer concentration was determined by varying its concentration and testing the change in the degree of bacterial kill.

Control experiments without ultrasound were also carried out simultaneously to assess the role of cavitation in the inactivation process. All experiments were performed in duplicate with two parallel samples and arithmetic means of parallel measurements were reported.

3.3.4.1. Continuous mode/homogeneous solutions. *E.coli* suspensions were sonicated for 20 minutes in System-1, and for 30 minutes in Systems 2 and 3. The volumes of the test solutions were 80 mL, 100 mL, and 300 mL in System-1, 2 and 3, respectively. All systems were operated in the batch mode. A 1 mL sample was withdrawn within certain time intervals during each experiment to monitor the change in bacterial concentration with time.

3.3.4.2. Pulse mode/homogeneous solutions. The above experiments were repeated under pulse sonication with 2 minutes of ultrasonic irradiation followed by 1 minute of pause (silent) period, which end up with 6.5 cycles in System-1; and 10 cycles in Systems-2 and 3.

3.3.4.3. Heterogeneous experiments. Experiments with solid particles with various particle sizes were performed in System-1, in continuous mode. The solids were sand and talc with regular sizes and grouped according to their particle sizes as

given in Table 3.1. Note that each group of solid particles has uniform size and regular shape.

Table 3.1. Particle sizes of solids used in heterogeneous experiments.

Type of Solid	Particle Size (d)
Sand	$d \geq 1$ mm
Sand	250μm<d<1mm
Sand	53μm<d<250μm
Talc	$d \leq 53$μm

The mass concentration of the particles was 0.12 g/L in each run, regardless of type and size of the solids. Test solutions were sonicated continuously for 20 minutes and samples were withdrawn from the reactor within certain time intervals to monitor the decrease in bacterial concentration with time.

Control experiments with each solid were carried out in the absence of ultrasound to detect whether or not any reduction in the cell concentration occurred by adsorption on the surface of the particles. Further, at the end of each heterogeneous experiment, solid particles were separated from the test effluent and incubated in growth medium to detect if there was any *E.coli* adsorption on the surface.

3.3.5. Analytical

3.3.5.1. Determination of the Ultrasonic Power in the Reactor. The power deposited in the test samples was determined calorimetrically, using the procedure described in the literature (Mason et al., 1992). The initial temperature rise induced by ultrasound was converted into energy input by using the following equation:

$$P = m_L . C_p . (dT/dt) \qquad (3.1)$$

where ‘’m_L’’ is the mass of liquid, ‘‘C_p’’ is the specific heat constant at constant pressure (4.184 J.g/^{0}C) and the term (dT/dt) is the slope of the curve Temperature versus time.

3.3.5.2. Enumeration of Bacteria. Enumeration of *E.coli* was made by ‘’Membrane Filtration Technique’’ in accordance with the Standard Methods (APHA, AWWA, WPCH, 1989). This procedure consisted of passing a known volume of a sample through a membrane filter that has pores smaller than the size of the species being analyzed. Vacuum was provided to accomplish filtration through a funnel.

1 mL of sample pipetted from the test solution was poured in a sterile glass bottle and 10 mL of sterilized filtration water was added in order to provide better spreading of bacteria on the entire surface of the membrane filter and to prevent any damage on cells during the application of vacuum through the filtration process. Then, all the liquid was poured through the sterile filter funnel, the membrane filter placed on an absorbent pad saturated with 2 mL of MLSB growth medium and placed in a sterile petri dish. After incubation at 44 ^{0}C for 24 hrs, colonies formed were counted visually. The technique assumes that each colony has derived from a single cell; the results were reported as ‘’ # of colonies/mL’’.

In every set of experiments, a membrane filter through which filtration water without any test solution was filtered and another dry membrane filter were placed on absorbent pads and incubated in order to detect if there was any contamination in filtration water or growth medium. Additionally, filtrates collected at the bottom of the funnel were also re-filtered so as to control if any bacteria could escape during the filtration process through the membrane.

3.3.5.3. Analysis of Hydrogen Peroxide. The concentration of H_2O_2 was determined by ‘’I_3^- method’’, which is based on the colorimetric determination of I_3^- formed when H_2O_2 is added to a concentrated solution of I^- (Klassen, 1994). The analysis of H_2O_2 at concentrations as low as 1 μM is possible by determining the yield of I_3^- formed when H_2O_2 reacts with KI in a buffered solution containing ammonium molybdate as a catalyst. Two solutions were prepared: Solution A

consisted of 33 g KI, 1g of NaOH and 0.1 g of ammonium molybdate tetrahydrate diluted to 500 mL with deionized water. Solution B, an aqueous buffer, contained 20 g KHP per 1000 mL. 2.5 mL of solution - A, 2.5 mL of solution - B and 1 mL sonicated sample were diluted to 10 mL by deionized water. The absorbance of the resulting solution was measured at 351.0 nm using a spectrophotometer. The increase in the absorbance of the solution was the direct indication of the accumulation of H_2O_2. The concentration of H_2O_2 was calculated by means of a calibration curve. The absorbance of the solutions and the calibration curve are presented in Tables A.1-3, and Figure A.1, respectively in Appendix A.

4. RESULTS AND DISCUSSION

4.1. Power Efficiencies of the Reactors

The applied and the deposited powers (as determined by calorimetrically) in each system are summarized in Table 4.1.

Table 4.1. Summary of applied and deposited powers in experimental systems.

SYSTEM	Power Applied (W)	Power Deposited (W)
SYSTEM-1 (20 kHz- 80 mL)	59	36.5
SYSTEM-2 (300 kHz- 100 mL)	25	14.7
SYSTEM-3 (520 kHz- 300 mL)	40	33.6

4.2. System-1 (20 kHz- 80 mL)

4.2.1. Continuous Sonication - Homogeneous Medium

Continuous sonication of *E.coli* suspensions with an initial cell concentration of $N_0=10^3$ colonies/mL was performed. The appearance of the colonies after 10 minutes of sonication is given in Figure 4.1. Samples were withdrawn in certain time intervals to monitor the residual surviving bacteria. The data are presented in Figure 4.2. It was found that the density of *E.coli* decreased sharply during the initial stages of sonication, but the reduction slowed down after 5 minutes. Complete disinfection could be accomplished in 20 minutes. No bacterial kill was recorded in control experiments without ultrasound. Additionally, no colony formation was observed in the filtrate, showing that bacteria could not escape during membrane filtration process.

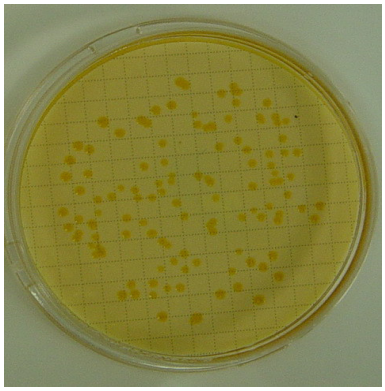

Figure 4.1. *E.coli* colonies incubated in petri dish at 44 ^{0}C for 24 h.

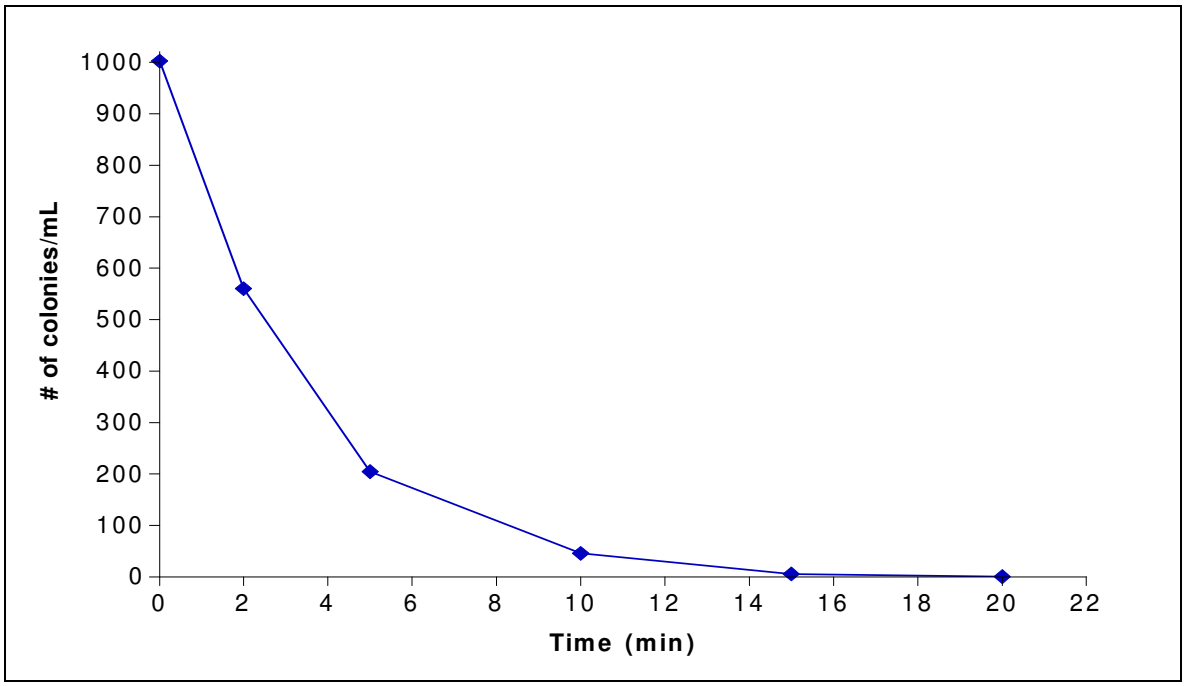

Figure 4.2. Decrease in *E.coli* concentration with time in System-1 during continuous- sonication (N_0=10^3 colonies /mL, 0.1 M KH_2PO_4).

4.2.1.1. Formation of H_2O_2. The presence of H_2O_2 can be considered as a rough indication of •OH radicals in ultrasonic systems, as a consequence of the reactions given by Eq. 2.1 and Eq. 2.2 in Section 2.3.3. Comparative profiles of H_2O_2 evolution in deionized water and in the *E.coli* solutions are shown in Figure 4.3. It can be seen from the figure that the presence of *E.coli* decreased the accumulation of H_2O_2, indicating the attack of uncombined •OH radicals to the cells as a chemical bacteriocide. This implies that chemical effects were also present (especially after 10 minutes of sonication) in addition to the mechanical

effects of power ultrasound. It should be also noted that H_2O_2 itself is a strong biocide as indicated by the decrease in its concentration in *E.coli* containing water.

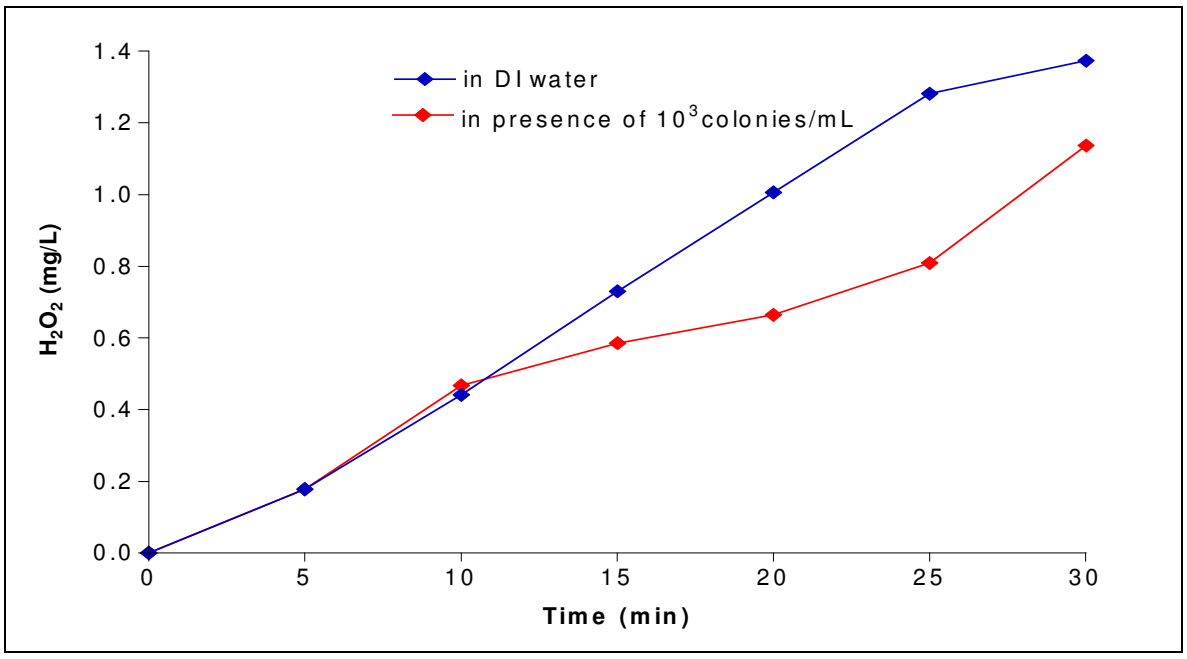

Figure 4.3. Concentration of H_2O_2 in deionized water and in presence of *E.coli* (N_0=10^3 colonies/mL) in System-1.

4.2.1.2. Effect of Buffer Concentration. Changing the buffer concentration did not result in any change in the performance of this system. Thus, the data were not presented and all experiments were performed in the presence of 0.1M KH_2PO_4.

4.2.1.3. Effect of Initial Cell Concentration. The impact of the input cell concentration was investigated by performing the experiments with different concentrations (N_0=640, 320, and 240 colonies/mL). Plots of survival ratio (per cent remaining) versus time at different cell concentrations are presented in Figure 4.4. All results are the average of the two data sets and differences between them are not significant. It is important to note that this is also valid for all data sets of each system.

According to the Figure 4.4, complete disinfection was accomplished at the end of 20 minutes, in all cases. It was found that the rate of removal was higher when the initial number of colonies was lower. If the second minute of sonication is

considered, 60% of cells were removed when the starting concentration was 240 colonies/mL; however only around 40% of the bacteria could be inactivated when it was 10^3 colonies/mL. The reasons for this observation might be the following:

(i) When the number of cells in a unit volume is high (high to medium), cells tend to agglomerate to form bacterial clusters, in which the resistance of cell to the mechanical shear forces and chemical attacks is high.

(ii) When the number of cells is low (low to medium).The probability of cluster formation is lower, so that cells are more vulnerable to outside effects.

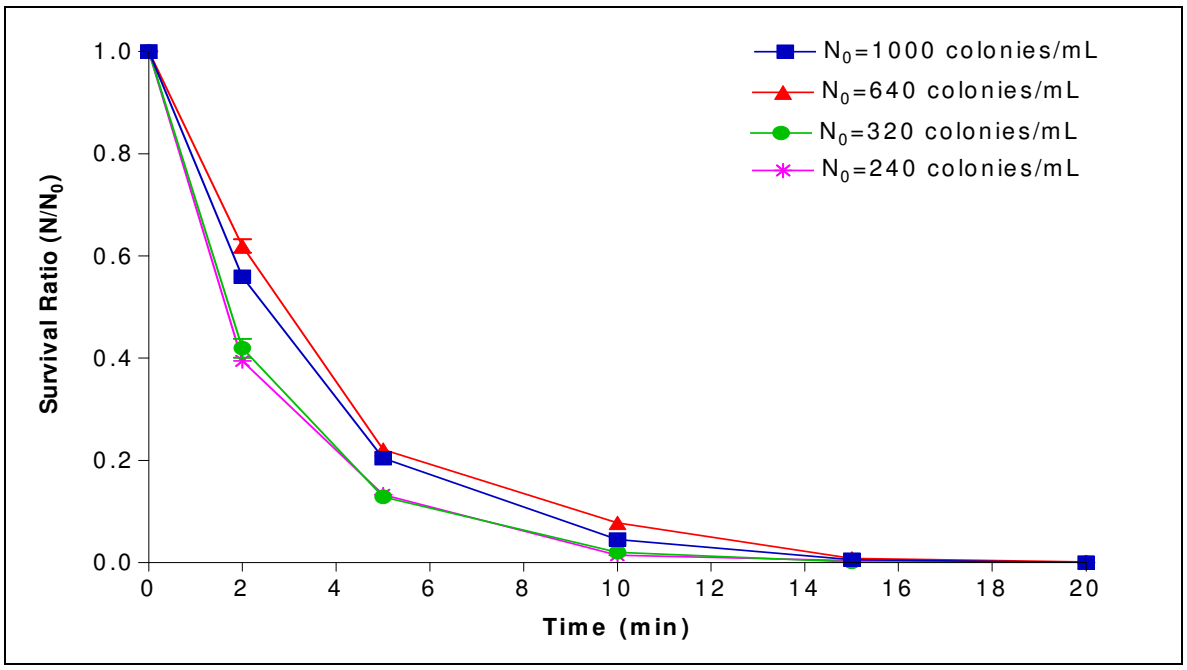

Figure 4.4. Profiles of survival ratios with different initial cell concentrations in System-1 during continuous sonication. (0.1 M KH_2PO_4).

4.2.2. Pulse Sonication - Homogeneous Medium

In order to investigate the effect of operational mode, *E.coli* solutions with $N_0=10^3$ colonies/mL were sonicated in pulse mode. The decrease in cell concentration with time is presented in Figure 4.5. It was observed that, the profile of inactivation was very similar to that in the continuous mode. Nearly complete decay could be achieved in 20 minutes. It is important to keep in mind that in the pulse mode, total time of contact with ultrasound was 14 minutes, while it was 20 minutes in the continuous operation.

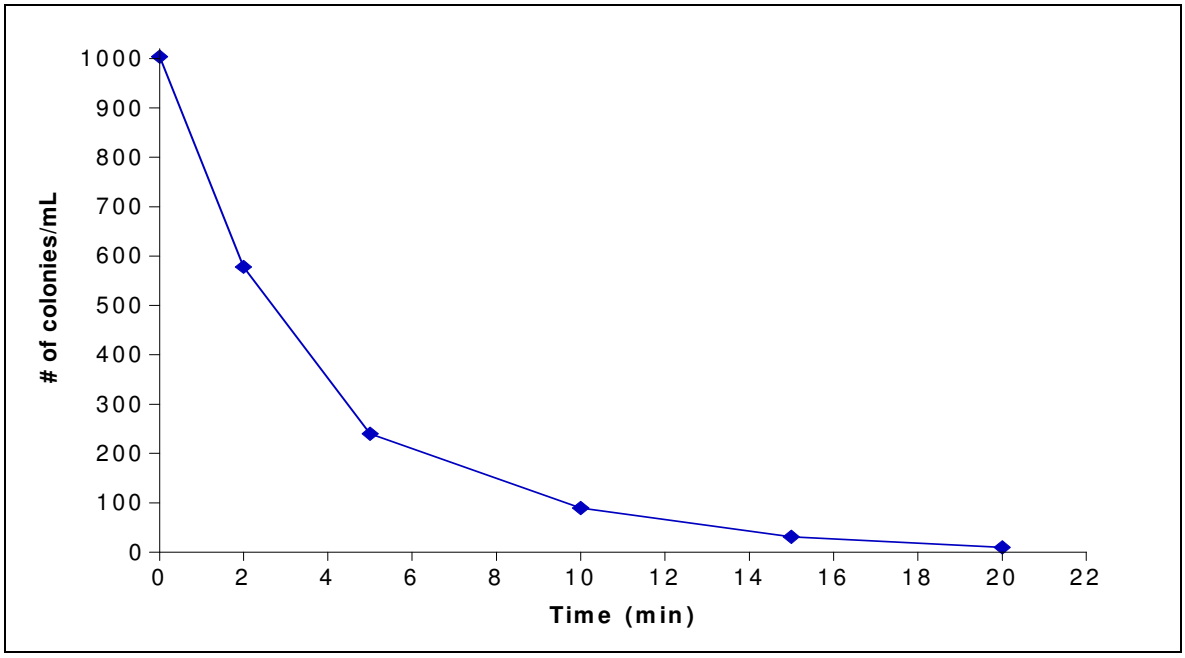

Figure 4.5. Decrease in *E.coli* concentration with time in System-1 during pulse-sonication ($N_0=10^3$ colonies /mL, 0.1 M KH_2PO_4).

4.2.2.1. Effect of Initial Cell Concentration. The impact of the input cell concentration was investigated by performing the experiments with different cell concentrations (N_0=610, 310, and 230 colonies/mL). The plots of survival ratio (per cent remaining) versus time are presented in Figure 4.6. The figure shows that, the tendency of the cells for inactivation is very similar to that observed in the continuous mode.

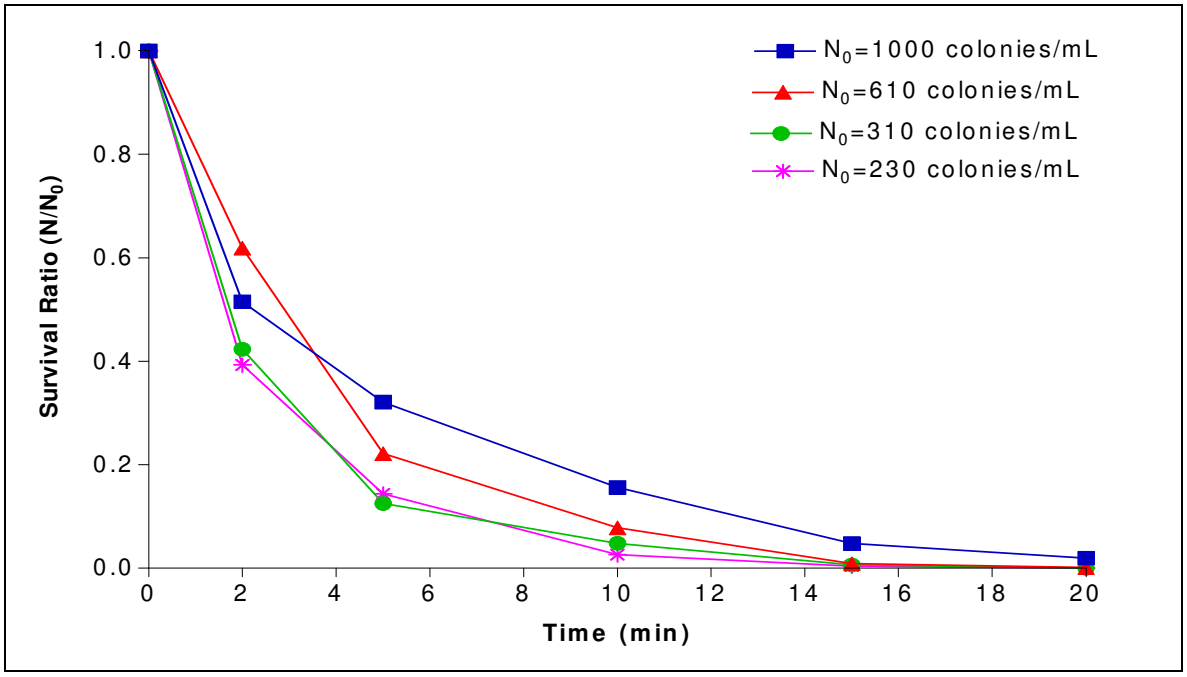

Figure 4.6. Profiles of survival ratios with different initial cell concentrations in System-1 during pulse - sonication (0.1 M KH_2PO_4).

4.2.3. Comparison of Operational Modes

The efficiency of sonochemical reactions can be enhanced by applying pulsed mode of operation. The mechanism of enhancement comes from the continuing effect of residual cavitation nuclei during the inactive period. However, we found that the rate of inactivation was lower in the pulse mode as shown in Figure 4.7.

The reason for the lack of enhancement in bacterial kill by pulse operation must be because of the long lag period we applied. During this long pause, it is possible that cavities were cushioned, such that the remaining colonies formed clusters again. The reason for our long lag period is the fact that it was not practical to turn off and turn on the generator too often and too fast without risk of damaging the system.

However, it should be noted that the efficiency of the pulse mode is still high, because total sonication time is 6 minutes less than that in the continuous mode. This matter will be discussed further in Section 4.6.

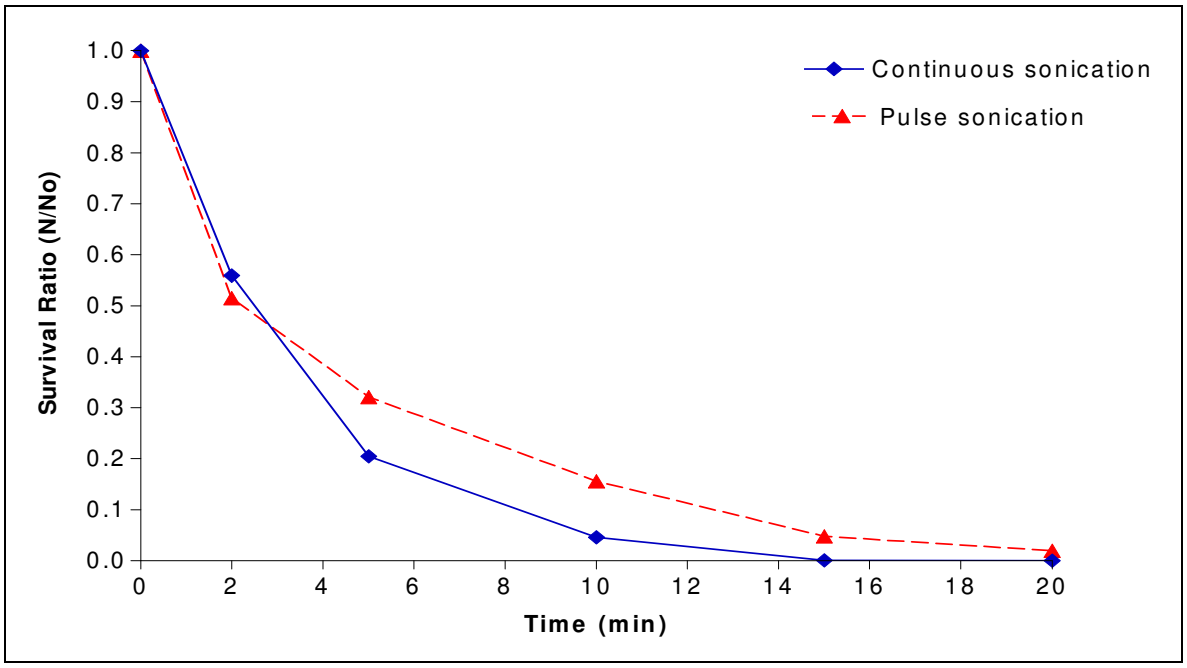

Figure 4.7. Comparative profiles of survival ratios with respect to operational mode in System-1 (N_0=10^3 colonies/mL, 0.1 M KH_2PO_4).

4.2.4. Continuous Sonication - Heterogeneous Medium

Addition of solids into sonicated solutions is a well known way of enhancing both mechanical and chemical effects of ultrasound. When cavitation occurs in a liquid near a solid surface, the dynamics of cavity collapse changes dramatically. In pure liquids, the cavity remains spherical during collapse because its surroundings are uniform. Close to a solid boundary, however, cavity collapse is very asymmetric, generating high-speed jets of liquid. Bubble size and collapse time may not be influenced by nature of the solid particles used, but the shape of bubbles may change. Hence, larger surface of the bubbles enable the ejection of more radical species into bulk liquid (Suslick, 1990).

For this purpose, heterogeneous experiments were run in solutions containing various sized sand and talc. All experiments were operated

continuously with No=10^3 colonies/mL and solid concentration was 0.12 g/L. The results of control experiments showed that there was no decrease in the *E.coli* concentration, and no colony formation was recorded in the petri dishes containing solids. These imply that adsorption on solid surfaces did not occur.

It is known that power ultrasound is effective in reducing the size of particles. Ince and Belen (2001) found that the solids (ceramic, zinc, and granular activated carbon) were converted into powder form after a very short period of sonication. However, in this study, we found that particle size was not reduced by ultrasound, to be attributed to the rigidness or non-brittle nature of the sand.

4.2.4.1. Sonication with Sand Particles. Comparative profiles of reduction in cell concentration in the presence and absence of sand particles (d>1mm) are given in Figure 4.8. In accordance with the figure, sand with this particle size did not improve the system performance; only a negligible enhancement can be noticed; because size reduction and effective dispersion of sand particles could not be achieved.

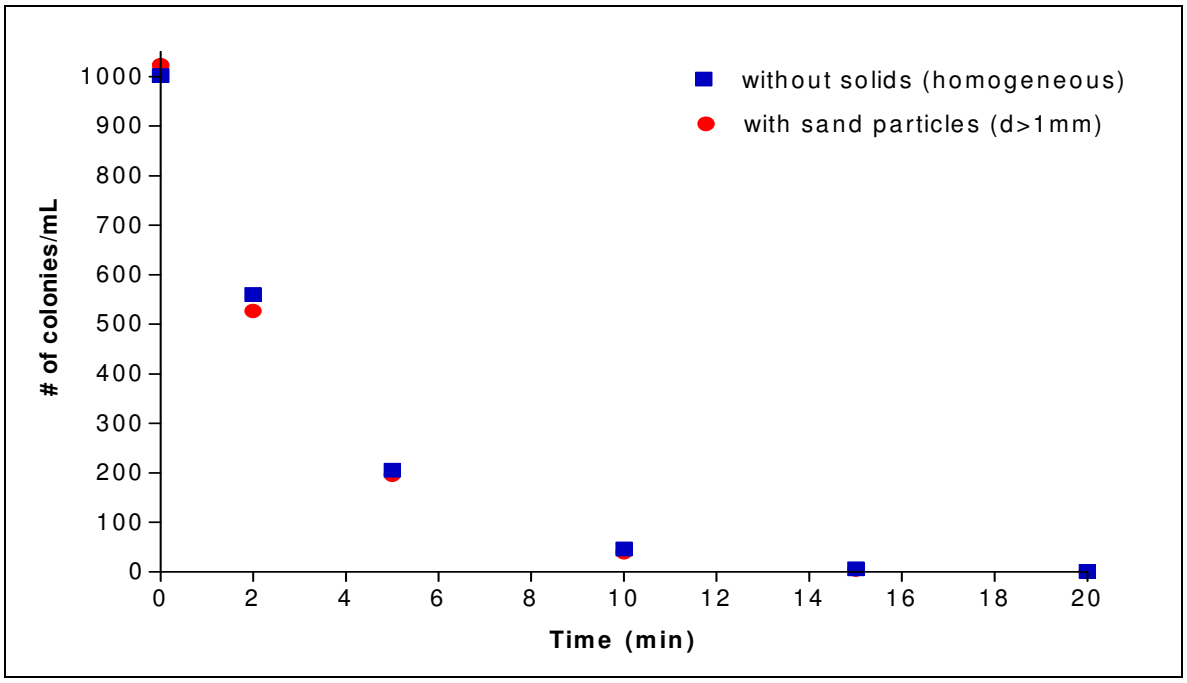

Figure 4.8. Decrease in *E.coli* concentration in the absence and the presence of sand in System-1.

4.2.4.2. Sonication with Talc. In the experiment, talc in powder form having a uniform size less than 53 µm was used. Comparative disinfection profiles with and without talc are presented in Figure 4.9. It was found that addition of talc markedly enhanced ultrasonic inactivation. Higher effectiveness of talc is due of considerably smaller particle size.

4.2.4.3. Effect of Particle Size. Experiments were performed with the addition of sand particles having different sieve sizes. Comparative profiles are presented in Figure 4.10. It was found that sand particles with a particle size of d>250 µm was not effective. However, the rate of kill was accelerated by 16.4 % and 16.7 % with sand at 53 µm<d<250 µm and with talc at d≤53 µm, respectively. Thus, it can be concluded that the smaller the size of the particles, the higher the enhancement in the rate of kill.

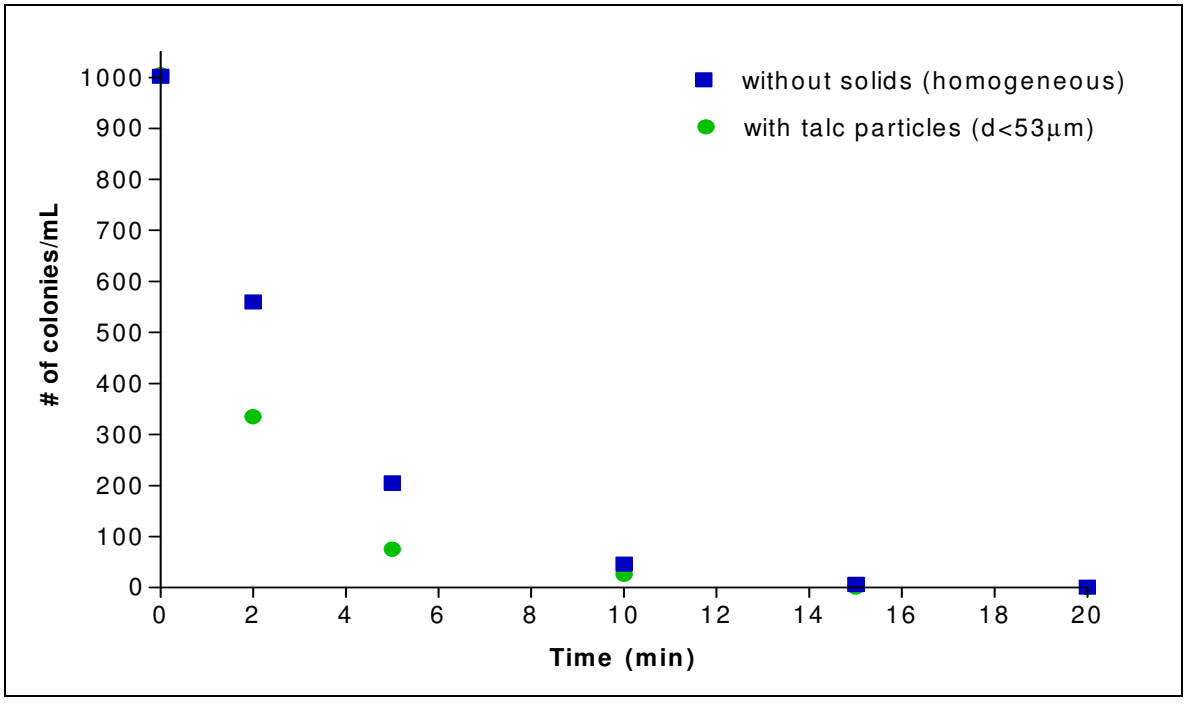

Figure 4.9. Decrease in *E.coli* concentration in the absence and the presence of talc in System-1.

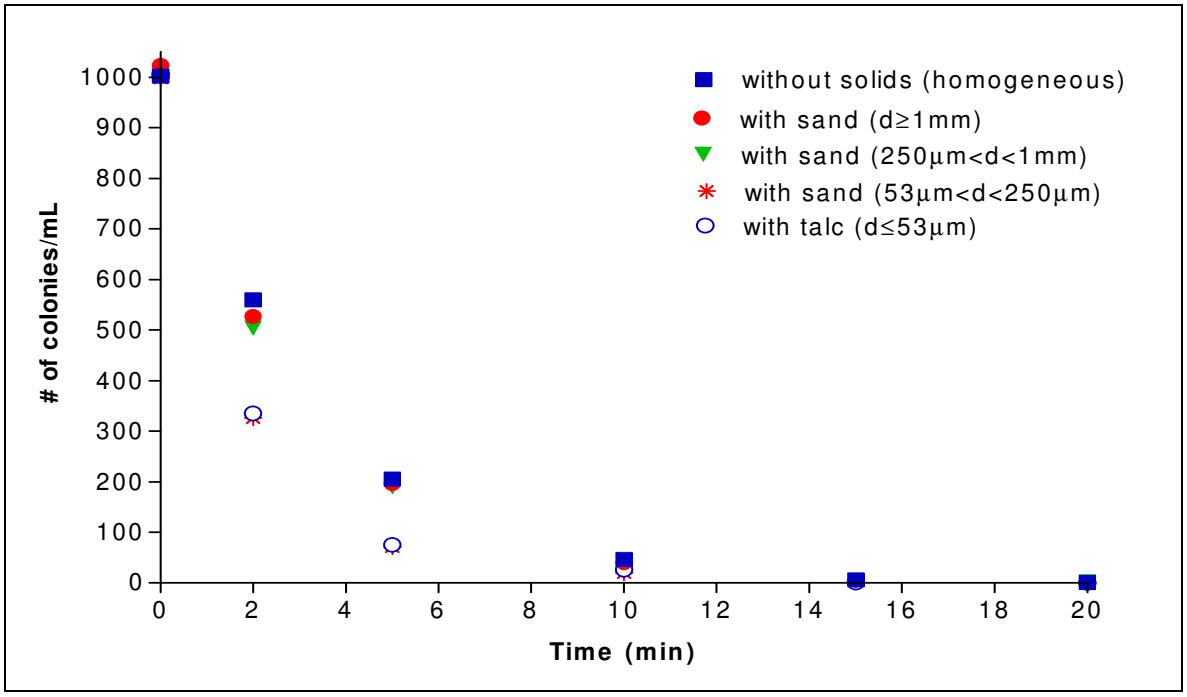

Figure 4.10. Profiles of decrease in *E.coli* concentration with solids having different sizes (N_0=10^3colonies/mL, C_{solid}= 0.12 g/L, 0.1M KH_2PO_4).

4.3. System-2 (300 kHz- 100 mL)

As was given in the Section 4.1, the applied power and the deposited power in this system were 25 W and 14.7 W, respectively. All experiments were performed in homogeneous medium both in continuous and pulse modes. Total experimental time was 30 minutes at each mode.

4.3.1. Continuous Sonication

E.coli suspensions with N_0=10^3 colonies/mL were sonicated for 30 minutes, and the decrease in cell concentration was monitored. The data are presented in Figure 4.11. It was found that only 45 % kill could be accomplished in 30 minutes, in contrast to 100% kill in System-1 in 20 minutes. This is because of the fact that at this frequency mechanical effects are low; the inactivation of cells is mainly governed by the chemical effects of ultrasound i.e. oxidative damage of •OH

radicals and H_2O_2. Moreover, availability of •OH radicals is affected by the concentration of phosphate buffer.

4.3.1.1. Effect of Buffer Concentration. The slow decrease in *E.coli* concentration in System-2 might be the result of •OH scavenging effect of phosphate ion in the buffer. This might considerably decrease the available •OH radicals which could attack bacterial cells. Scavenging effect is generally based on the reaction rates between the scavenger and the radical and the concentration of the scavenger. The chemical reaction of $H_2PO_4^-$ with •OH is as given in following equation (Rincon and Pulgarin, 2004):

$$H_2PO_4^- + \bullet OH \rightarrow H_2PO_4^{\bullet} + OH^- \qquad (4.1)$$

Hence, when the concentration of $H_2PO_4^-$ was 0.1 M, it competed with •OH in the solution, thus lowering the rate of bacterial kill. Rincon and Pulgarin (2004) reported that $H_2PO_4^{\bullet}$ radicals are not reactive as •OH radicals.

Comparative rates of kill in the presence of 0.1 M and 0.02 M KH_2PO_4 are presented in Figure 4.12. In accordance with the figure, the performance of System-2 was considerably improved by lowering the buffer concentration to 0.02 M; removal of cells was increased from 45% to 95.5%. This indicates that chemical attack of radicals was an important mechanism in the disinfection process in this system. Hence, more radicals were available to attack bacterial cells, when the concentration of scavenging ions ($H_2PO_4^-$) was decreased. Therefore, all experiments in System-2 were carried out with a test medium containing 0.02 M KH_2PO_4. Control experiments were conducted to see if 0.02 M KH_2PO_4 was still effective in cell protection from osmotic pressure or not. These experiments consisted of monitoring the cell concentration in 0.02 M KH_2PO_4 solution without sonication. The results showed that this concentration was still protective.

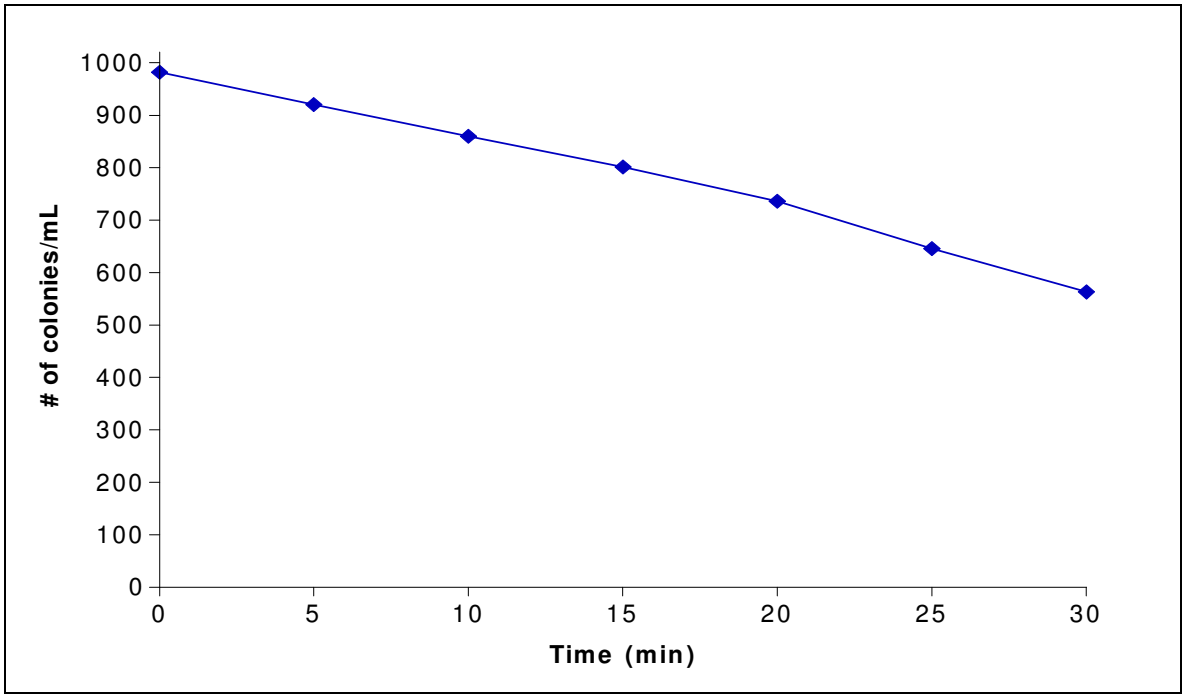

Figure 4.11. Decrease in *E.coli* concentration with time in System-2 during continuous- sonication ($N_0=10^3$ colonies /mL, 0.1 M KH_2PO_4).

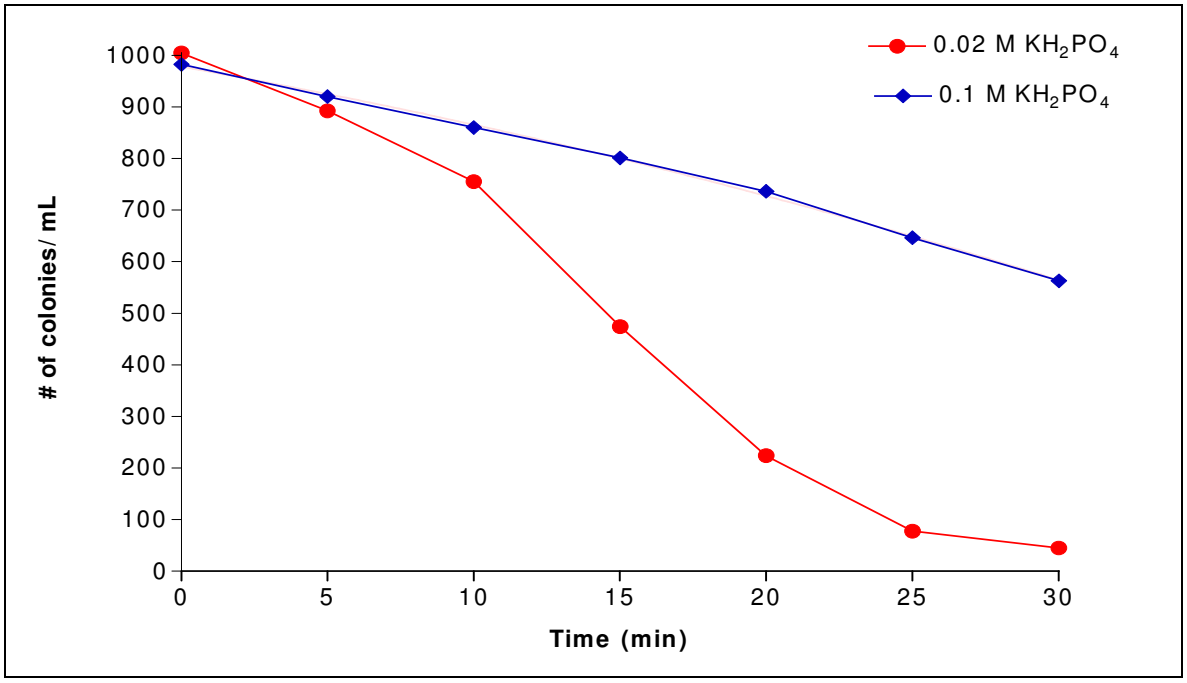

Figure 4.12. Effect of phosphate buffer concentration on the disinfection performance of System-2 ($N_0=10^3$ colonies /mL).

4.3.1.2. Formation of H_2O_2. As stated previously, formation of H_2O_2 was considered as an indication of •OH radical yield. Its formation was monitored in deionized water, in solutions of 0.1 M and 0.02 M KH_2PO_4 and in the presence of E.*coli* cells are as presented in Figure 4.13. It was found that H_2O_2 accumulation in 0.02 M KH_2PO_4 was higher than that in 0.1 M KH_2PO_4, indicating the scavenging effect of phosphate ions on •OH radicals. This more clearly explains the enhancement of disinfection performance in System-2 by decreasing the buffer concentration. Moreover, since H_2O_2 is also a disinfectant, increase in its accumulation was improved the efficiency of the system. The decrease in H_2O_2 accumulation in *E.coli* solution was because of the fact that •OH radicals and H_2O_2 were consumed by the cells.

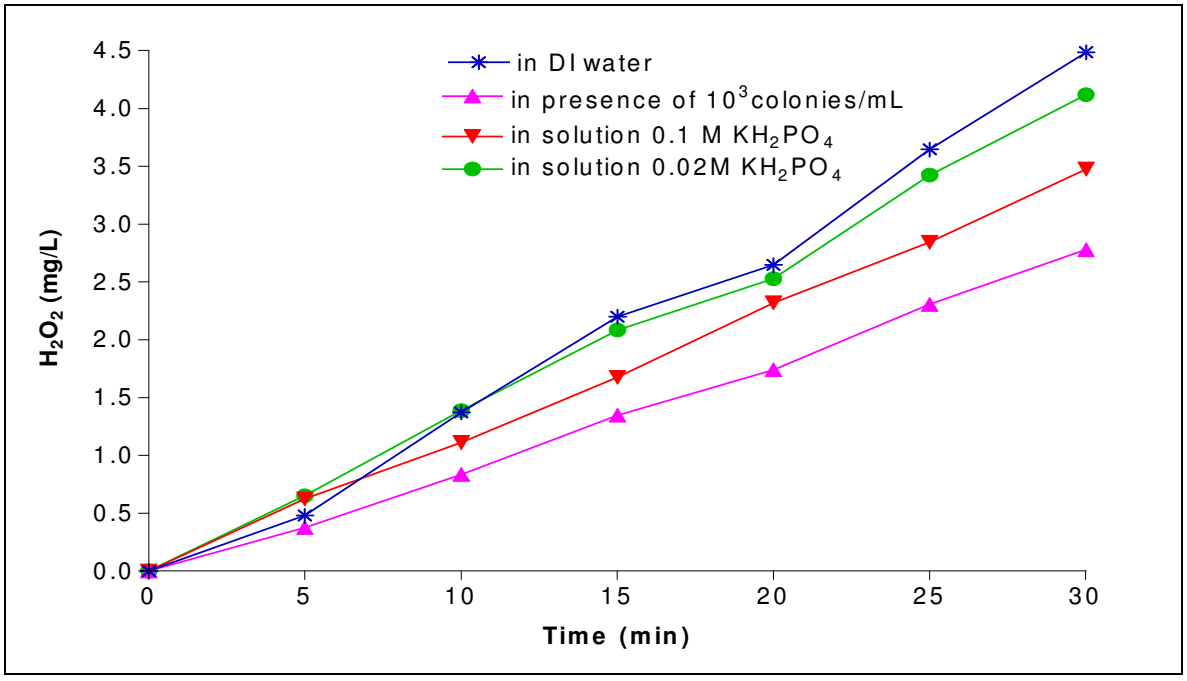

Figure 4.13. H_2O_2 concentration in deionized water, buffer solutions and in presence of *E.coli* (N_0=10^3 colonies/mL) in System-2.

4.3.1.3. Effect of Initial Cell Concentration. The impact of the input cell concentration was investigated by performing the experiments with different cell concentrations (N_0=590, 360, and 180 colonies/mL). The plots of survival ratio (per cent remaining) versus time are presented in Figure 4.14. As observed from the figure, more than 90% removal could be accomplished in 30 minutes of sonication,

in all cases. However at very high and very low cell concentrations the rate was slower. Highest removal was detected at N_0= 360 colonies/mL. This might be due to the combination of interactive phenomena as follows:

(i) In case of low initial cell concentration, the cells were randomly distributed such that the probability of being exposed to extreme conditions (the chance of contacting bubbles) was very low.

(ii) In case of high initial cell concentration, cells tend to be in the form of clusters, and the rapture of cell wall at the inner part of the cluster is difficult. However, in this case, the probability of contacting bubbles and/or being attached by •OH radicals is much higher.

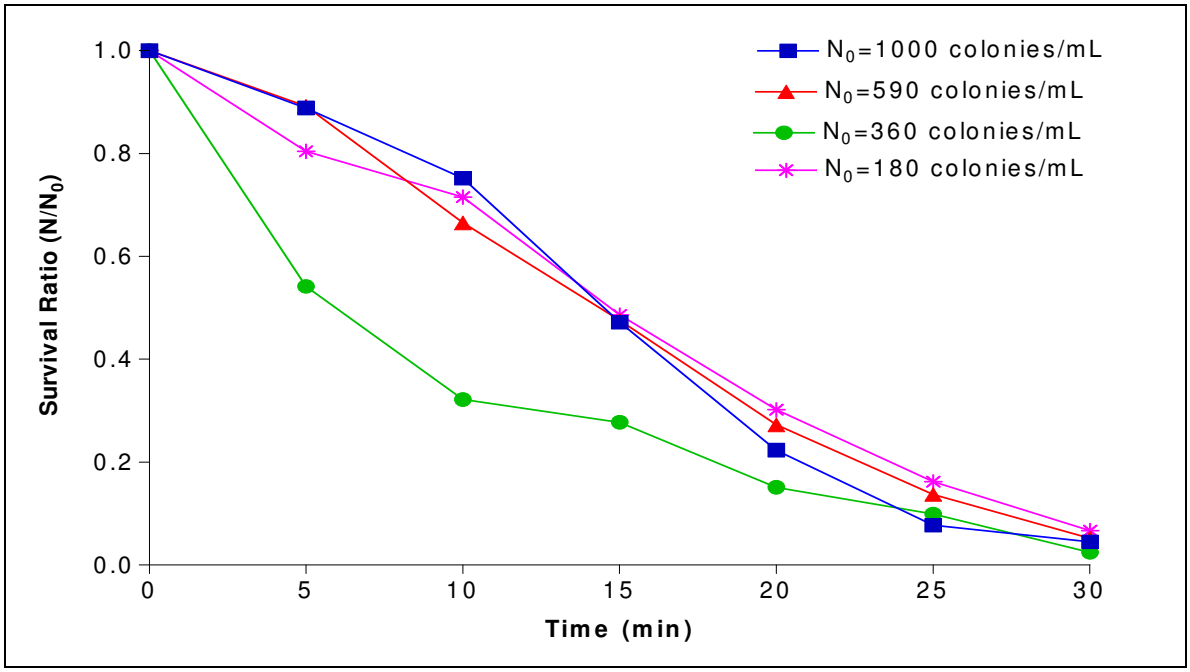

Figure 4.14. Profiles of survival ratios with different initial cell concentrations in System-2 during continuous - sonication (0.02 M KH_2PO_4).

4.3.2. Pulse Sonication

E.coli suspensions ($N_0=10^3$ colonies/mL) were sonicated in the pulse mode. Decrease in cell concentration with time during pulse sonication is represented in Figure 4.15. The profile was similar to that observed in the continuous mode. It was found that 83 % of the cells were inactivated in 30 minutes of operation.

4.3.2.1. Effect of Initial Cell Concentration. The impact of the input cell concentration was investigated by performing the experiments with different cell concentrations (N_0=600, 360, and 180 colonies/mL). Plots of survival ratio (per cent remaining) versus time are presented in Figure 4.16. In all cases, more than 80% of the cells were destroyed at the end of 30 minutes. Similar to continuous sonication, maximum performance could be achieved when the initial concentration was 360 colonies/mL.

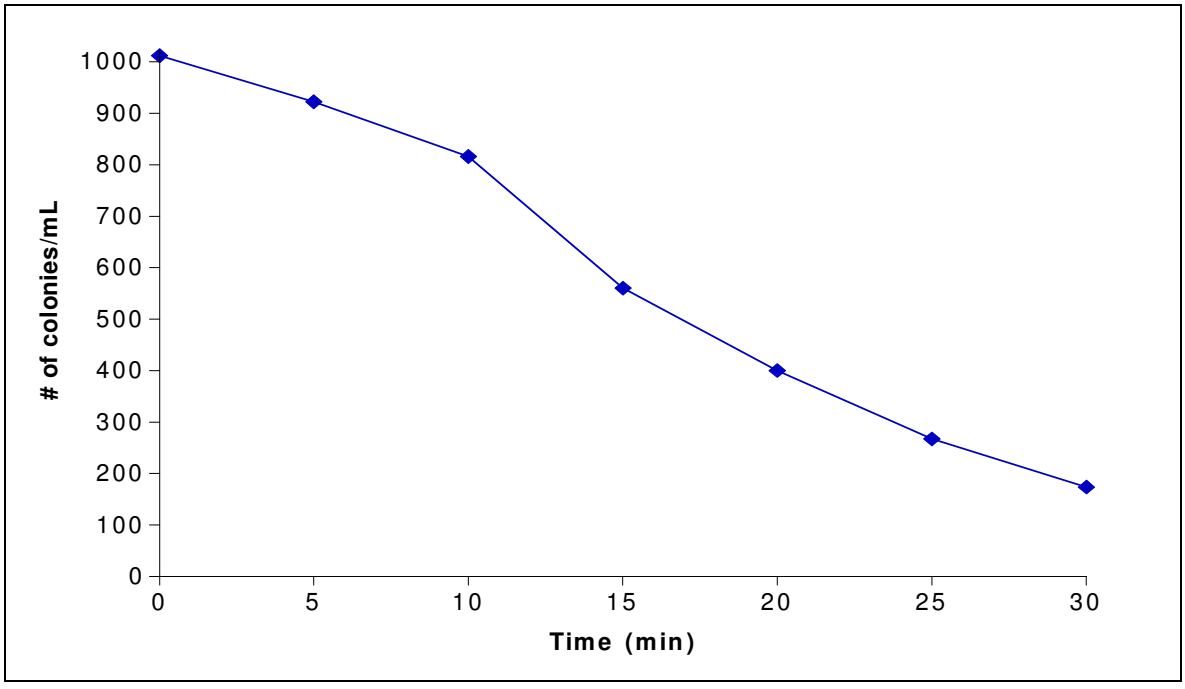

Figure 4.15. Decrease in *E.coli* concentration with time in System-2 during pulse-mode irradiation ($N_0=10^3$ colonies/mL, 0.02 M KH_2PO_4).

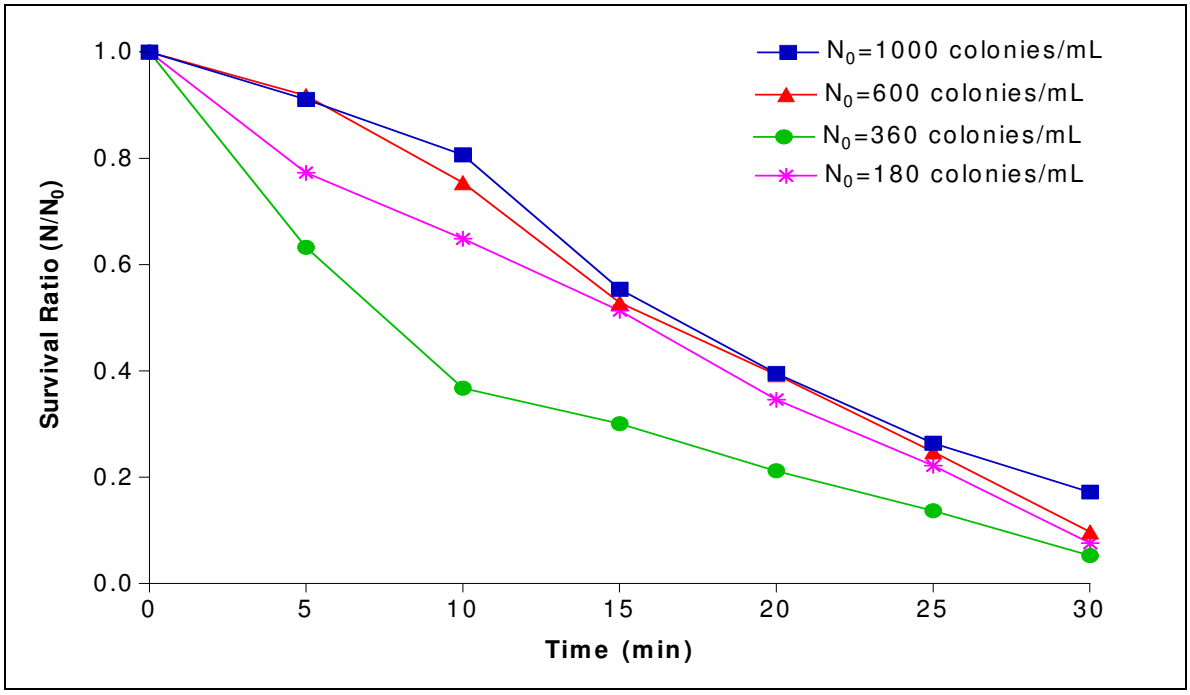

Figure 4.16. Profiles of survival ratios with different initial cell concentrations in System-2 during pulse - sonication (0.02 M KH_2PO_4).

4.3.3. Comparison of Operational Modes

Profiles of survival ratios in continuous and pulse mode operations are presented in Figure 4.17. It was found that pulse mode of sonication slightly decreased the system performance (per cent kill reduced from 95.5 to 83). This can be attributed to the fact that the effects created by ultrasound diminished and single cells deagglomorated during the lag periods. It is also important to note that the difference between rates of inactivation was larger as the time of operation proceeded. In both cases, relatively faster rate was observed in between 10^{th} and 15^{th} minutes of irradiation. This might be because of decrease in cell concentration.

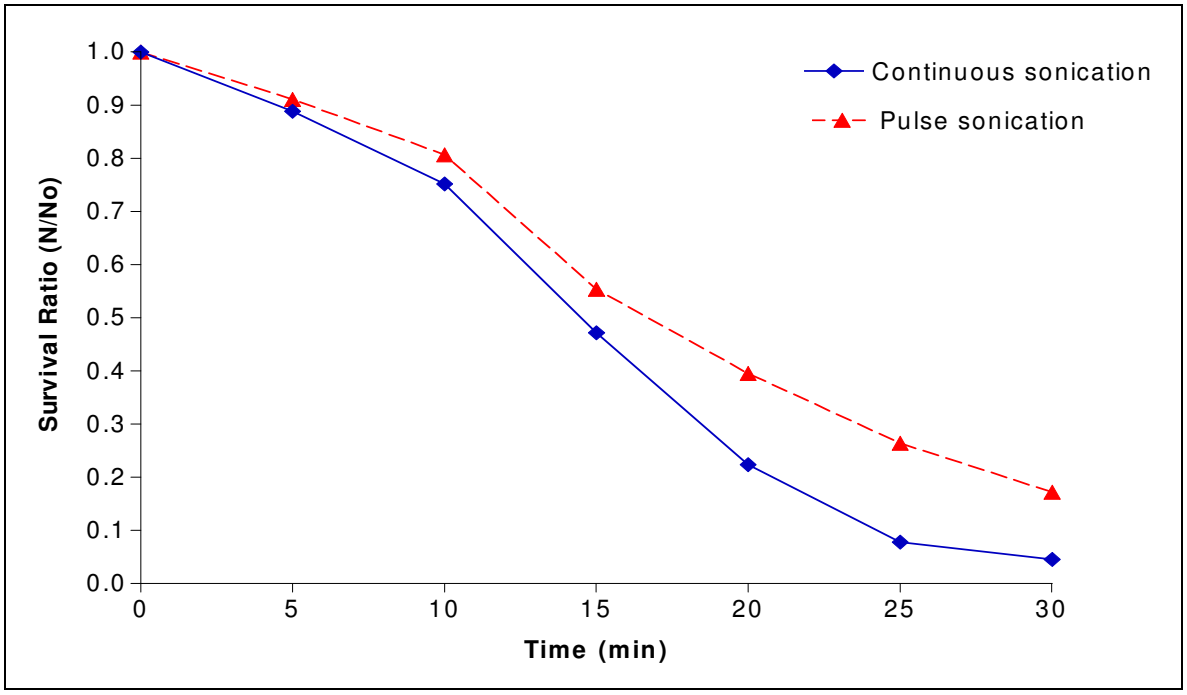

Figure 4.17. Comparative profiles of survival ratios with respect to operational mode in System-2 ($N_0=10^3$ colonies/mL, 0.02 M KH_2PO_4).

4.4. System-3 (520 kHz- 300 mL)

As presented in the Section 4.1, the applied power and the deposited power in this system were 40 W and 33.6 W, respectively. All experiments were performed in homogeneous medium in continuous and pulse modes. Total experimental time was 30 minutes.

4.4.1. Continuous Sonication

E.coli suspensions with $N_0=10^3$ colonies/mL was sonicated for 30 minutes. Decrease in the number of colonies with time is presented in Figure 4.18. It was found that only 11% reduction in the cell concentration could be observed in 30 minutes. The performance of this system is very low when compared to other two systems. This can be attributed to the fact that collapse of bubbles at this frequency is less energetic (Petrier and Francony, 1997). In addition, solution volume in this system is much larger than the others, so that the power deposited

per unit volume is quite small. Hence, System-3 can be considered ‘’ineffective’’ both for inducing mechanical and chemical effects of ultrasound.

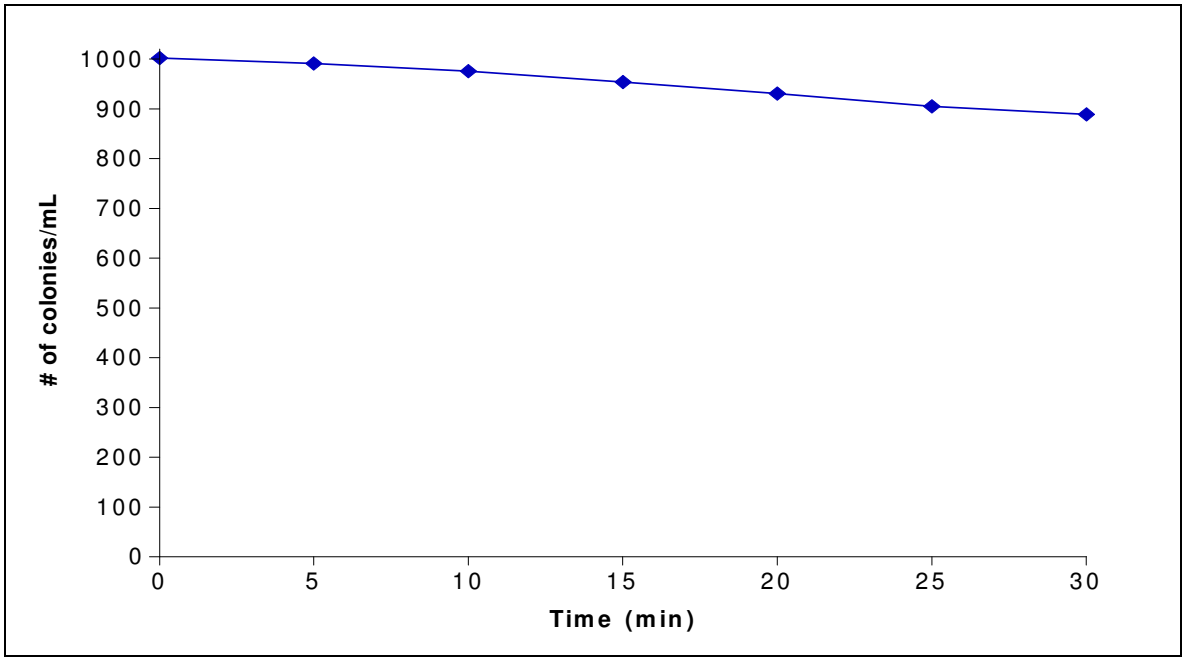

Figure 4.18. Decrease in *E.coli* concentration with time in System-3 during continuous- sonication ($N_0=10^3$ colonies/mL, 0.1 M KH_2PO_4).

4.4.1.1. Effect of Buffer Concentration. The buffer concentration was reduced to 0.02 M to check the performance of System-2. Comparative profiles of decrease in number of colonies are presented in Figure 4.19. A slight improvement could be observed such that the per cent removal increased to 19 from 11. This indicates that yield of •OH radicals was very low in the system. Although the improvement was lower than was expected, all experiments in this system were performed with 0.02 M KH_2PO_4.

4.4.1.2. Formation of H_2O_2. Its formation was monitored in deionized water, in 0.1 M and 0.02 M KH_2PO_4 and in the presence of *E.coli* cells. The data are presented in Figure 4.20. According to the figure, H_2O_2 accumulation was less in the presence of bacteria, which indicates the attack of radicals and H_2O_2. However reduction in buffer concentration could not increase the H_2O_2 accumulation. This is the indication of why the expected improvement in the system performance could not be achieved by reducing the buffer concentration.

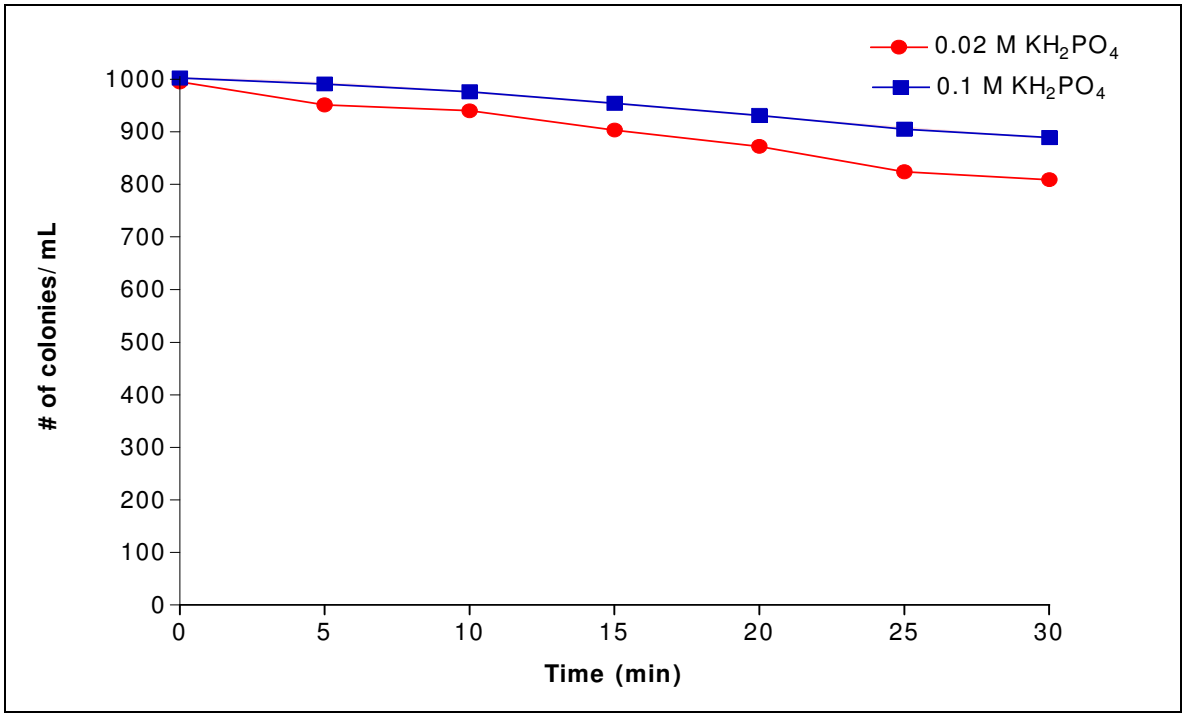

Figure 4.19. Effect of phosphate buffer concentration on the disinfection performance of System-3 (N_0=10^3 colonies/mL).

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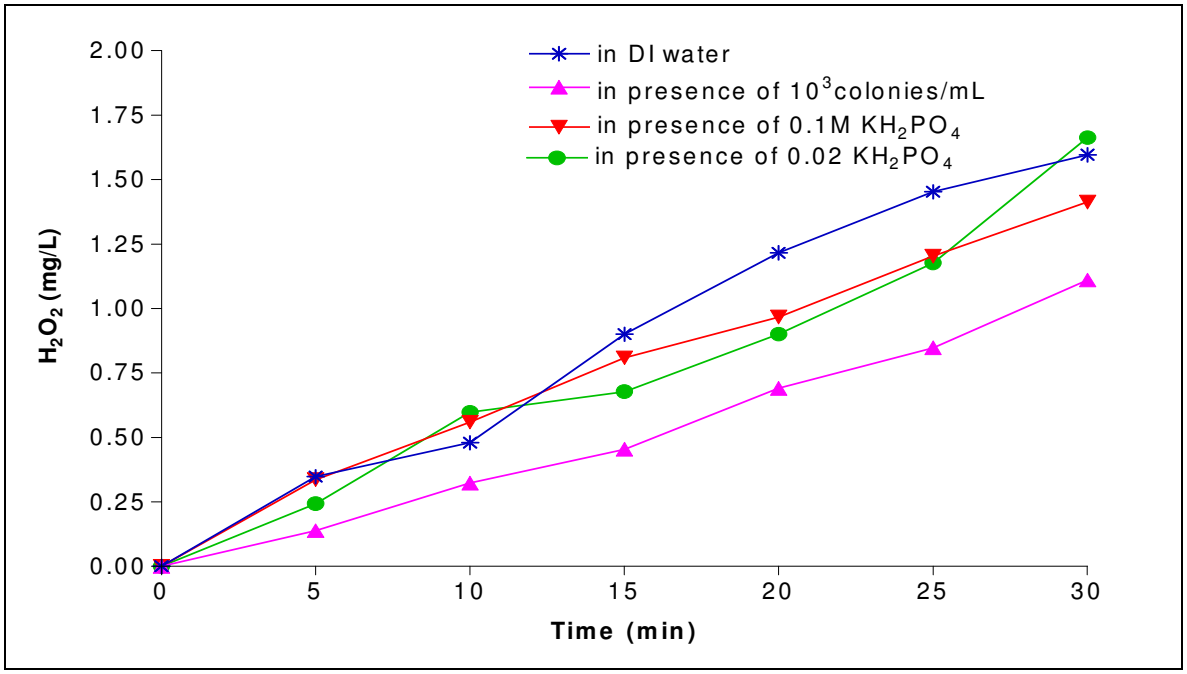

Figure 4.20. H_2O_2 concentration in deionized water, buffer solutions and in presence of *E.coli* (N_0=10^3 colonies/mL) in System-3.

4.4.1.3. Effect of Initial Cell Concentration. The impact of the input cell concentration was investigated by performing the experiments with different cell concentrations (N_0=650, 320, and 150 colonies/mL). Plots of survival ratio (per cent remaining) are presented in Figure 4.21. It was observed that the initial concentration of cells did not affect the removal rate in the range of N_0= 320 – 10^3 colonies/mL. However it was slightly enhanced when the initial number of colonies was low. This might be the result of that the tendency of the cells to agglomerate is low, thus single cells are more likely to be exposed to effects created by cavitation.

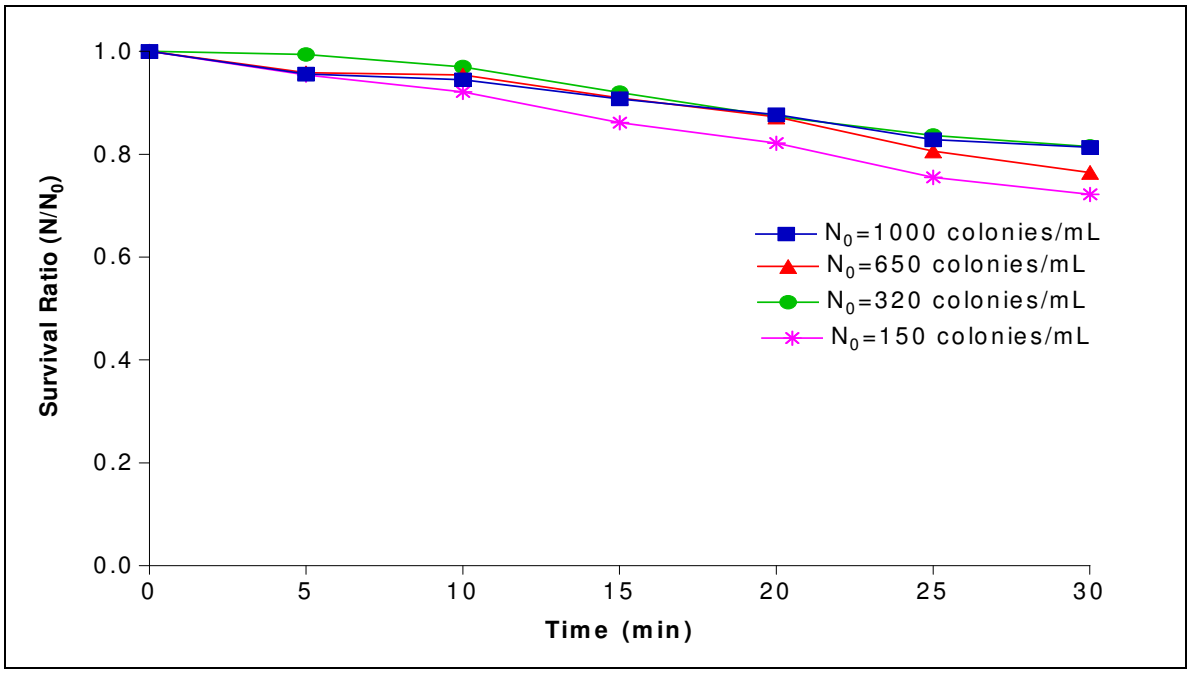

Figure 4.21. Profiles of survival ratio with different initial cell concentrations in System-3 during continuous - sonication (0.02 M KH_2PO_4).

4.4.2. Pulse Sonication

E.coli suspensions (N_0=10^3 colonies/mL) were sonicated in pulse mode for 30 minutes. Decrease in cell concentration with time is presented in Figure 4.22. Similar to continuous operation, a slight decrease in number of colonies were observed during pulse sonication; and only 17% reduction could be accomplished in 30 minutes.

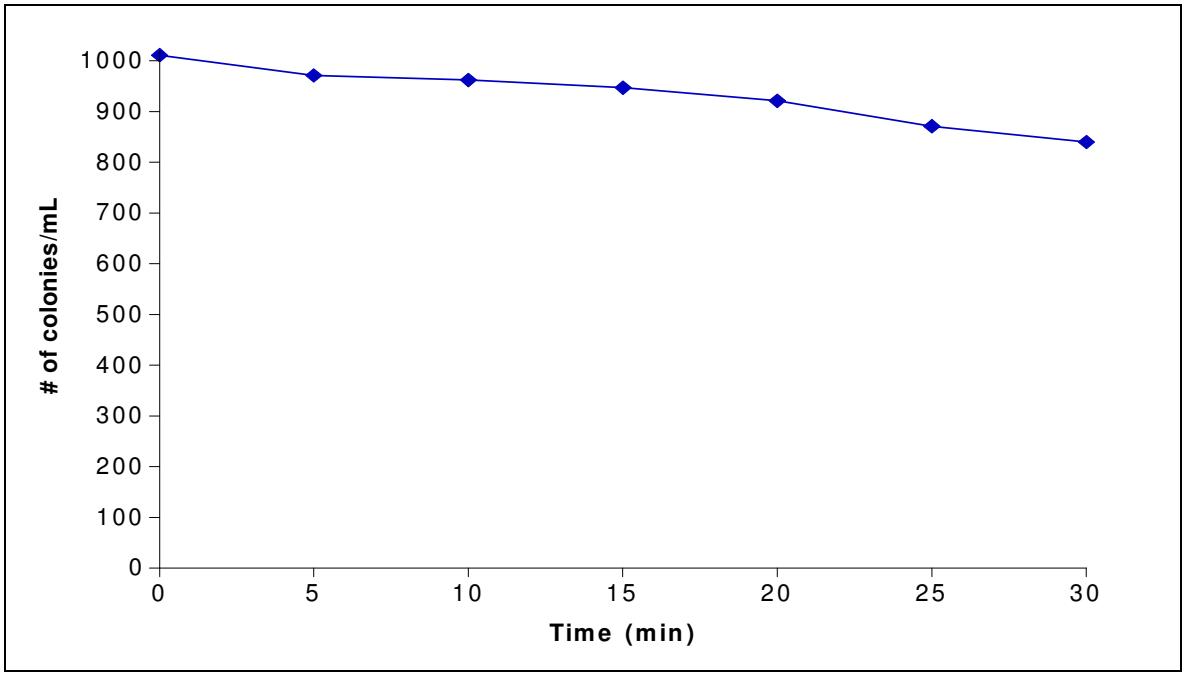

Figure 4.22. Decrease in *E.coli* concentration with time in System-2 during pulse-sonication (N_0=10^3 colonies /mL, 0.02 M KH_2PO_4).

4.4.2.1. Effect of Initial Cell Concentration. The impact of the input cell concentration was investigated by performing the experiments with different cell concentrations (N_0= 630, 320, and 150 colonies/mL) in pulse mode. Plots of survival ratio (per cent remaining) versus time are presented in Figure 4.23. The results in this figure indicate that there was no significant change in the system performance in the initial concentration range of N_0=320–10^3 colonies/mL. Similar to continuous operation, only a slight improvement could be observed at the lowest concentration.

4.4.3. Comparison of Operational Modes

The plots of survival ratio in continuous and pulse mode are presented in Figure 4.24. According to the figure, decay profiles are very similar. The rate of removal could not be improved. When it is compared with other systems, decrease in the performance of the system in pulse mode was low. Overall, the effects created by ultrasound were very low in both operational modes.

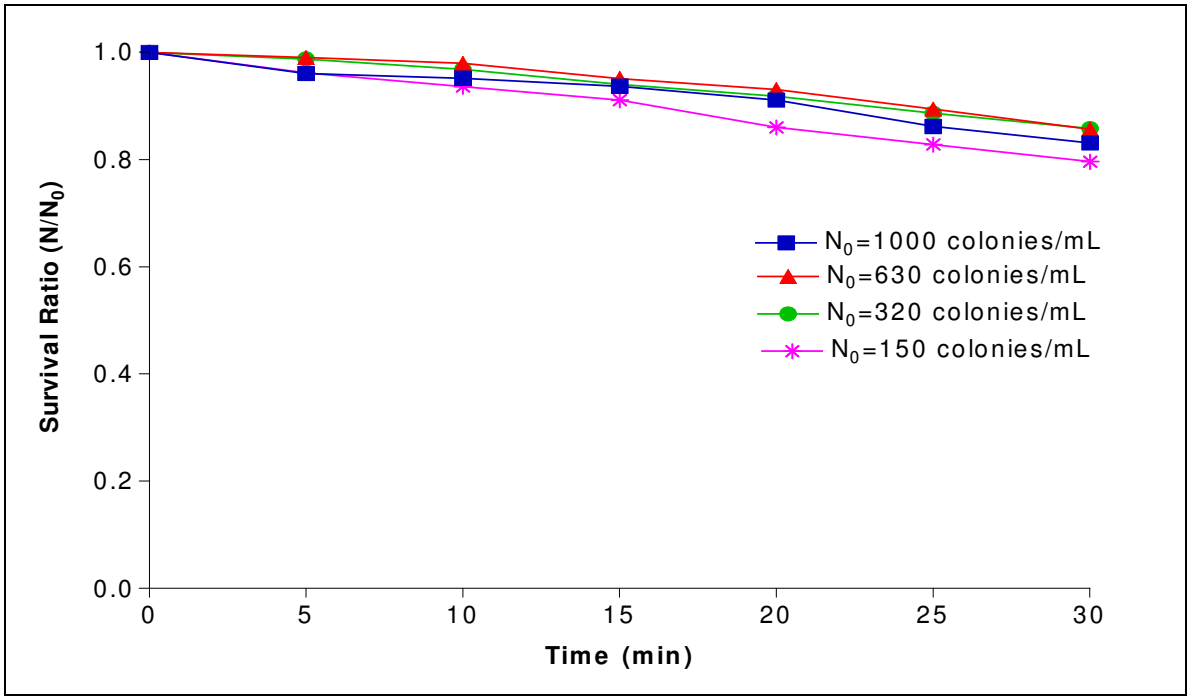

Figure 4.23. Profiles of survival ratios with different initial cell concentrations in System-3 during pulse - sonication (0.02 M KH_2PO_4).

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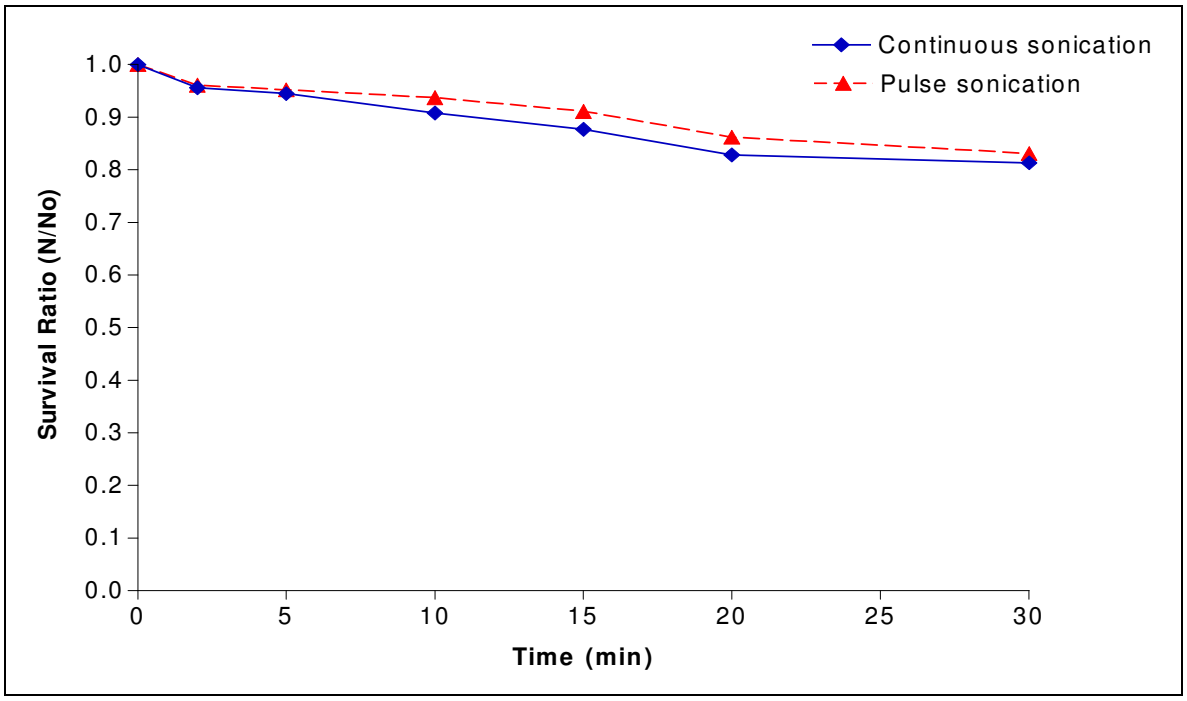

Figure 4.24. Comparative profiles of survival ratios with respect to operational mode in System-3 ($N_0=10^3$ colonies/mL, 0.02 M KH_2PO_4).

4.5. Ultrasonic Disinfection Kinetics

Ince and Belen (2001) analyzed bacterial survival – time data under 20 kHz ultrasound by nonlinear regression techniques to establish the representative process kinetics. They found that the concentration of *E.coli* was given by:

$$\ln N = a + bt^n \tag{4.2}$$

where ‘’N’’ is the number of *E.coli* colonies in unit volume (# of col./mL), ‘’t’’ is the contact time (min) and ‘’a’’, ‘’b’’, and ‘’n’’ are parameters of the model. The predicted model was indeed the integrated form of a rate expression proposed by chlorination kinetics:

$$\frac{dN}{dt} = kNt^m \tag{4.3}$$

where ‘’dN/dt’’ is the time rate of change in bacterial number per unit volume, ‘’k’’ is the observed rate constant in time$^{-(m+1)}$, and ‘’m’’ is an empirical constant. It is representing the time dependency of the rate. The greater the value of ‘’m’’, the higher is the effect of contact time the rate of disinfection. If ‘’m’’ is zero, the rate is independent of time, and is first order, as given by Chick’s Law:

$$\frac{dN}{dt} = kN \tag{4.4}$$

The integration of Eq. 4.3 between the limits N_O at t=0 and N at t yields:

$$\ln N = \ln No + \frac{k}{m+1} t^{m+1} \tag{4.5}$$

The predicted model in Eq. 4.2 and the integrated expression in Eq. 4.5 are identical, so that the parameters a, b, and n of the former are exchangeable with the constants of the latter, i.e., ln(No), k/(m+1) and (m+1), respectively. The similarity between the predicted model and the model for chlorination kinetics

points out the similarity of the destruction mechanisms. The implication is that the contribution of secondary biocides (e.g. H_2O_2, and OH radicals) should be accounted for in the overall process analysis in ultrasonic reactors.

4.5.1. System-1 (20 kHz- 80 mL)

4.5.1.1. Homogeneous Kinetics. The results of regression analysis and model parameters ‘’a’’, ‘’b’’, and ‘’n’’ are presented in Figure 4.25 for continuous mode and Figure 4.26 for pulse mode of irradiation. The values of ‘’b’’ and ‘’n’’ were substituted into k=b (m+1) and m=n-1, respectively to determine the kinetic coefficients listed in Table 4.2 for continuous mode and Table 4.3 for pulse mode. Note that ‘’k’’ and ‘’m’’ were estimated for 20 minutes of operation.

In continuous mode of operation, values of ‘’m’’ are negative for lower initial cell concentrations, indicating the rate of disinfection was decreasing with time. The order of reaction was higher at high cell concentration range, and it was slightly lower in the medium range. In pulse mode, the rate was decreasing with time in all cases, and effect of contact time on the rate of kill was low, when the initial cell concentration is low.

Since the overall reaction orders and units of rate coefficients are different, the evaluation and comparison of process performances were made based on the time requirements for 25, 50, 95 and 99.9 % reduction. The results are the extrapolation of the data obtained in 20 minutes of sonication. The findings are listed in Table 4.4 and 4.5 for continuous and pulse modes, respectively.

When the time required to achieve 25% kill was considered (initial stages of the experiments), it can be concluded that rate of removal was higher when the initial cell concentration was in medium level in both modes. The rate of removal in pulse mode was very similar to that in continuous mode at the beginning; however it became slower after 50% of the cells were destroyed. This is because of the randomness effect discussed previously.

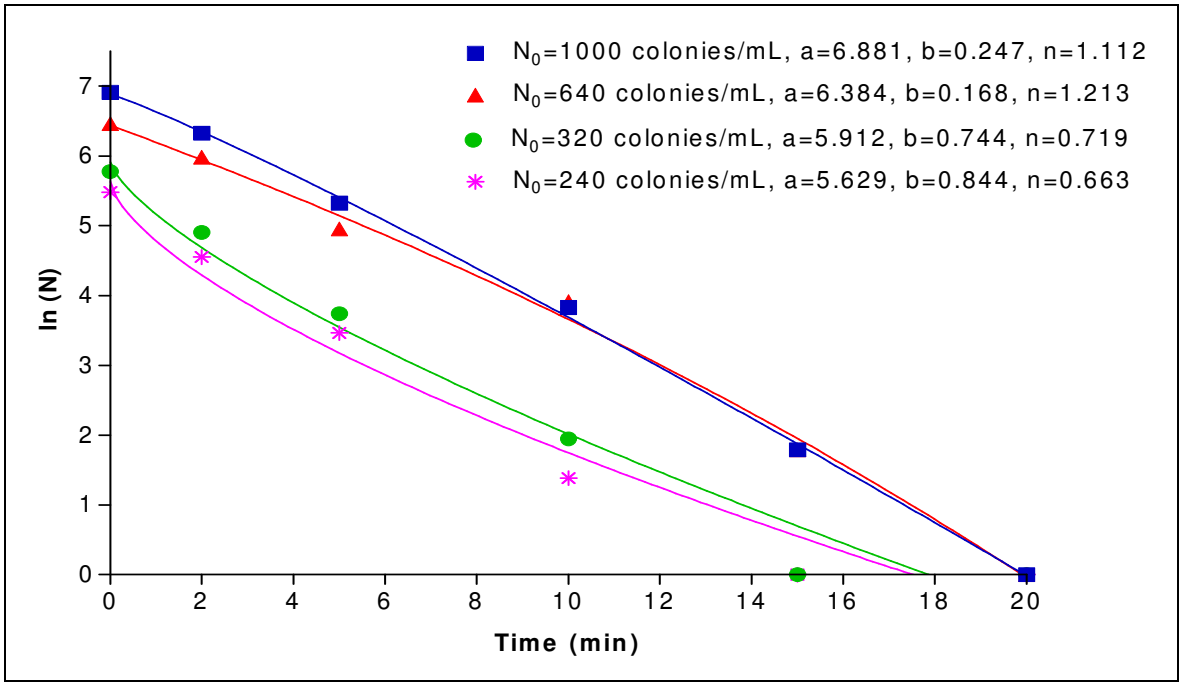

Figure 4.25. Reduction of *E.coli* with time in System-1 during continuous-sonication (0.1M KH_2PO_4). Solid lines represent the fit of Eq. 4.2 to the data by non-linear regression.

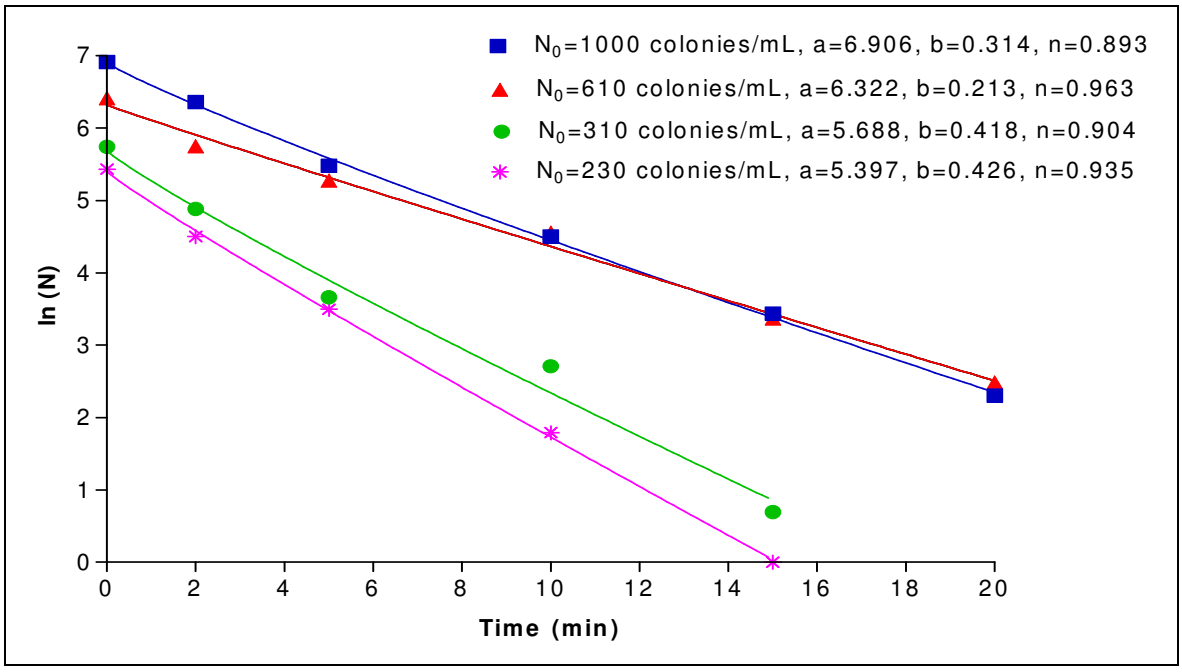

Figure 4.26. Reduction of *E.coli* with time in System-1 during pulse-sonication (0.1M KH_2PO_4). Solid lines represent the fit of Eq. 4.2 to the data by non-linear regression.

Table 4.2. Coefficients of process kinetics in System-1 during continuous sonication (0.1 M KH_2PO_4).

Initial Concentration, N_0 (# of colonies/mL)	Kinetic Coefficients		R^2
	k^* (min^{-n})	m =(n-1)	
1000	-0.274 (±0.018) $min^{-1.1}$	0.112	1.00
640	-0.204 (±0.079) $min^{-1.2}$	0.213	0.99
320	-0.534 (±0.129) $min^{-0.7}$	-0.281	0.97
240	-0.559 (±0.706) $min^{-0.7}$	-0.337	0.97

* The numbers in parentheses are 95% confidence intervals.

Table 4.3. Coefficients of process kinetics in System-1 during pulse sonication (0.1 M KH_2PO_4).

Initial Concentration, N_0 (# of colonies/mL)	Kinetic Coefficients		R^2
	k^*(min^{-n})	m =(n-1)	
1000	-0.280 (±0.019) $min^{-0.9}$	-0.107	1.00
610	-0.205 (±0.087) $min^{-1.0}$	-0.038	1.00
310	-0.377 (±0.736) $min^{-0.9}$	-0.096	0.99
230	-0.398 (±0.041) $min^{-0.9}$	-0.065	1.00

* The numbers in parentheses are 95% confidence intervals

Table 4.4. System performance for constant ratios of bacterial removal in System-1 (continuous-homogeneous).

Initial Concentration, N_0 (# of colonies/mL)	Kill Time (min)			
	25%	50%	95%	99.9%
1000	1.1	2.4	9.4	19.9
640	1.2	2.9	10.5	21.2
32	0.5	1.2	7.4	22.8
240	0.4	1.0	7.3	24.6

Table 4.5. System performance for constant ratios of bacterial removal in System-1 (pulse -homogeneous).

Initial Concentration, N_0 (# of colonies/mL)	Kill Time (min)			
	25%	50%	95%	99.9%
1000	0.9	2.4	12.5	31.6
610	0.9	2.9	15.1	36.6
310	0.5	1.6	8.7	22.1
230	0.5	1.6	7.9	19.6

4.5.1.2. Heterogeneous Kinetics. The same model was applied for the data obtained from the sonication of heterogeneous medium. The results of regression analysis and curve fittings are presented in Figure 4.27. The kinetic coefficients ‘’k’’ and ‘’m’’ were estimated for 20 minutes of operation. The findings are listed in Table 4.6 for all test conditions.

It was observed that addition of solids resulted in a reduction in the ‘’m’’ value, this means that the time dependency of the rate was decreased. The order of reaction was decreased when the solid particles having smaller diameter than 1 mm introduced into medium. The ‘’m’’ values are negative in the experiments carried out with solid particles having particle size of less than 1 mm, indicating that the rate was decreasing with time.

The time requirements for 25, 50, 95 and 99.9% removal were listed in Table 4.7. Note that the results are the extrapolation of the data obtained in 20 minutes of sonication. It was found that the effect of solid particles is more pronounced at the initial stages of the sonication, at higher concentration of the bacteria. With the prolonged irradiation and at lower bacterial concentration, enhancement of the system performance is less prominent.

The decrease in the effects of solids could be explained by that during early exposure, the probability of bacterial contact with the solid – liquid interface was high, therefore number of bacteria destroyed was higher, however as the irradiation progressed, the rate of kill was limited by the number of bubbles in the

bulk liquid, approaching to the rate observed in homogeneous medium. Additionally, catalytic effects may fade away by vibrational erosion of solid surfaces (Ince and Belen, 2001).

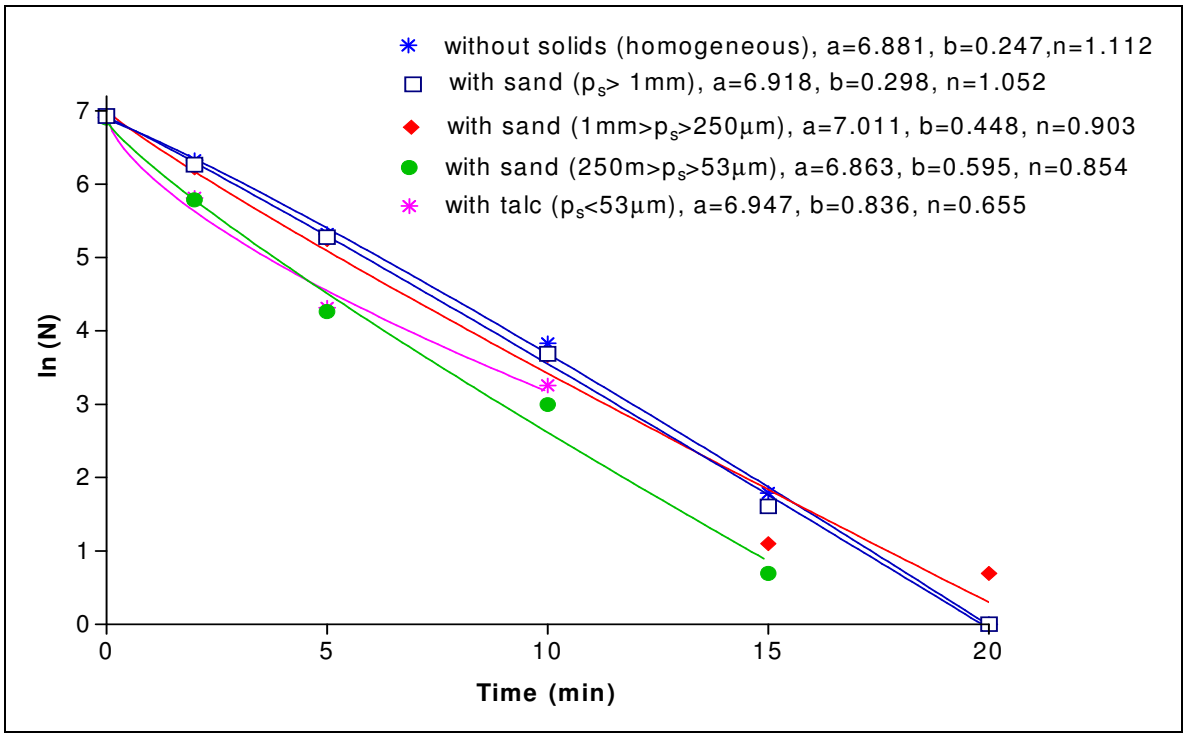

Figure 4.27 Reduction of *E.coli* with time in System-1 in heterogeneous medium (0.1M KH_2PO_4). Solid lines represent the fit of Eq. 4.2 to the data by non-linear regression.

Table 4.6 Coefficients of process kinetics in heterogeneous experiments ($N_0=10^3$colonies/mL, C_{solid}= 0.12 g/L, 0.1M KH_2PO_4).

Test Condition	Kinetic Coefficients		R^2
	$k^*(min^{-n})$	m =(n-1)	
Without solids (homogeneous)	-0.274 (±0.018) $min^{-1.1}$	0.112	1.00
With sand (d≥1mm)	-0.314 (±0.028) $min^{-1.1}$	0.052	1.00
With sand(1mm>d>250µm)	-0.405 (±0.550) $min^{-0.9}$	-0.097	0.98
With sand (250µm>d>53µm)	-0.508 (±0.634) $min^{-0.8}$	-0.147	1.00
With talc (d≥53µm)	-0.547 (±0.652) $min^{-0.7}$	-0.346	0.99

* The numbers in parentheses are 95% confidence intervals

Table 4.7. Comparative system performance for constant ratios of bacterial removal ($N_0=10^3$ colonies/mL) in homogeneous and heterogeneous medium.

Process	Kill Time (min)			
	25%	50%	95%	99.9%
Ultrasound	1.1	2.4	9.4	19.9
Ultrasound + sand (d>1mm)	1.0	2.3	9.0	19.8
Ultrasound + sand (1mm>d>250μm)	0.9	1.9	8.5	21.0
Ultrasound + sand (250μm>d>53μm)	0.4	1.1	6.5	17.5
Ultrasound + talc (d<53μm)	0.2	0.8	7.2	25.0

4.5.2. System-2 (300 kHz- 100 mL)

The same kinetic model was applied for the data obtained in System-2. The results of regression analysis and the model parameters ‘’a’’, ‘’b’’, and ‘’n’’ are presented in Figure 4.28 and Figure 4.29 for continuous and pulse sonication, respectively. The kinetic coefficients ‘’k’’ and ‘’m’ were estimated for 30 minutes of sonication. The findings are listed in Table 4.8 for continuous mode and Table 4.9 for pulse mode. It was observed that at lowest concentration, the order of reaction and the ‘’m’’ value was highest in both irradiation modes.

Time requirements for constant ratios of bacterial removal listed for continuous and pulse modes in Table 4.10 and 4.11, respectively. Note that the results are the extrapolation of the data obtained in 30 minutes of sonication. The maximum rate was achieved at the initial concentration of 360 colonies/mL in both modes of irradiation. At very high concentration, the rate of kill was low as a result of that bacteria tend to form clusters; at very low concentration, however, the chance of being contact with the bubble or reactive species was low.

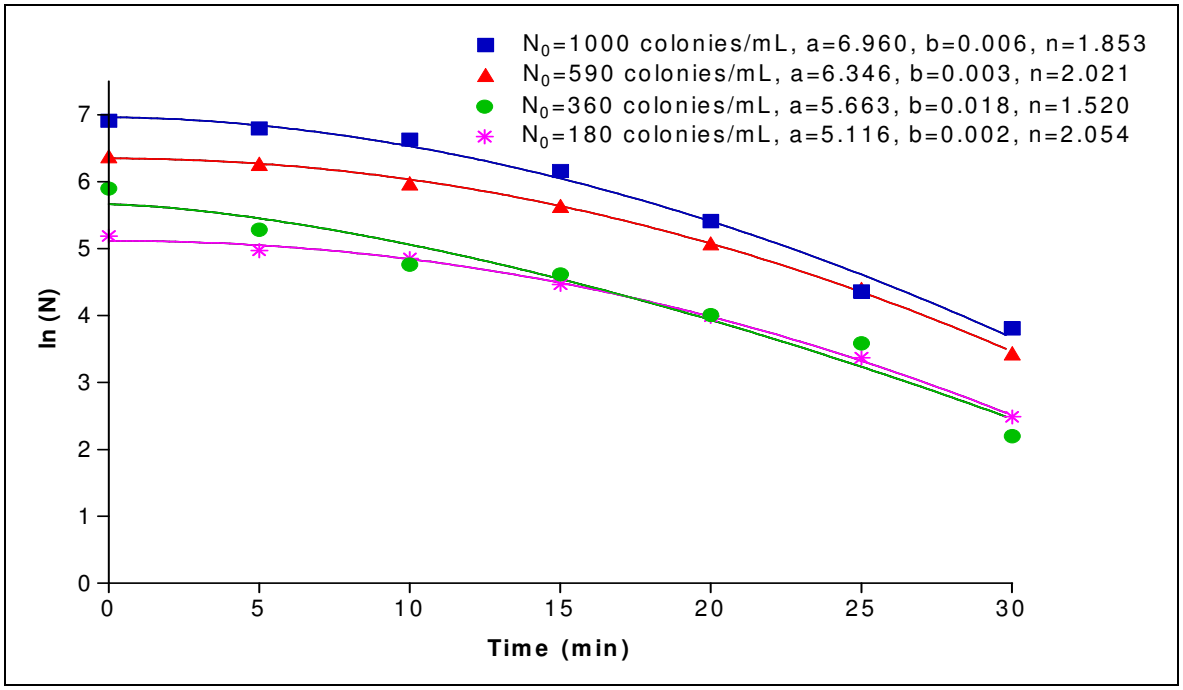

Figure 4.28. Reduction of *E.coli* with time in System-2 during continuous-sonication (0.02 M KH_2PO_4). Solid lines represent the fit of Eq. 4.2 to the data by non-linear regression.

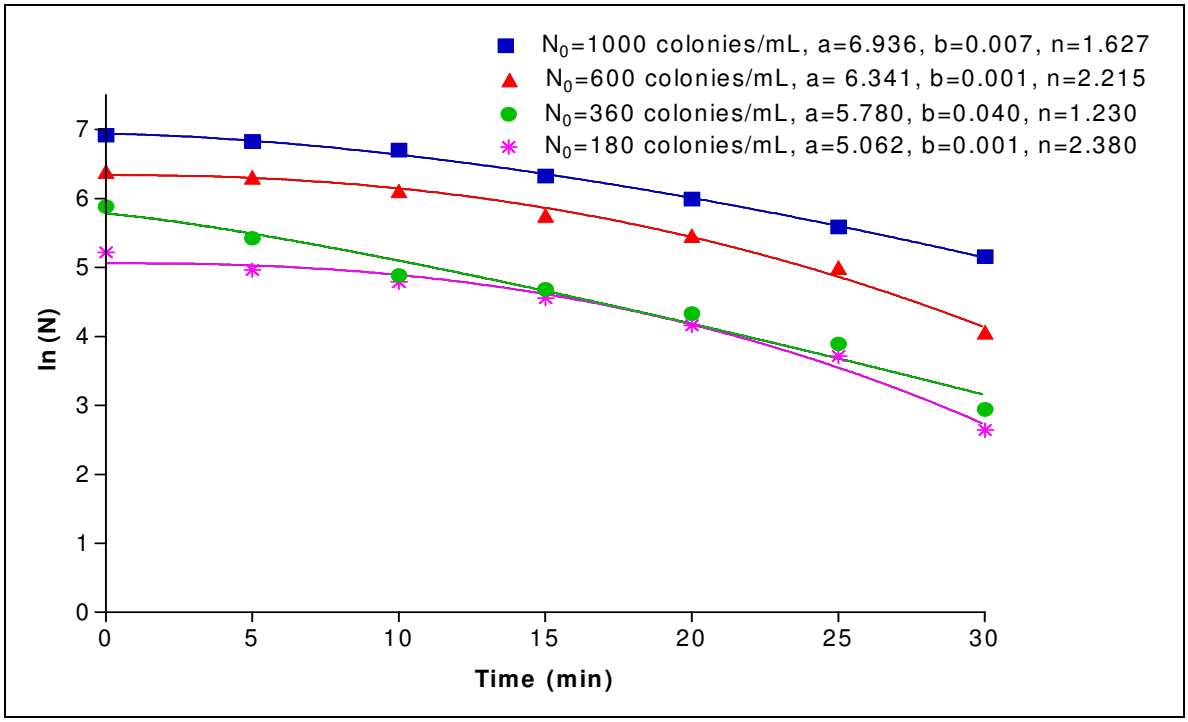

Figure 4.29. Reduction of *E.coli* with time in System-2 during pulse-sonication (0.02 M KH_2PO_4). Solid lines represent the fit of Eq. 4.2 to the data by non-linear regression.

Table 4.8. Coefficients of process kinetics in System-2 during continuous sonication (0.02 M KH_2PO_4).

Initial Concentration, N_0 (# of colonies/mL)	Kinetic Coefficients		R^2
	$k^*(min^{-n})$	m =(n-1)	
1000	-0.011 (±0.010) $min^{-1.9}$	0.852	0.99
590	-0.006 (±0.000) $min^{-2.0}$	1.021	1.00
360	-0.028 (±0.074) $min^{-1.5}$	0.522	0.96
180	-0.005 (±0.001) $min^{-2.0}$	1.054	0.97

* The numbers in parentheses are 95% confidence intervals

Table 4.9. Coefficients of process kinetics in System-2 during pulse sonication (0.02 M KH_2PO_4).

Initial Concentration, N_0 (# of colonies/mL)	Kinetic Coefficients		R^2
	$k^*(min^{-n})$	m =(n-1)	
1000	-0.012 (±0.002) $min^{-1.6}$	0.627	1.00
600	-0.003 (±0.002) $min^{-2.2}$	1.215	0.99
360	-0.049 (±0.073) $min^{-1.2}$	0.230	0.97
180	-0.002 (±0.003) $min^{-2.4}$	1.380	0.98

* The numbers in parentheses are 95% confidence intervals

Table 4.10. System performance for constant ratios of bacterial removal in System-2 (continuous - sonication).

Initial Concentration, N_0 (# of colonies/mL)	Kill Time (min)			
	25%	50%	95%	99.9%
1000	8.8	13.5	28.9	45.1
590	9.0	14.4	30.3	46.0
360	2.3	8.6	27.5	49.1
180	14.6	19.5	36.3	53.6

Table 4.11. System performance for constant ratios of bacterial removal with respect to initial concentration in System-2 (pulse - sonication).

Initial Concentration, N_0 (# of colonies/mL)	Kill Time (min)			
	25%	50%	95%	99.9%
1000	10.4	17.4	41.7	69.4
600	11.7	18.5	36.8	53.9
360	3.4	8.9	32.5	65.1
180	8.4	14.3	28.4	40.7

4.5.3. System-3 (520 kHz- 300 mL)

The kinetic model used for System-1 and System-2 was also applied for the data obtained in System-3. The results of regression analysis and model parameters are presented in Figures 4.30 and 4.31 for continuous and pulse mode of irradiation, respectively. The values of kinetic coefficients were estimated for 30 minutes sonication. The findings are listed in Table 4.12 for continuous mode and Table 4.13 for pulse mode.

In continuous mode, time dependency of the rate was slightly lower as compared to pulse mode. The order of reaction was lowest for the lowest initial concentration, and it was highest for the initial cell count of 630 per mL, during both modes of irradiation.

Times required for 25, 50, 95 and 99.9% removal were calculated and listed for continuous and pulse modes in Table 4.14 and 4.15, respectively. Note that the results are the extrapolation of the data obtained in 30 minutes of sonication. It was observed that the rate of kill was very low for all cases, such that in order to accomplish 99.9% reduction of bacteria, the solution should be sonicated for a very long time.

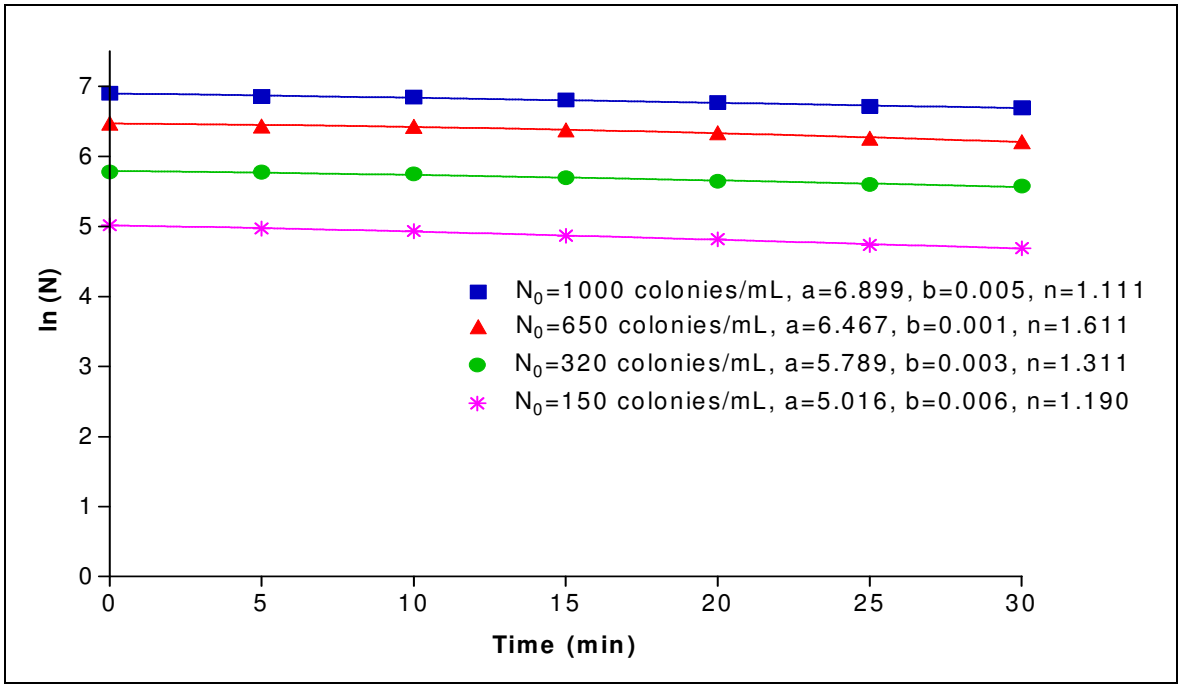

Figure 4.30. Reduction of *E.coli* with time in System-3 during continuous-sonication (0.02 M KH_2PO_4). Solid lines represent the fit of Eq. 4.2 to the data by non-linear regression.

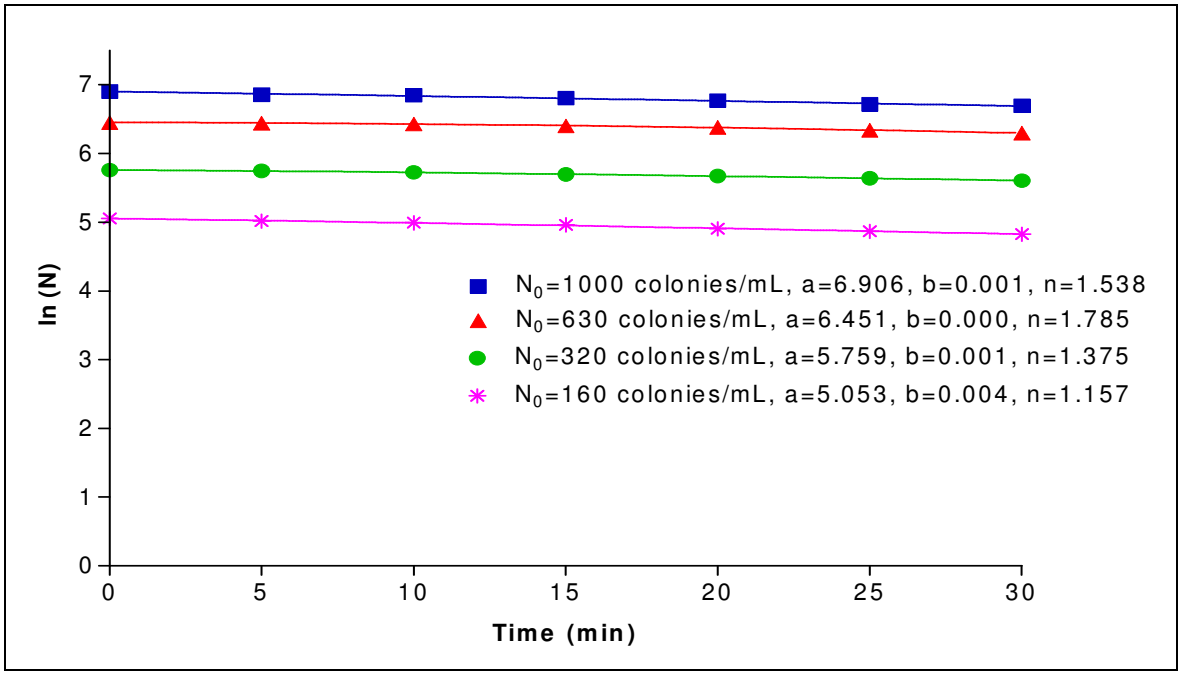

Figure 4.31. Reduction of *E.coli* with time in System-3 during pulse-sonication (0.02 M KH_2PO_4). Solid lines represent the fit of Eq. 4.2 to the data by non-linear regression.

Table 4.12. Coefficients of process kinetics in System-3 during continuous sonication (0.02 M KH_2PO_4).

Initial Concentration, N_0 (# of colonies/mL)	Kinetic Coefficients		R^2
	$k^*(min^{-n})$	m =(n-1)	
1000	-0.005 (±0.003) $min^{-1.1}$	0.111	0.99
650	-0.002 (±0.001) $min^{-1.6}$	0.611	0.99
320	-0.003 (±0.003) $min^{-1.3}$	0.311	0.98
150	-0.007 (±0.001) $min^{-1.2}$	0.190	1.00

* The numbers in parentheses are 95% confidence intervals

Table 4.13. Coefficients of process kinetics in System-3 during pulse sonication (0.02 M KH_2PO_4).

Initial Concentration, N_0 (# of colonies/mL)	Kinetic Coefficients		R^2
	$k^*(min^{-n})$	m =(n-1)	
1000	-0.001 (±0.002) $min^{-1.5}$	0.537	0.97
630	-0.001 (±0.000) $min^{-1.8}$	0.785	1.00
320	-0.002 (±0.000) $min^{-1.4}$	0.375	1.00
160	-0.005 (±0.001) $min^{-1.2}$	0.157	0.99

* The numbers in parentheses are 95% confidence intervals

Table 4.14. System performance for constant ratios of bacterial removal in System-3 (continuous - sonication).

Initial Concentration, N_0 (# of colonies/mL)	Kill Time (min)			
	25%	50%	95%	99.9%
1000	37.3	83.7	315.4	670.1
650	32.9	57.5	143.6	241.4
320	34.2	65.0	195.1	367.8
150	26.3	54.5	185.4	373.9

Table 4.15. System performance for constant ratios of bacterial removal with respect to initial concentration in System-3 (pulse- sonication).

Initial Concentration, N_0 (# of colonies/mL)	Kill Time (min)			
	25%	50%	95%	99.9%
1000	54.9	101.5	281.4	503.1
630	35.5	57.8	130.8	208.8
320	60.0	115.3	336.8	619.2
160	37.6	83.7	303.1	626.3

4.6. Comparison of Systems

Disinfection performances of each system are presented in Figure 4.32 for a comparative review. The results indicate that, power ultrasound has a considerably higher performance and a dramatic effect on the viability of the bacteria. Mechanical effects of cavitational collapse together with the production of radical species provided high rates of bacterial kill in System-1. In systems operated with high frequency, the mechanical effects are less significant, hence the performances of the systems were limited with extend of chemical reactions.

As shown in Table 4.16, diameter of the cavity bubbles are biggest in the System-1, this means that collapse time is longer and build-up of energy at the final stages is more elevated. With an increase of frequency, acoustic periods become shorter, size of bubbles decreases; therefore, the cavitation intensity decreases.

The ultrasonic disinfection performance of System-3 is very low, since the diameter of cavity was smallest and duration of collapse is very short, resulting less energetic collapse of bubbles.

The ultrasonic power or intensity has been considered as one of the important factors and only the input electric power is not always informative to indicate ultrasonic power for sonication, since the energy conversion of ultrasound is transducer dependent. In these systems, the power dissipated and the volume irradiated were different. Therefore, it is more meaningful to compare the power densities, which is the actual power deposited (measured calorimetrically, referring to Section 4.1) per sonicated volume. According to Table 4.17, it can be concluded that the system having highest power density has highest ultrasonic disinfection performance.

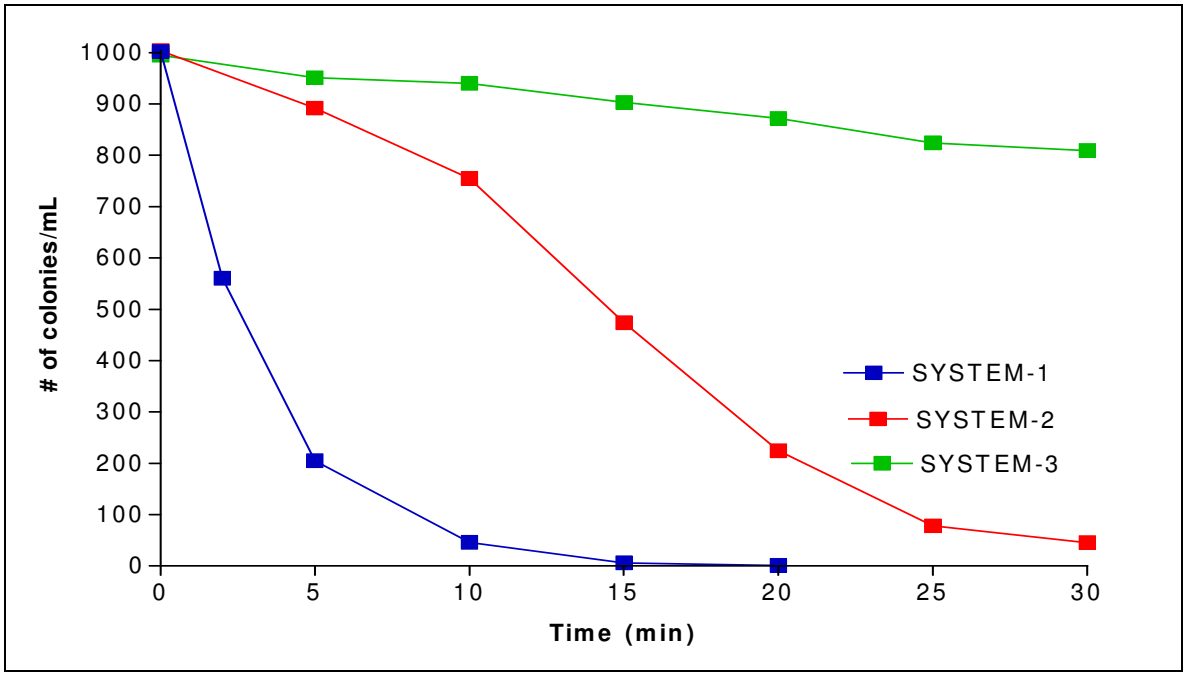

Figure 4.32. Comparative profiles of decrease in *E.coli* concentration in systems during continuous - mode irradiation ($N_0=10^3$ colonies/mL).

Table 4.16. Diameter of bubble and collapse time versus frequency (Petrier and Francony, 1997).

Frequency (kHz)	Diameter of Pulsating Bubble (mm)	Duration of the Collapse (μs)
20	0.33	12.5
300	0.022	0.83
520	0.013	0.5

Table 4.17. List of power densities in the experimental systems.

System	Power Deposited (W)	Sonicated Volume (mL)	Power Density (W/mL)
1	36.5	80	0.456
2	14.7	100	0.147
3	33.6	300	0.112

As observed in the Figure 4.33, the formation of H_2O_2 was low in Systems-1 and 3, which indicates lower •OH production in these systems. The reason for this

in 20 kHz is due to the fact that at such frequencies the time to reach the resonating radius of the bubble is long, and so is the bubble life time. Accordingly, radicals such as •OH that are produced during the long-lasting collapse process have sufficient time for combination in the gas phase (or at the interface) before they are ejected into the liquid bulk. On the other hand, at high frequency irradiation the duration of cavity collapse is much shorter so that a larger fraction of radical species will have the chance to escape into the bulk liquid. In fact, short bubble life (short collapse duration) is favorable for radical production and the likelihood of their ejection out of the gas phase, but unfavorable for the "quality" or the violence of collapse (Colarusso and Serpone, 1996). In most cases, there exists an optimum frequency, at which the rate of radical production and the duration of cavity collapse provide the "best" conditions. In our case 300 kHz is the optimum, because here longer lived bubble advantages (longer than those at 520 kHz) for more violently collapsing cavities seem to dominate over shorter lasting but less energetic cavity collapse (at 520 kHz) that allows larger spread of •OH radicals into solution.

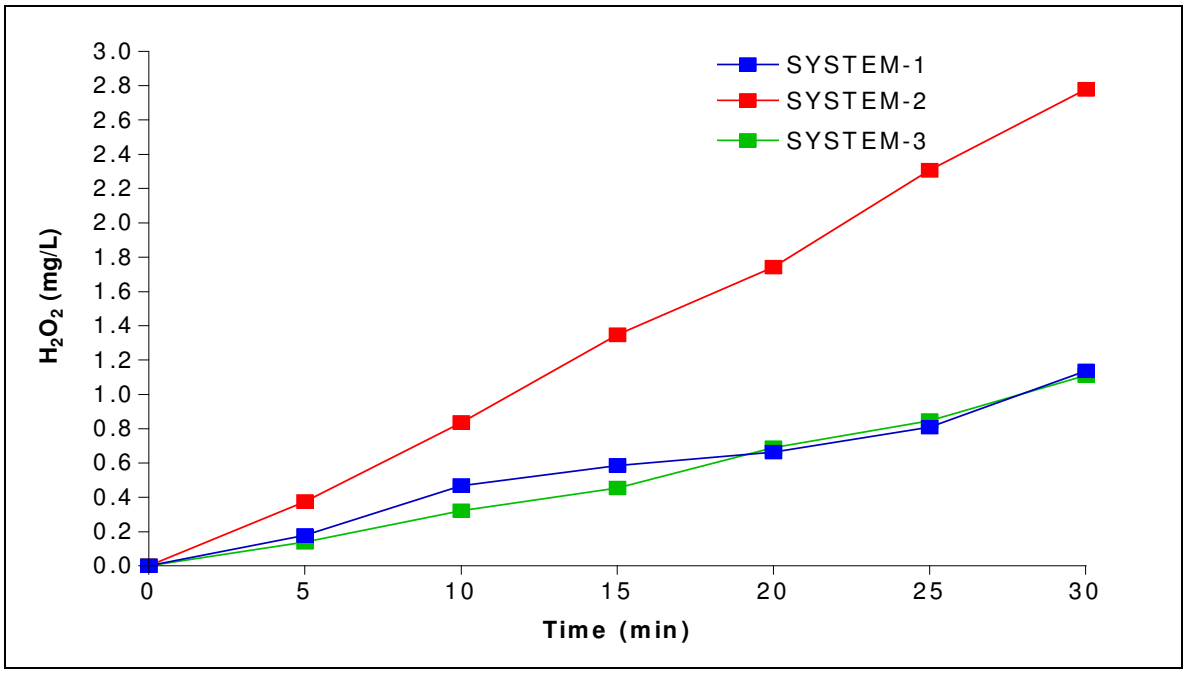

Figure 4.33. Comparative profiles of formation of H_2O_2 in systems in presence of 10^3 colonies/mL.

Although the radicals present in the bulk liquid were lower in System-1 (formation of H_2O_2 was low) than in System-2, the disinfection performance of the former was markedly higher, implying that the destruction of cells occurred mainly by mechanical effects of ultrasound such as jetting liquids and shear forces.

Comparative kinetics of bacterial destruction in systems operated with $N_0=10^3$/mL are presented in Figure 4.34. it can be concluded that the rate of inactivation in System-2 is more related to the contact time than in others.

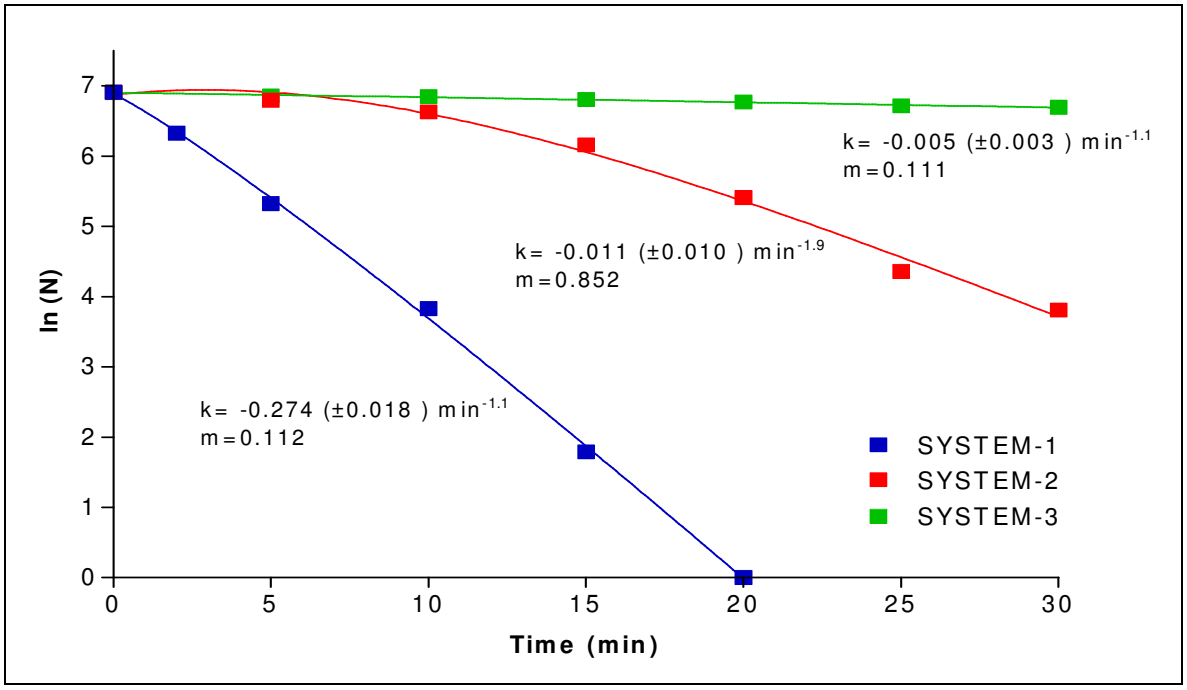

Figure 4.34. System comparison of the ultrasonic disinfection. Solid lines represent the fit of Eq. 4.2 to the data by non-linear regression.

The time requirements for constant removal ratios were estimated for 20 minutes in System-1, and 30 minutes in System-2 and 3. The findings are listed in Table 4.18.

Table 4.18. Comparative system performance for constant ratios of bacterial removal in homogeneous medium ($N_0=10^3$ colonies/mL).

System	Kill Time (min)			
	25%	50%	95%	99.9%
1	1.1	2.4	9.1	19.3
2	8.8	13.5	28.9	45.1
3	37.3	83.7	315.2	669.6

The results show that more than 99.9% removal of bacteria could be accomplished in System-1 in 20 minutes. On the other hand, nearly 40 minutes of sonication were needed to have the same amount of reduction in System-2 and more than 11 hours required in System-3, which is not economic and technically feasible.

4.6. Ultrasonic Yields and Relative Efficiencies of the Systems

Energy efficiency and the cost play a vital role in the selection of a suitable disinfection technique, which in turn would affect the overall economics of a water treatment scheme. An ideal disinfection technique is one, which is able to bring down the bacterial population to the required level, and is also energy efficient or economical.

The relative efficiencies of all systems operated with different modes were assessed by comparing their Ultrasonic Yields, which can be defined as the change in bacterial concentration in the irradiated volume per power deposited in that volume. Therefore, it can be formulated as (Tauber et al., 2000):

$$G = \frac{\Delta N \, x \, V}{P_d} \tag{4.6}$$

where G is the Ultrasonic Yield, (colonies/W), ΔN is the reduction in colony counts (colonies/mL); V is the total sonicated volume (mL) and P_d is the sonic energy deposited in a given volume (W).

The overall rate of disinfection in terms of colonies killed per unit time can be obtained by multiplying the rate (ΔN/Δt) by the volume treated in any experimental set-up at any operating condition. This product is referred as the efficacy of each equipment and/or method for treating a specified volume. The smaller the product, the lower is the efficiency of the disinfection process. As the scales of the operation, i.e. quantity of the water treated are different in the case of different equipments and conditions, such a comparison is more relevant and necessary to assess the suitability of the method for a desired scale of operation.

Furthermore, since the power consumption is the most designating factor of the cost, extend of disinfection based on the energy dissipated (colonies/ W.s = colonies killed/J) was calculated and the ‘’Efficiency’’, E is defined as:

$$E = \frac{\text{no. of bacteria killed}}{\text{energy dissipated}} \tag{4.7}$$

Ultrasonic yield ‘’G’’, and the efficiency of disinfection ‘’E’’ for the systems are summarized in Table 4.19. For a meaningful comparison, only 20 minutes operation was considered.

Table 4.19 shows that although System-1 provided fastest disinfection, System-2 at the pulse mode was the most energy efficient. Hence, even if pulse mode of operation could not improve the rate of disinfection, the saving of energy in the pause (silent) periods made System-2 the most energy efficient.

Table 4.19 Comparative system yields and energy efficiencies.

SYSTEM[a]	Mode[b]	N_{20}[c] (col./mL)	ΔN[d] (col./mL)	G, (bacteria killed/W)	E (bacteria killed/joule)	$t_{99.9\%}$[e] (min)
System-1 (Homogeneous)	Continuous	1	999	2189.5	1.83	19.3
	Pulse	10	990	2224.7	2.65	31.9
System-1sand (ps> 1mm)	Continuous	1	999	2189.6	1.83	19.8
System-1 sand (1mm>ps> 250 µm)		0	1000	2191.8	1.83	21.0
System-1 sand (250 µm>ps> 53 µm)		2	998	2187.4	1.83	17.5
System-1 talc (ps< 53 µm)		0	1000	2191.8	1.83	25.0
System-2	Continuous	224	776	5278.9	4.40	45.1
	Pulse	400	600	4081.6	4.86	69.4
System-3	Continuous	872	128	1139.5	0.95	670.1
	Pulse	921	79	703.2	0.84	503.1

[a] Buffer concentrations: 0.1 M KH_2PO_4 in System-1, 0.02 M KH_2PO_4 in Systems-2 and 3. Refer to Table 4.17 for P_d (Power dissipated) and V (irradiation volume).

[b] Δt= 20 min for continuous mode, Δt= 14 min for pulse mode

[c] N_{20}= # of colonies/mL after 20 minutes of operation.

[d] N_0= 10^3 col./mL

[e] Time required to achieve 99.9 % kill for No=10^3 col./mL

5. CONCLUSIONS AND RECOMMENDATIONS

In this study, the operational and kinetic parameters of ultrasonic disinfection were investigated in three different systems. As a result of the evaluation and interpretation of the experimental data, the following conclusions were derived:

1. The rate of bacterial kill in System-1 is considerably higher than that in System 2 and 3. This indicates that power ultrasound is a more effective technique for ultrasonic disinfection. This was attributed to the significance of the mechanical effects of power ultrasound, such as shear forces and jetting phenomena. Actually, System-3 can not be considered as a viable technique of ultrasonic disinfection, because of its poor radical yield and energy efficiency.

2. The presence of phosphate ions in solution is a significant parameter affecting the overall kill performance in high frequency systems. This was justified by the accumulation of H_2O_2, which was lower when the $H_2PO_4^-$ was high.

3. The lag periods in pulse mode of sonication were too long for cavitation effects to continue. Hence the pulse mode of operation did not improve the rate of kill as was expected.

4. In general, the rate of kill was low at high and low initial cell concentrations; and highest at the medium level. In System-3, however, the rate was highest when the initial cell concentration was lowest.

5. In heterogeneous experiments. It was observed that sand particles having a diameter greater than 1 mm could not enhance the system performance. Decrease in particle size enhanced bacterial kill; to be attributed to increase in surface area; thus increase in the number of cavity nuclei.

6. The kinetics of the process was described by the following model:

$$\ln N = \ln No + \frac{k}{m+1} t^{m+1}$$

It was found that the time dependency of the rates changed by the mode of sonication, initial cell concentration, and solids. It was observed that the rate of disinfection was highest in System-1, followed by System-2 and 3. The rate was accelerated with solids addition (d≤53μm) in System-1.

7. The ‘’ultrasonic yield, G’’ and the ‘’efficiency, E’’ of the systems were in the order:

G: Sys-2 (pulse) > Sys-2(continuous) > Sys-1 > Sys-3
E: Sys-2 (pulse) > Sys-2(continuous) > Sys-1 > Sys-3

The following are recommended for future studies:

1. More research on pulse mode of operation, including heterogeneous medium.
2. More experiments with different solid particles with varying sizes.

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APPENDIX A

Analysis of H_2O_2: Calibration Curve and Absorbance of the Solutions

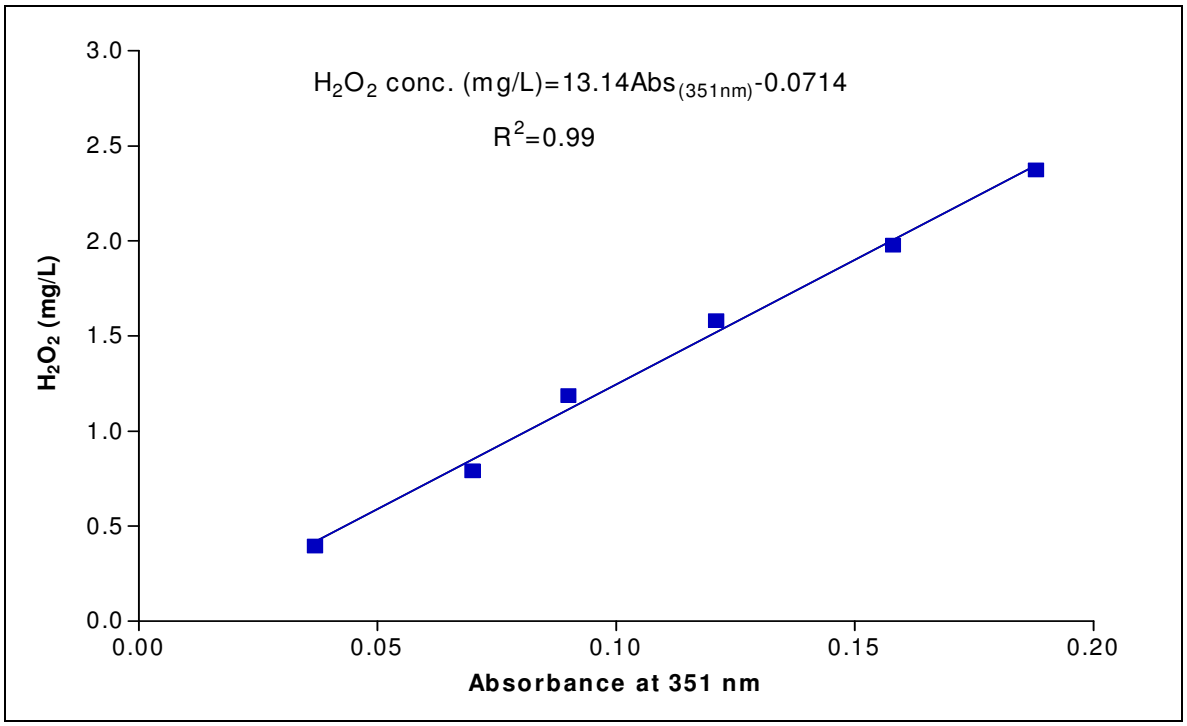

Figure A.1. H_2O_2 calibration curve.

Table A.1. Absorbance of the solutions (351 nm) in System-1

Time (min)	In Deionized Water	In presence of 0.02 M KH_2PO_4	In presence of 0.1 M KH_2PO_4	In presence of 10^3col./mL
0	0	0	0	0
5	0.019	0.029	0.022	0.019
10	0.039	0.045	0.043	0.041
15	0.061	0.057	0.054	0.05
20	0.082	0.08	0.067	0.056
25	0.103	0.087	0.075	0.067
30	0.11	0.113	0.089	0.092

Table A.2. Absorbance of the solutions (351 nm) in System-2

Time (min)	In Deionized Water	In presence of 0.02 M KH_2PO_4	In presence of 0.1 M KH_2PO_4	In presence of 10^3col./mL
0	0	0	0	0
5	0.042	0.055	0.053	0.034
10	0.11	0.111	0.09	0.069
15	0.173	0.164	0.133	0.108
20	0.207	0.198	0.182	0.138
25	0.283	0.266	0.222	0.181
30	0.347	0.319	0.27	0.217

Table A.3. Absorbance of the solutions (351 nm) in System-3

Time, min	In Deionized Water	In presence of 0.02 M KH_2PO_4	In presence of 0.1 M KH_2PO_4	In presence of 10^3col./mL
0	0	0	0	0
5	0.032	0.024	0.031	0.032
10	0.042	0.051	0.048	0.042
15	0.074	0.057	0.067	0.074
20	0.098	0.074	0.079	0.098
25	0.116	0.095	0.097	0.116
30	0.127	0.132	0.113	0.127