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Sonochemical disinfection of municipal wastewater

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Abstract

The application of high intensity, low frequency ultrasound for the disinfection of simulated and septic tank wastewaters is evaluated in this work. Laboratory scale experiments were conducted at 24 and 80 kHz ultrasound frequency with horn-type sonicators capable of operating in continuous and pulsed irradiation modes at nominal ultrasound intensities up to 450 W. For the experiments with simulated wastewaters, *Escherichia coli* were used as biological indicator of disinfection efficiency, while for the experiments with septic tank wastewaters, the total microbiological load was used. Complete elimination of *E. coli* could be achieved within 20–30 min of irradiation at 24 kHz and 450 W with the efficiency decreasing with decreasing intensity and frequency. Moreover, continuous irradiation was more effective than intermittent treatment based on a common energy input. Irradiation of the septic tank effluent prior to biological treatment at 24 kHz and 450 W for 30 min resulted in a three-log total microbiological load reduction, and this was nearly equal to the reduction that could be achieved during biological treatment. Bacterial cell elimination upon irradiation was irreversible as no reappearance of the microorganisms occurred after 24 h.
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Keywords: Disinfection; *E. coli*; Ultrasound; Wastewaters

1. Introduction

Disinfection has become a challenging aspect of water treatment because of the rapid elevation of health standards and the growing concern for pollution-free water resources. The most commonly used disinfection methods utilize cheap and effective chemicals such as chlorine and its products, which unfortunately, have a number of serious drawbacks. They produce chlorinated organic products, which are dangerous for mankind as well as the environment, since they are toxic, carcinogenic, and mutagenic [1]. Additionally, they cannot totally inactivate all the pathogenic microorganisms that can be present in wastewaters or the drinking water due to their low oxidant action [2]. Due to these problems alternative methods for water disinfection are being investigated, and amongst these is the application of ultrasound.

Sonochemical oxidation techniques involve the use of ultrasound waves to produce an oxidative environment via cavitation that yields localized microbubbles and supercritical regions in the aqueous phase. The collapse of these bubbles leads to extremely high local temperatures and pressures. These conditions are very short-lived, but have shown to result in the generation of highly reactive radicals [3], which are capable of initiating or promoting many fast reduction–oxidation reactions. Sonochemistry is an example of advanced oxidation process (AOP). AOP owe their enhanced reactivity, at least in part, to the generation of reactive free radicals, the most important of which is the excited hydroxyl radical ($^{\bullet}$OH) [4].

Ultrasound is able to inactivate bacteria and deagglomerate bacterial clusters through a number of physical, mechanical, and chemical effects arising from acoustic cavitation. On collapse, cavitation bubbles produce enough energy to mechanically weaken or disrupt bacteria or biological cells via a number of processes [5]:

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

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

Sonication alone can provide powerful disinfection. However, to achieve 100% kill rates using only ultrasound is necessary to use high ultrasonic intensities. Unfortunately, this makes the technique expensive to use for general large-scale microbiological decontamination. Nevertheless, there is a drive towards the use of ultrasound in decontamination as an adjunct to other techniques because some microorganisms are becoming resistant to existing disinfection techniques involving biocides, ultraviolet light, and heat treatment. In this view, recent studies have dealt with the use of low frequency ultrasound (in the range 20–40 kHz) alongside ozone [6], ultraviolet irradiation [7], hydrodynamic cavitation [8], electrolysis [9], chlorination [10–12], and heterogeneous catalysts (i.e. activated carbon, ceramic, zinc, and titanium dioxide) [13,14], and reported that enhanced disinfection efficiencies could be achieved with the combined treatments. Process efficiency may also be dictated by operating conditions such as ultrasound intensity, frequency, and conduct time as well as the water matrix (i.e. pH, alkalinity, suspended solids, dissolved gases) [5,13,15,16].

This paper addresses the disinfection of municipal wastewater using sonication alone in order to determine the fundamental effects of changes in frequency, power, and sonication time on bacterial elimination.

2. Materials and methods

2.1. Wastewaters

Experiments were conducted at laboratory scale using both synthetic municipal and septic tank wastewaters. The synthetic wastewater, whose composition is shown in Table 1, was prepared according to an OECD recipe [17], and has a mean dissolved organic carbon concentration of about 40 mg L^{-1} (100 mg L^{-1} COD). The septic tank wastewater with an initial COD and total microbiological load of 1200 mg L^{-1} and $\sim 10^6$ col ml^{-1} respectively, was taken from the Michaniona wastewater treatment plant at the Greater Thessaloniki Area. Samples were collected from the equalization tank of the plant, as well as from the outlet of the secondary treatment unit.

Table 1
Composition of the synthetic municipal wastewater

Substance	Concentration (mg L^{-1})
Peptone	64
Meat extract	44
Urea	12
K_2HPO_4	11.2
NaCl	2.8
$CaCl_2 \cdot 2H_2O$	1.6
$MgSO_4 \cdot 7H_2O$	0.8

Table 2
Composition of the nutrient broth LB-agar

Substance	Concentration (mg L^{-1})
Bactrotryptone	10,000
Yeast extract	5000
NaCl	10,000
Agar	15,000

2.2. Sonication experiments

An Ultrason 250 (LabPlant, UK) ultrasound generator operating at a fixed frequency of 80 kHz and a variable electric power output up to 150 W, connected to a titanium-made horn with a 7 mm tip was used. For those experiments carried out at a frequency of 24 kHz, a UP 400S (Dr Hielscher GmbH, Germany) horn-type sonicator operating at a variable electric power output up to 450 W was used instead. Both sonicators can operate either continuously or in pulse (i.e. on–off) mode. Reactions were carried out in an open to the atmosphere cylindrical all-glass reaction vessel. In all cases, 100 ml of wastewater were subjected to ultrasonic irradiation.

2.3. Assessment of disinfection efficiency

A suspension of *Escherichia coli XL-1 Blue* was prepared by overnight growth at 37 °C in a shaking incubator in broth LB, whose composition in shown in Table 2. *E. coli XL-1 Blue* can grow in the presence of the antibiotic tetracycline. By that, only the added *E. coli* can grow, ensuring that there would be no external infection. The bacterial cell concentration present in a given sample can be determined by measuring the optical density (OD) (turbidity) of the suspension, and therefore, OD measurement was used for bacterial calibration (600 nm). For reproducibility between experiments, bacterial cell suspensions were prepared to an OD of 0.7. Finally, a specific volume of the above suspension was added to the synthetic wastewater.

The disinfection efficacy of ultrasound irradiation was assessed by measuring the microorganism colonies present before and after treatment. Enumeration of bacteria was done by viable plate counts [18]. This is a standard plate counting technique, which accurately reflects the number of viable colony forming units (CFU) remaining after sonication. For this purpose, plate count agar was used as the growth medium. Serial dilutions of the sonicated bacterial suspension samples were prepared using the synthetic wastewater, and analyzed by viable count (spread plate technique 1 ml) on nutrient agar, in the presence of 12 $\mu g\ ml^{-1}$ of the antibiotic tetracycline. Following incubation at 37 °C for 24 h, the number of CFU could then be correlated with the amount of viable bacterial cells per milliliter of suspension.

For the experiments with septic tank wastewater, the extent of disinfection of the total microbiological load was estimated by using broth LB-agar, as the growth medium, in the absence of the antibiotic tetracycline.

3. Results and discussion

The effect of ultrasound intensity and frequency on bacterial elimination is shown in Fig. 1, where experiments with the synthetic wastewater were performed at 24 kHz ultrasound frequency and nominal intensities varying between 90 and 450 W as well as at 80 kHz, and a nominal intensity of 150 W. As can be clearly seen, the rate of bacterial removal increases with increasing ultrasound power at a constant frequency, and for instance, the removal is complete approximately after 15 and 90 min at 450 and 90 W, respectively at 24 kHz. The beneficial impact of increasing ultrasound intensity on elimination rates has also been demonstrated in several previous studies [5,7,10,15,16]. At a constant nominal applied power of 150 W, increasing ultrasound frequency from 24 to 80 kHz has little effect on microbial elimination with only 170 and 135 col ml^{-1} surviving respectively after 30 min, and complete elimination occurring after 45 min. Samples taken 24 h after the end of the sonication runs were reanalyzed with respect to the bacterial population that might have been regenerated. In all cases, no reappearance was observed. Interestingly, the effect of changing frequency on bacterial elimination has received little attention in the literature. Hua and Thompson [15] reported that increasing ultrasound frequency from 205 to 358 to 618, and finally to 1071 kHz at common operating conditions (e.g. applied power of about 128 W emitted by a plate-type sonicator in 300 ml oxygenated solutions) had a considerable adverse effect on *E. coli* elimination rates, which decreased from 0.078 min^{-1} at 205 kHz to 0.03 min^{-1} at 1071 kHz. The authors speculated that the increased hydrogen peroxide production rates (due to water sonolytic cleavage) observed at lower frequencies would result in increased bacterial removal since hydrogen peroxide, and its cleavage reactive radicals behave as disinfectants.

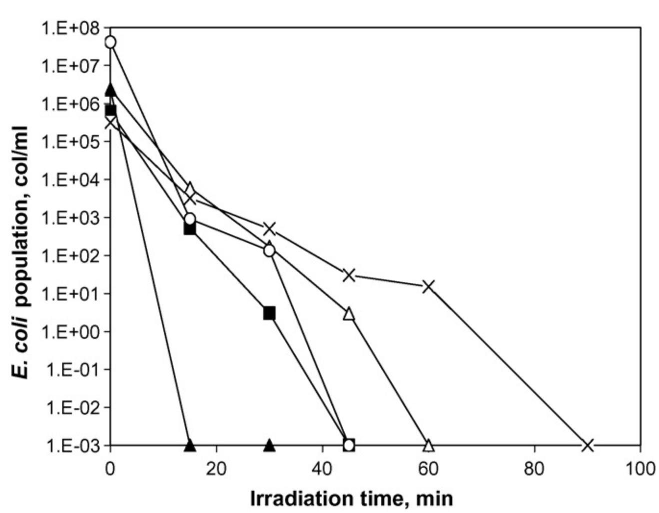

Fig. 1. Temporal change of *E. coli* population during sonication of synthetic wastewater at various ultrasound intensities and frequencies. (–▲–) 24 kHz, 450 W; (–■–) 24 kHz, 300 W; (–△–) 24 kHz, 150 W; (–×–) 24 kHz, 90 W; (–○–) 80 kHz, 150 W.

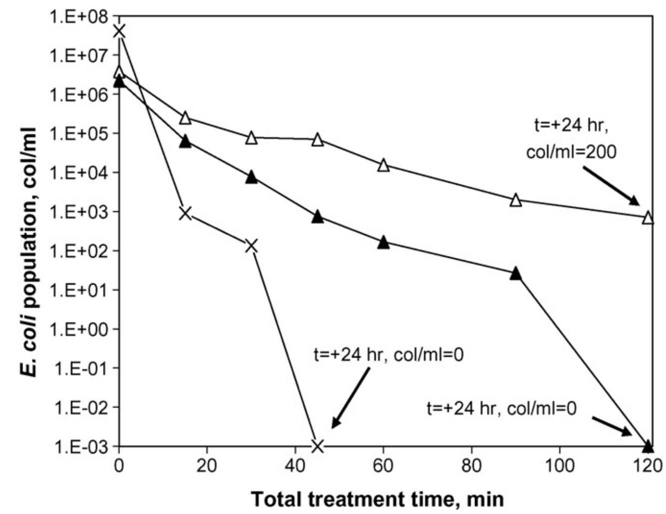

Fig. 2. Temporal change of *E. coli* population during sonication of synthetic wastewater at 80 kHz, 150 W, and various cycles of irradiation. –×–, continuous irradiation; –▲–, 9 s on–1 s off; –△–, 5 s on–5 s off.

In further experiments, the effect of irradiation mode (i.e. continuous *versus* pulsed) was examined at 80 kHz and 150 W of ultrasound frequency and intensity, respectively, and the results are shown in Fig. 2. It should be noted that the timescale of Fig. 2 corresponds to the overall treatment time (i.e. including the “off” period), and not only the irradiation time, for example, for the run performed at 5 s on–5 s off cycle, the actual irradiation time is only 60 min. As can be clearly seen, complete bacterial elimination can rapidly occur during continuous irradiation, and this is accompanied by the lack of any reappearance of bacterial colonies over the following 24 h (inset numbers in Fig. 2 show 24-h reappearance populations). On the other hand, a short pause of irradiation (i.e. 1 s in every 10 s of operation) has a substantial detrimental effect on disinfection with complete elimination occurring only after 120 min of treatment (this corresponds to 108 min of irradiation). For the 5 s on–5 s off operation, about 710 col ml^{-1} survive after 60 min of irradiation, and this is still higher than the surviving population after the same period of irradiation for the 9 s on–1 s off mode of operation. These results show that continuous irradiation is clearly more effective than intermittent operation for the same energy input presumably due to the fact that the submission of bacteria to physical, mechanical, and chemical effects arising from continuous cavitation results in severe and irreversible cell damage.

In a final set of experiments, both the untreated and biologically treated septic tank wastewaters were subjected to sonication at 24 kHz and 300 or 450 W, and the change of the total microbiological load was followed as a function of irradiation time.

As seen in Fig. 3, the untreated effluent has a microbiological load in the order of 10^5–10^6 col ml^{-1}, and this is reduced to about 5000 col ml^{-1} after secondary treatment. By means of ultrasound irradiation, this reduction can be achieved after 30 min of sonication at 450 W (dotted line in Fig. 3). Increasing intensity from 300 to 450 W has only a marginal effect on disinfection efficiency.

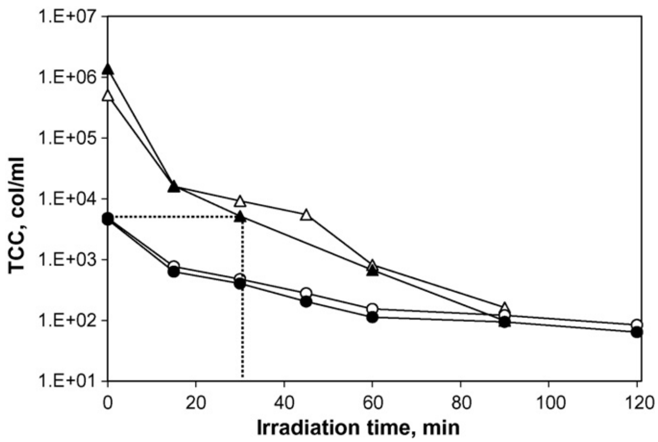

Fig. 3. Temporal change of total microbiological load during sonication of septic tank wastewater at 24 kHz. (–▲–) untreated effluent, 450 W; (–△–) untreated effluent, 300 W; (–●–) biologically treated effluent, 450 W; (–○–) biologically treated effluent, 300 W.

From Figs. 1 and 3, it can be seen that disinfection of synthetic wastewater at 24 kHz and 300 or 450 W proceeds substantially faster than that of untreated septic tank effluent at common conditions. This is possibly due to the fact that the water matrix of the actual effluent is considerably different from that of the synthetic effluent, and this affects sonochemical efficiency. For instance, the organic load, in terms of COD, of the untreated effluent is about 1200 mg L^{-1}, that is, 10 times more than that of the synthetic wastewater. It is well known that the organic additives compete the bacteria on the $OH^{\bullet}$ capture, lowering so the disinfection efficiency of the method. Previous studies have shown that water quality such as suspended solids and alkalinity [16], dissolved gases [15], as well as the presence of radical scavengers [13] may partly affect disinfection efficiency.

4. Conclusions

In this work the sonolytic disinfection of synthetic and real wastewater has been demonstrated. It was observed that, high power and low frequency ultrasound is capable of eliminating nearly completely the *E. coli* colonies in synthetic municipal wastewaters, as well as the total microbiological load in actual municipal wastewaters at relatively short irradiation times.

Besides the irradiation time, other factors that affect disinfection efficiency are ultrasound frequency and intensity, type of operation (i.e. continuous or pulsed), as well as the water matrix (i.e. concentration and composition of the effluent).

Finally, it was observed that cell elimination induced by ultrasound irradiation is permanent, as denoted by the zero reappearance rates of the disrupted bacteria.

References

[1] T.K. Nissinen, I.T. Miettinen, P.J. Martikainen, T. Vartiainen, Disinfection by-products in Finnish drinking waters, Chemosphere 48 (2002) 9–20.
[2] C.H. King, E.B. Shotts, R.E. Wooley, K.G. Porter, Survival of coliforms and bacterial pathogens within protozoa during chlorination, Appl. Environ. Microbiol. 54 (1988) 3023–3033.
[3] Y.G. Adewuyi, Sonochemistry: environmental science and engineering applications, Ind. Eng. Chem. Res. 40 (2001) 4681–4715.
[4] L.H. Thompson, L.K. Doraiswamy, Sonochemistry: science and engineering, Ind. Eng. Chem. Res. 38 (1999) 1215–1249.
[5] E. Joyce, S.S. Phull, J.P. Lorimer, T.J. Mason, The development and evaluation of ultrasound for the treatment of bacterial suspensions. A study of frequency, power, and sonication time on cultured *Bacillus* species, Ultrason. Sonochem. 10 (2003) 315–318.
[6] K.K. Jyoti, A.B. Pandit, Ozone and cavitation for water disinfection, Biochem. Eng. J. 18 (2004) 9–19.
[7] T. Blume, U. Neis, Improved wastewater disinfection by ultrasonic pre-treatment, Ultrason. Sonochem. 11 (2004) 333–336.
[8] K.K. Jyoti, A.B. Pandit, Effect of cavitation on chemical disinfection efficiency, Water Res. 38 (2004) 2249–2258.
[9] E. Joyce, T.J. Mason, S.S. Phull, J.P. Lorimer, The development and evaluation of electrolysis in conjunction with power ultrasound for the disinfection of bacterial suspensions, Ultrason. Sonochem. 10 (2003) 231–234.
[10] H. Duckhouse, T.J. Mason, S.S. Phull, J.P. Lorimer, The effect of sonication on microbial disinfection using hypochlorite, Ultrason. Sonochem. 11 (2004) 173–176.
[11] T.J. Mason, E. Joyce, S.S. Phull, J.P. Lorimer, Potential uses of ultrasound in the biological decontamination of water, Ultrason. Sonochem. 10 (2003) 319–323.
[12] T. Blume, U. Neis, Improving chlorine disinfection of wastewater by ultrasound application, Water Sci. Technol. 52 (2005) 139–144.
[13] M.F. Dadjour, C. Ogino, S. Matsumura, S. Nakamura, N. Shimizu, Disinfection of *Legionella pneumophila* by ultrasonic treatment with TiO_2, Water Res. 40 (2006) 1137–1142.
[14] N.H. Ince, R. Belen, Aqueous phase disinfection with power ultrasound: process kinetics and effect of solid catalysts, Environ. Sci. Technol. 35 (2001) 1885–1888.
[15] I. Hua, J.E. Thompson, Inactivation of *Escherichia coli* by sonication at discrete ultrasonic frequencies, Water Res. 34 (2000) 3888–3893.
[16] B.A. Madge, J.N. Jensen, Disinfection of wastewater using a 20-kHz ultrasound unit, Water Environ. Res. 74 (2002) 159–169.
[17] Current Protocols in Molecular Biology 1 (1994) (Chapters 1.03–1.15).
[18] OECD Simulation Test-Aerobic Sewage Treatment 303A (1999).