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Original Article

Inactivation of Coliforms in Sludge Through Cavitation Phenomena by Ultrasonic Waves

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Abstract

Background: One of the most challenging and critical processes in wastewater treatment is sludge treatment. This study aimed to investigate the effects of low frequency ultrasound and high level of energy on inactivation rate of total coliform of sludge and ascertain the optimal operating parameters of the ultrasound waves.
Methods: In this research, the density of ultrasound (W/mL) and time (minutes) were investigated. The effect of these parameters on the inactivation of total coliform in sludge was also investigated.
Results: The results revealed that the optimum operating time and ultrasound density were 30 minutes and 2.5 W/mL, respectively. Also, the frequency of 20 kHz of total coliform removal rate in these conditions was 99.44% .
Conclusion: Ultrasound waves as well as micro and nano bubbles could remove total coliform and decontaminate the sludge, thereby incrementing the rate of treatment.
Keywords: Cavitation, Nano-bubbles, Sludge, Total coliform, Ultrasound density

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Introduction

Disinfection is a crucial part of a wastewater treatment plant. As the health standards of people's life increases, its role becomes more significant.[1,2] There are different types of pathogens in the sludge such as viruses, bacteria, parasites, and fungi. There are also different indicator pathogens such as fecal coliforms, clostridium perfringens, helminth ova, salmonella, enteric viruses, etc.[3] Coliforms are the major indicators of pathogens in sludge or wastewater. The primary goal of this process is to remove pathogenic microorganisms to alleviate their health hazard.[4,5]

Different techniques are applied for disinfection purpose. Chlorination, ultraviolet (UV) irradiation and ozonation are the most widely used and typical approaches.[6-8] Despite the acceptable efficiency of these techniques, their limitation and side products vitalize emerging, safer and more promising technologies. Major drawbacks of conventional disinfection methods are their hazards such as iodate, bromate and THMs that are considered as carcinogenic agents by application of chlorination and ozonation, respectively.[9-11] Additionally, application of UV technique is limited due to the requirement of highly light scattering or absorbing medium and self-repair of disinfected microorganisms.[11]

Ultrasound waves are one of the alternative methods for conventional disinfection techniques, which not only do not produce undesirable by-products but also have good efficiency in inactivation of microbes and viruses from water and wastewater.[12,13] The disinfection of ultrasound is based on the presence of cavitation occurrences.[14]

Several physical, chemical and mechanical processes are taking place during cavitation phenomena. During the cavitation process, micro and Nanobubbles are produced that is followed by expansion and implosion. Contraction and refraction cycle of ultrasound wave causes the reduction and expansion of the average diameter between molecules in a liquid. If the refraction force of ultrasonic waves is intense enough to break the attraction force of molecules, cavitation bubbles are produced.[15] The generated bubbles also experience contraction and refraction cycle. If the expansion is sufficient to reach an unstable phase, the implosion will occur which unleash high energy and causes sonochemical reactions.[16,17] By implosion of gas bubbles, physical and mechanical impact of cavitation process are imposed by increase in pressure and temperature (locally up to 5000°C and 2000 atmosphere) as well as shear stress and turbulence that can break cell membranes and release intercellular molecules in the external environment.[18,19]

Cavitation phenomena has also chemical effect which is produced by the OH^- and H^+ radicals that lacerate cell wall by oxidation and pathogenic microorganism.[19,20] The final product of oxidation is H_2O_2 and H_2O that not only are hazardous, but also H_2O_2 is an oxidizing agent which enhances the oxidation process. One of the most critical indicators of existing pathogenic agents in water and wastewater is total coliform, elimination of which is an essential target of disinfection. Several studies have examined the application of ultrasonic waves.[19,21] Vázquez-López et al studied the removal of total coliform in wastewater by ultrasound, and reached to almost complete removal of total coliform in frequency of 26 kHz and time of 30 minutes.[21] In another research, 99.99% of coliform removal rate was achieved in 0.2250 KW.h/m^3 of specific energy consumption.[13]

The objective of present study was to determine the impact of ultrasound on disinfection of sludge and find the optimal range of operating parameters by ultrasound to reduce the total coliform bacteria in the sludge by response surface methodology.

Materials and Methods

To estimate the efficiency of ultrasound on sludge disinfection, the samples of sludge were taken after digesters from sewage treatment plant of the south of Tehran. For this purpose, COD was measured by Hatch DR 5000 spectrophotometer based on standard procedures test number of 5220. Total coliform and *E. coli* experiments were analyzed by MPN standard method and test number of 9221. Also, pH and temperature were measured by Metrom 691 device according to test numbers of 2310 and 2550. All tests were carried out by the standard methods.[22] Table 1 shows the sludge samples characters.

A homogenizer ultrasound with a 5-mm sonotrode was used. The output of electric power was reached up to 750 W, and the frequency of operating was set to 20 kHz.

Data analysis was done by Design Expert 10.2.1. For experiment's design, Box–Behnken design based on the RSM was used to evaluate the impact of variables on coliform inactivation.

Box-Behnken design is more proficient and powerful than other designs such as the three-level full factorial, central composite design (CCD) and Doehlert design. Box–Behnken designs can sharply reduce the number of experimental sets without decreasing the accuracy of optimization compared with other factorial design methods.[23-26]

The tests were evaluated at times of 30, 15, 10, 5 and 1 minute. Also, the density of ultrasound levels was 2.5, 1.3, 1, 0.75 and 0.375 W/mL.

Results and Discussion

This research was performed to determine ultrasonic wave's effect on disinfection of sludge and find the optimal operating parameters range using ultrasound waves to inactivate total coliform of sludge. Table 2 shows the response values of total coliform inactivation.

The maximum coliform inactivation was 99.44% in ultrasonic density of 2.5 W/mL (P=0.0001) and time of 30 minutes.

Table 3 shows the results of variance analysis and regression. A and B are time (minutes) and density of ultrasonic (W/mL), respectively.

Equation 1 shows the expected second order polynomial equation. High coefficient of correlation (R^2 = 98.95) indicated that the model was reproducible.

$$Y_1 = 21.33843 + 9.24075\ A - 8.58969\ B + 1.46878\ AB - 0.31898\ A^2 - 0.28880\ B^2 \quad (1)$$

Table 1. Sludge Samples Characteristics

Parameter	Unit	Value
COD	mg/L	20675 ± 267
Temp	°C	21 ± 1
pH	-	7.88 ± 0.1
Escherichia coli	-	3.24×10^8
Total coliforms	mg/L	8.65×10^8

Table 2. Response Values of Total Coliform Inactivation

Run	Factor A, Time (Min)	Factor B, Ultrasonic Density (W/mL)	Total Coliform Inactivation (%)
1	30	2.5	99.44
2	10	1	88.20
3	10	1	90.85
4	10	1	90.23
5	15	0.75	95.81
6	15	1.3	97.50
7	10	0.375	88.15
8	10	1	89.57
9	10	2.5	93.89
10	5	0.75	47.90
11	1	1	21.70
12	5	1.3	64.46
13	10	1	90.38

Table 3. Variance Results of RSM Analysis for Inactivation of Total Coliform

Source	Sum of Squares	*df*	Mean Square	F value	*P* value
Model	6054.25	5	1210.85	30.95	0.0001
Time (A)	160.39	1	160.39	4.10	0.0825
Ultrasonic density (B)	112.14	1	112.14	2.87	0.1343
AB	47.18	1	47.18	1.21	0.3085
A^2	567.53	1	567.53	14.51	0.0066
B^2	0.12	1	0.12	3.173E-003	0.9567
Residual	273.82	7	39.12		
Lack of fit	269.59	3	8.98	8.5	0.4
Pure error	4.23	4	1.06		
Cor total	6328.07	12			

Based on Table 2 and the statistical analysis, there was no significant differences between time of sonification and ultrasound density (p>0.05). This may be due to heterogeneity of physico-chemical composition of the samples between the repetitions. The studies conducted by Gholami et al,[27] Lazarotto et al[28] and Mahvi et al[29] found similar results.

Figure 1 shows the changes in total coliform population during time by different ultrasound densities. During the test period and formation of micro and nano-bubbles, more than 2 log reduction in total coliform at 30 minutes was observed. Some mechanisms that affect the total coliform inactivation are as follow:

A) The temperature of samples will rise by increase in time of sonication which accelerates the ions diffusion process in the cell membranes.

B) Decomposition of pollutants using ultrasonic waves occur under the oxidization process by the formation of $OH^{\cdot}$ radicals as a result of ultrasonic dynamic mixing, thermal destruction, and shearing process.[16,27,30]

As seen in Figure 1, with increase in time of sonication up to 30 minutes, the removal of total coliform reaches their maximum level. With increase in time of sonication, this reduction continued, though the optimal time was 30 minutes due to the cost-effectiveness of the process.

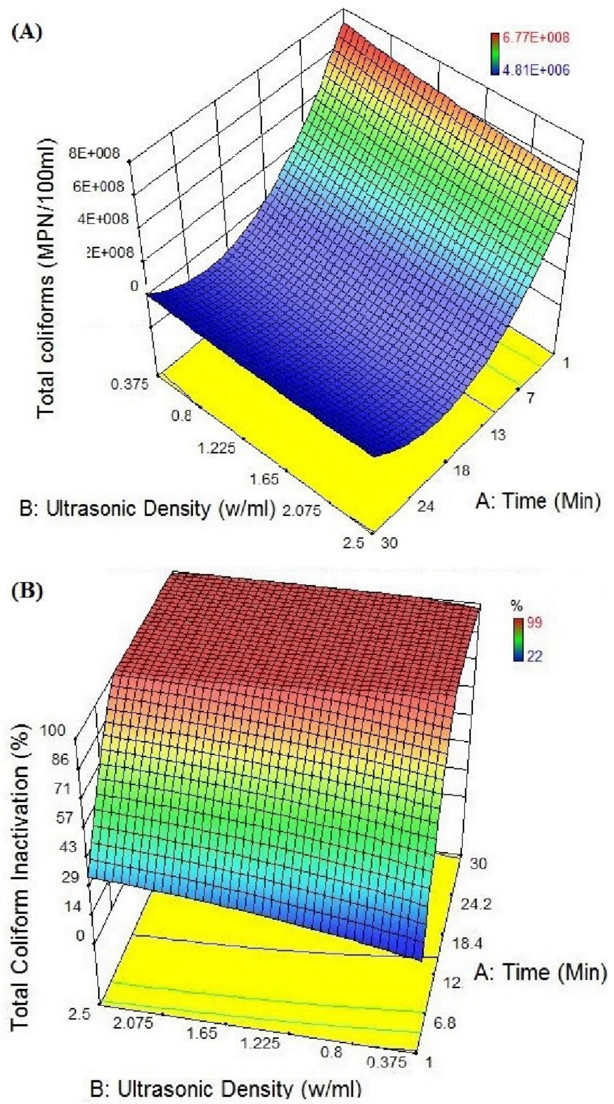

Figure 1. The effect of time on total coliform population (A) and inactivation (B).

A research on the total coliform inactivation established that by increase in time of ultrasonic waves up to 45 minutes, elimination of the total coliform bacteria would be over 99%.[31] Al-Juboori's study findings confirm the results of our study by showing the relationship between increase in time and the rise in total coliform inactivation.[10] In a similar study conducted by Ali et al, ultrasonic wave was used for inactivation of coliforms of water. In the study, experiments showed that higher contact time up to 60 minutes and higher frequency of 60 Hz have the most effectiveness in removal of coliforms.[32]

Figure 1 indicates a significant reduction of total coliform population by the increase in ultrasonic density from 0.375 to 2.5 W/mL. The optimum removal of total coliform was 99.44 percent which is equal to total coliform inactivation. This was achieved at ultrasonic density of 2.5 W/mL.

The pulse cycle in this research was 9 seconds, and according to the results of previous studies, more time is need to the active surface of bacteria ultrasonic cavitation of micro and nano-bubbles that lead to more bacteria inactivation.[16,31]

Ultrasound waves on coliforms punctures their cell walls and produces $OH^{\cdot}$ radicals, and extrusion of the intracellular matrix ultimately kills the coliforms. In general, different factors such as volume of sample, type of microorganism, wave amplitude, time and ultrasonic power have the most important role on removal of coliforms by sonification.[33]

A research by Ayyildiz et alon inactivation of total coliform using ultrasound waves observed that the removal rate was increased over 90 percent in 10 minutes when the ultrasonic frequency reached to 20 kHz and ultrasonic density was between 75 to 300 W/L.[34] Zhou et al[35] investigated the impact of increasing ultrasonic density on inactivation of total coliform. The results revealed that by increasing ultrasonic density from 2 to 12 W/mL, the rate of inactivation enhanced considerably

Conclusion

In this study, we investigated the pilot scale sonolytic disinfection and inactivation of total coliform in wastewater sludge. The results revealed that inactivation of almost all the total coliform could be reached by applying high power and low-frequency ultrasound waves at relatively short irradiation times through formation of micro and nano cavitation bubbles. Moreover, the critical parameters of ultrasonic disinfection of sludge were ultrasound density and irradiation time.

According to the results, the maximum of total coliform inactivation was 99.44% in operating conditions of ultrasonic density, irradiation time and ultrasonic frequency of 2.5 W/mL, 30 minutes and 20 kHz respectively. The results also proved the apparent effectiveness of ultrasound waves on inactivation of the total coliform bacteria in wastewater sludge.

Authors' Contribution

Conceptualization: Farshad Golbabaei Kootenaei.
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Project administration: Nasser Mehrdadi.
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Visualization: Farshad Golbabaei Kootenaei.
Writing–original draft: Farshad Golbabaei Kootenaei.
Writing–review & editing: Farshad Golbabaei Kootenaei.

Competing Interests

The authors declared no conflict of interest.

Ethical Approval

This study was approved by University of Tehran.

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References

1. Alimoradzadeh R, Assadi A, Afshar F, Rahmani AR, Samarghandi MR. Photocatalytic removal of *Pseudomonas aeruginosa* from water using titanium dioxide nanoparticles and UV irradiation. J Hum Environ Health Promot. 2015;1(1):28-33. doi: 10.29252/jhehp.1.1.4.
2. Collivignarelli MC, Abbà A, Miino MC, Caccamo FM, Torretta V, Rada EC, et al. Disinfection of wastewater by UV-based treatment for reuse in a circular economy perspective. Where are we at? Int J Environ Res Public Health. 2020;18(1):77. doi: 10.3390/ijerph18010077.
3. Li M, Song G, Liu R, Huang X, Liu H. Inactivation and risk control of pathogenic microorganisms in municipal sludge treatment: a review. Front Environ Sci Eng. 2022;16(6):70. doi: 10.1007/s11783-021-1504-5.
4. Naddeo V, Cesaro A, Mantzavinos D, Fatta-Kassinos D, Belgiorno V. Water and wastewater disinfection by ultrasound irradiation-a critical review. Glob Nest J. 2014;16(3):561-77.
5. Yadav M, Gole VL, Sharma J, Yadav RK. Biologically treated industrial wastewater disinfection using synergy of US, LED-UVS, and oxidants. Chem Eng Process. 2021;169:108646. doi: 10.1016/j.cep.2021.108646.
6. Hu ZT, Chen Y, Fei YF, Loo SL, Chen G, Hu M, et al. An overview of nanomaterial-based novel disinfection technologies for harmful microorganisms: mechanism, synthesis, devices and application. Sci Total Environ. 2022;837:155720. doi: 10.1016/j.scitotenv.2022.155720.
7. Declerck P, Vanysacker L, Hulsmans A, Lambert N, Liers S, Ollevier F. Evaluation of power ultrasound for disinfection of both Legionella pneumophila and its environmental host *Acanthamoeba castellanii*. Water Res. 2010;44(3):703-10. doi: 10.1016/j.watres.2009.09.062.
8. Nam-Koong H, Schroeder JP, Petrick G, Schulz C. Preliminary test of ultrasonically disinfection efficacy towards selected aquaculture pathogens. Aquaculture. 2020;515:734592. doi: 10.1016/j.aquaculture.2019.734592.
9. Li Y, Li W, Zhang X, Jiang J. Effects of ultrasonication on the DBP formation and toxicity during chlorination of saline wastewater effluents. J Environ Sci (China). 2022;117:326-35. doi: 10.1016/j.jes.2022.05.029.
10. Al-Juboori RA, Aravinthan V, Yusaf T. Impact of pulsed ultrasound on bacteria reduction of natural waters. Ultrason Sonochem. 2015;27:137-47. doi: 10.1016/j.ultsonch.2015.05.007.
11. Al-Juboori RA, Yusaf T. Biofouling in RO system: mechanisms, monitoring and controlling. Desalination. 2012;302:1-23. doi: 10.1016/j.desal.2012.06.016.
12. Zhou X, Yan Y, Li Z, Yin J. Disinfection effect of a continuous-flow ultrasound/ultraviolet baffled reactor at a pilot scale. Ultrason Sonochem. 2017;37:114-9. doi: 10.1016/j.ultsonch.2017.01.003.
13. Cao P, Hao C, Ma C, Yang H, Sun R. Physical field simulation of the ultrasonic radiation method: an investigation of the vessel, probe position and power. Ultrason Sonochem. 2021;76:105626. doi: 10.1016/j.ultsonch.2021.105626.
14. Cao T, Tong W, Feng F, Zhang S, Li Y, Liang S, et al. H2O2 generation enhancement by ultrasonic nebulisation with a zinc layer for spray disinfection. Chem Eng J. 2022;431:134005. doi: 10.1016/j.cej.2021.134005.
15. Stack LJ, Carney PA, Malone HB, Wessels TK. Factors influencing the ultrasonic separation of oil-in-water emulsions. Ultrason Sonochem. 2005;12(3):153-60. doi: 10.1016/j.ultsonch.2003.10.008.
16. Dehghani MH. Effectiveness of ultrasound on the destruction of *E. coli*. Am J Environ Sci. 2005;1(3):187-9.
17. Mohammadi AR, Mehrdadi N, Nabi Bidhendi G, Torabian A. Excess sludge reduction using ultrasonic waves in biological wastewater treatment. Desalination. 2011;275(1-3):67-73. doi: 10.1016/j.desal.2011.02.030.
18. Joyce E, Phull SS, Lorimer JP, Mason TJ. 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. 2003;10(6):315-8. doi: 10.1016/s1350-4177(03)00101-9.
19. Wang F, Wang Y, Ji M. Mechanisms and kinetics models for ultrasonic waste activated sludge disintegration. J Hazard Mater. 2005;123(1-3):145-50. doi: 10.1016/j.jhazmat.2005.03.033.
20. Cui X, Talley JW, Liu G, Larson SL. Effects of primary sludge particulate (PSP) entrapment on ultrasonic (20 kHz) disinfection of *Escherichia coli*. Water Res. 2011;45(11):3300-8. doi: 10.1016/j.watres.2011.03.034.
21. Vázquez-López M, Amabilis-Sosa LE, Moeller-Chávez GE, Roé-Sosa A, Neumann P, Vidal G. Evaluation of the ultrasound effect on treated municipal wastewater. Environ Technol. 2019;40(27):3568-77. doi: 10.1080/09593330.2018.1481889.
22. American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater. Washington, DC: APHA; 2005.
23. Ferreira SL, Bruns RE, Ferreira HS, Matos GD, David JM, Brandão GC, et al. Box-Behnken design: an alternative for the optimization of analytical methods. Anal Chim Acta. 2007;597(2):179-86. doi: 10.1016/j.aca.2007.07.011.
24. Robinson T. Box-Behnken design. In: Encyclopedia of Statistics in Quality and Reliability. USA: John Wiley & Sons; 2007.
25. Baş D, Boyacı İH. Modeling and optimization I: usability of response surface methodology. J Food Eng. 2007;78(3):836-45. doi: 10.1016/j.jfoodeng.2005.11.024.
26. Pham TT, Brar SK, Tyagi RD, Surampalli RY. Ultrasonication of wastewater sludge--consequences on biodegradability and flowability. J Hazard Mater. 2009;163(2-3):891-8. doi: 10.1016/j.jhazmat.2008.07.091.
27. Gholami M, Mirzaei R, Mohammadi R, Zarghampour Z, Afshari A. Destruction of *Escherichia coli* and *Enterococcus faecalis* using low frequency ultrasound technology: a response surface methodology. Health Scope. 2014;3(1):e14213. doi: 10.17795/jhealthscope-14213.
28. Lazarotto JS, Júnior EPM, Medeiros RC, Volpatto F, Silvestri S. Sanitary sewage disinfection with ultraviolet radiation and ultrasound. Int J Environ Sci Technol (Tehran).

2022;19(11):11531-8. doi: 10.1007/s13762-021-03764-7.

29. Mahvi AH, Dehghani MH, Vaezi F. Ultrasonic technology effectiveness in total coliforms disinfection of water. J Appl Sci. 2005;5(5):856-8. doi: 10.3923/jas.2005.856.858.
30. Bigelow TA, Northagen T, Hill TM, Sailer FC. The destruction of *Escherichia coli* biofilms using high-intensity focused ultrasound. Ultrasound Med Biol. 2009;35(6):1026-31. doi: 10.1016/j.ultrasmedbio.2008.12.001.
31. Amabilis-Sosa LE, Vázquez-López M, Rojas JL, Roé-Sosa A, Moeller-Chávez GE. Efficient bacteria inactivation by ultrasound in municipal wastewater. Environments. 2018;5(4):47. doi: 10.3390/environments5040047.
32. Ali N, Kamel Z, Wahba SZ. Ultrasonic as green chemistry for bacterial and algal control in drinking water treatment source. Egypt J Chem. 2020;63(10):4055-62. doi: 10.21608/ejchem.2020.42173.2852.
33. Starek A, Kobus Z, Sagan A, Chudzik B, Pawłat J, Kwiatkowski M, et al. Influence of ultrasound on selected microorganisms, chemical and structural changes in fresh tomato juice. Sci Rep. 2021;11(1):3488. doi: 10.1038/s41598-021-83073-8.
34. Ayyildiz O, Sanik S, Ileri B. Effect of ultrasonic pretreatment on chlorine dioxide disinfection efficiency. Ultrason Sonochem. 2011;18(2):683-8. doi: 10.1016/j.ultsonch.2010.08.008.
35. Zhou Z, Yang Y, Li X. Optimization of ultrasound-induced inactivation of model bacterial mixture using response surface methodology. J Water Supply Res Technol Aqua. 2015;65(1):54-63. doi: 10.2166/aqua.2015.045.