

Influence of Low-Frequency Ultrasound on the Disintegration of Coliform and Fecal Coliform Bacteria

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ABSTRACT

Wastewater contains an abundance of pathogenic microorganisms, which, if they infiltrate into the environment, can cause many bacterial diseases. The lack of widespread use of the wastewater disinfection process raises more and more concerns about the sanitary quality of the wastewater leaving treatment plants. This article aimed to test the effectiveness of the ultrasonic disintegration process against coliform bacteria and fecal coliforms in the treated wastewater. The paper presents the research on the use of ultrasound with a frequency of 20 and 40 kHz, with a variable and constant operating mode of an ultrasonic cleaner. The obtained test results confirm the effectiveness of the applied method. In most of the analyzed cases, 25-minute sonication reduced the number of tested microorganisms by over 90% compared to the control sample. Ultrasonic disintegration can therefore be successfully used in municipal wastewater treatment plants. The use of ultrasound for disinfection can improve the sanitary safety of the wastewater discharged into water or soil.

Keywords: coliform bacteria, ultrasonic disintegration, low-frequency ultrasound, treated wastewater.

INTRODUCTION

In raw municipal wastewater, there is a large percentage of domestic wastewater from municipal farms, the microbiological composition of which is determined by the population of the human intestinal microbiome [Frank et al., 2007]. The prevalence of pathogenic microorganisms in raw municipal wastewater is very diverse, often specific to a given country or region. Their primary source is urban and industrial wastewater, animal feces rinsed with rainwater from the surface, and also the feces of rats inhabiting sewage systems. Although the pathogenic microorganisms present in sewage constitute a relatively small percentage of the total number of microorganisms, some of them may pose a direct threat to the health of the wastewater treatment plant workers [Bray et al., 2015]. The effectiveness of removing microorganisms, including pathogenic microorganisms, depends on the features of the treatment system: the type of processes used, their duration and

the type, amount, and forms of microorganisms [Zhang et al., 2012]. Classic wastewater treatment processes ensure a high level of bacteria reduction, even up to 99%. However, despite such a high removal efficiency in wastewater, they are still present, among others, in coliform bacteria ranging from 10^4 to $10^6/100 \text{ cm}^3$ [Michałkiewicz et al., 2011; Butarewicz, 2013]. The US Environmental Protection Agency data indicate that the survival of bacteria in soil and on the surface of plants may reach even one year [US EPA, 2003].

According to the Polish legislation, the quality of treated wastewater leaving municipal wastewater treatment plants is determined by the Regulation of the Minister of Maritime Economy and Inland Navigation of July 12, 2019, on substances particularly harmful to the aquatic environment and the conditions to be met when discharging sewage into waters or ground, as well as in the discharge of rainwater or meltwater to waters or water facilities [Journal of Laws 2019, item 1311]. This regulation specifies the number

of physicochemical parameters that wastewater must meet after passing all stages of treatment processes. However, it does not address the issue of sanitary requirements — in terms of microbiological conditions, only in the case of the wastewater intended for agricultural use there is an obligation to determine whether the wastewater contains the bacteria of the *Salmonella* genus and eggs of intestinal parasites belonging to *Ascaris* sp., *Trichuris* sp., *Toxocara* sp. [Journal of Laws 2019, item 1311]. Therefore, wastewater treatment plants are not required to investigate the number of microorganisms in the wastewater or what types of microorganisms are there. Therefore, because a total 1,343,275.5 dam³ of sewage was discharged in Poland in 2019 [Central Statistical Office, 2021], the question about their sanitary safety arises.

The most common recipient of treated wastewater is surface water, from which microorganisms can then be transferred to humans as a result of using the water for recreational purposes and the production of drinking water [De Boeck et al., 2012; Drudge et al., 2012], as well as infection with *Escherichia coli* [Figueira et al., 2012]. Due to the lack of widespread use of the disinfection process of the wastewater leaving treatment plants, it was decided to carry out the tests aimed at checking the effectiveness of the ultrasonic disintegration process against the bacteria in the treated wastewater.

MATERIALS AND METHODS

The study of the impact of ultrasound on the disintegration of coliforms and fecal coliforms present in treated wastewater from the Municipal Wastewater Treatment Plant in Białystok was carried out in the microbiological laboratory of the Department of Chemistry, Biology, and Biotechnology of the Faculty of Civil Engineering and Environmental Sciences of the Białystok University of Technology.

The experiment involved determining the influence of low-frequency ultrasound (20 and 40 kHz) on the survival of coliforms and fecal coliforms in the treated wastewater. For disintegration, Polsonic ultrasonic cleaners with the ultrasound frequency of 20 and 40 kHz were used, and the determinations were based on the method of quantifying coli and *E. coli* bacteria following the PN-EN ISO 9308-2:2014-06 standard.

This method is based on the culture in a liquid medium containing a chromogenic substrate that is hydrolyzed by a specific enzyme and thus confirms the coliform bacteria (or *E. coli*). The reporting of results consists of calculating the value of the most probable number of bacteria (MPN) from the MPN tables.

The treated wastewater from the Wastewater Treatment Plant in Białystok was successively sonicated in an ultrasonic cleaner generating ultrasound with a frequency of 20 kHz. The tests were performed with the continuous and pulsating operation of the device. A similar process was carried out in a second washer, generating ultrasound at a frequency of 40 kHz in continuous operation. The total sonication time of treated wastewater was 25 minutes.

Before disintegration, the numbers of coliforms and fecal coliforms were determined in a control sample (cleaned walls without ultrasound treatment). After 10, 15, 20, and 25 minutes of the sonication process, the determinations were made in all variants, identical to those in the reference test. In order to determine the number of bacteria, the tubes with the chromogenic medium were incubated in an incubator at 37 °C and 44 °C for 48 hours. After incubation, the obtained test results were interpreted. On the basis of the NPL tables, the number of coliforms and fecal coliforms was calculated and expressed as NPL/100 cm³ (the most probable number of bacteria in 100 cm³ of treated wastewater). Each time during the determinations, the temperature of the wastewater subjected to the sonication process was monitored.

RESEARCH RESULTS AND DISCUSSION

Escherichia coli rods are among the fundamental indicators of the sanitary quality of water, sewage, and sewage sludge [Naidoo and Olaniran, 2014]. At the same time, strains of *E. coli* are among the most important pathogenic bacteria detected in municipal wastewater [Bitton, 2011]. Under certain conditions, these bacteria are pathogenic for humans and cause diseases of the digestive and urinary systems [Bray et al., 2015]. Coliform bacteria and *E. coli* (fecal coliform) bacteria also include *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Serratia*, *Hafnia* [Leclerc et al., 2001]. All coliform bacteria belong to the *Enterobacteriaceae* family, and the current definition [WHO, 2011] specifies that they are the microorganisms capable

of producing the β -D-galactosidase enzyme, the activity of which is based on most of the currently used methods of detecting these bacteria.

Table 1 shows the changes in the number of coliforms and fecal coliforms in the wastewater treated with ultrasound at the frequency of 20 and 40 kHz along with the temperature increase recorded during the process., Figures 1 and 2 show

the percentage changes in the number of these microorganisms dependent on sonication time.

On the basis of the obtained test results, a significant reduction in the number of coliforms and fecal coliforms in municipal wastewater treated with ultrasound was found.

The operation of an ultrasonic cleaner with a frequency of generating waves at the level of 20

Table 1. Most likely count of coliforms and fecal coliforms in ultrasonically treated wastewater

Sonication time [min]	20 kHz - continuous operation			20 kHz - pulse operation			40 kHz - continuous operation		
	Coliform bacteria [CFU/100 cm ³]	Faecal coliforms [CFU/100 cm ³]	Temperature [°C]	Coliform bacteria [CFU/100 cm ³]	Faecal coliforms [CFU/100 cm ³]	Temperature [°C]	Coliform bacteria [CFU/100 cm ³]	Faecal coliforms [CFU/100 cm ³]	Temperature [°C]
0	1.3*10 ⁴	0.7*10 ⁴	19	1.3*10 ⁴	0.7*10 ⁴	19	1.3*10 ⁴	0.7*10 ⁴	19
10	9.5*10 ³	2.4*10 ³	23	6.2*10 ³	2.1*10 ³	21	7.0*10 ³	2.4*10 ³	22
15	7.0*10 ³	1.3*10 ³	25	2.4*10 ³	1.3*10 ³	22	2.4*10 ³	0.7*10 ³	25
20	2.4*10 ³	0.62*10 ³	28	2.4*10 ³	0.62*10 ³	24	2.1*10 ³	0.62*10 ³	28
25	1.3*10 ³	0.62*10 ³	30	0.7*10 ³	0.2*10 ³	27	0.62*10 ³	0.2*10 ³	31

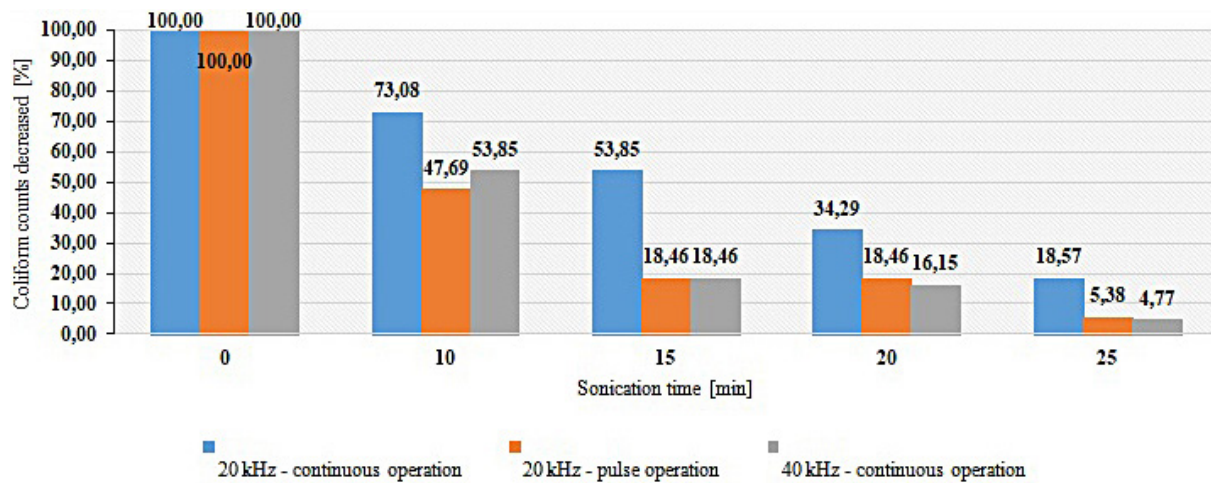


Figure 1. Percentage changes in the number of coliforms in the ultrasonically treated wastewater

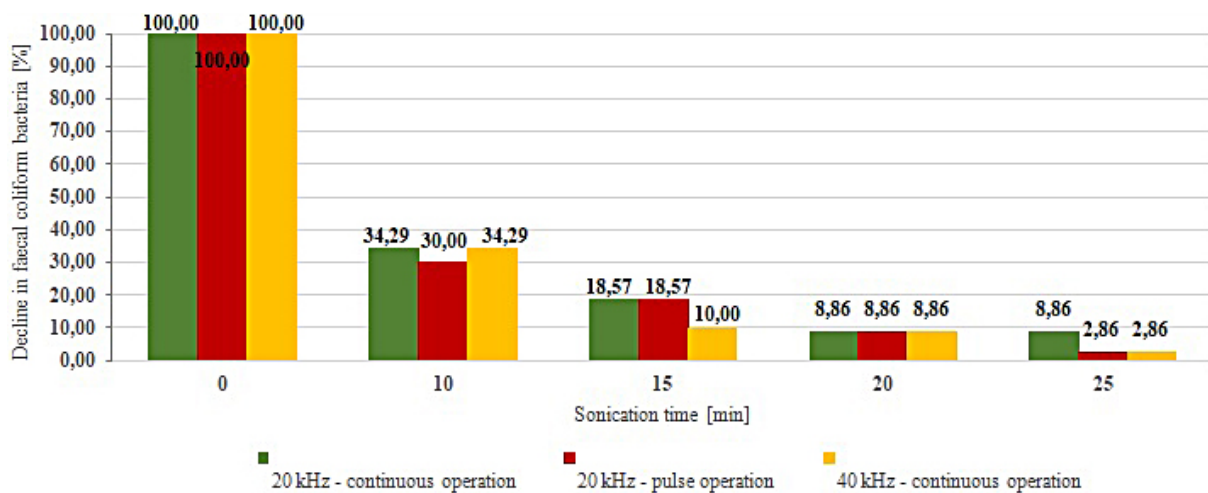


Figure 2. Percentage changes in the number of faecal coliform bacteria in the ultrasonically treated wastewater

kHz in a continuous mode resulted in a progressive decrease in the number of tested microorganisms. Ten minutes of sonication reduced the number of bacteria by nearly 27% in the case of coliform bacteria and by over 65% in the case of fecal coliforms. The maximum time of sonication (25 minutes) tested resulted in a reduction in the number of tested microorganisms by more than 81% (coliform bacteria) and 91% (fecal coliform bacteria), respectively. The temperature rose by 11 °C during the entire process.

After 10 minutes of sonication with 20 kHz ultrasound in the pulsed mode of operation of the device, a decrease in the number of microorganisms by over 52% (coliform bacteria) and 70% (fecal coliform bacteria) was noted, with a simultaneous increase in temperature by 2 °C. Along with the prolongation of the ultrasound treatment process, the number of bacteria decreased, reaching an efficiency of over 90% after 25 minutes of the process for coliform bacteria and after 20 minutes for fecal coliforms. After 25 minutes of sonication, the temperature rose by 8 °C.

The effect of ultrasound with a frequency of 40 kHz resulted in the best disintegration effect in the case of coliform bacteria and an effect comparable to the 20 kHz ultrasound wave (pulsed mode) for fecal coliforms. After 15 minutes of the process, a decrease in the number of microorganisms was observed within 80-90%, and after 25 minutes, it exceeded 95%. The temperature during the process rose by 12 °C, reaching a maximum of 31 °C.

Due to the general slight increase in temperature during all tested variants of the sonication process, it can be assumed that it had no significant impact on the destruction of microorganisms.

A properly functioning wastewater treatment plant, apart from the required reduction in the concentration of suspended solids, organic and biogenic substances, also achieves a high level of indicator bacteria removal, even above 99%. However, due to the high initial number of microorganisms in the raw sewage, their amount in the outflow is still high [Bray et al., 2015], which translates into a growing interest in the issues of wastewater disinfection. This process is most often based on the use of classical physicochemical methods: chlorination, ozonation, or ultraviolet radiation. However, an alternative may be the use of ultrasound.

The literature is more and more interested in the issue of ultrasonic disintegration but mainly focuses on the use of ultrasound in the treatment of sewage sludge. It is easy to find

the papers describing the relationship of sludge sonication to an increase in biogas production or a reduction in the amount of digested sludge [Simonetti et al., 2014, Bragugia et al., 2015, Zawieja et al., 2019; Dauknys et al., 2020; Wolski, 2020]. However, the number of items focusing on ultrasonic disintegration of microorganisms in sewage or sewage sludge is relatively small. Similar to the present study, the results were obtained by Foladori et al. [2007], who demonstrated high efficiency in removing the bacteria from the *E. coli* species by sonication with ultrasound at a frequency of 20 kHz. Butarewicz also obtained a reduction in the number of *Escherichia coli* in treated sewage [2016]. Rusin and Machnicka [2011] proved the effect of ultrasound with a frequency of 25 and 40 kHz on the reduction of the number of bacteria belonging to the *Enterobacteriaceae* family, while Pathki et al. [2014] drew the attention to the almost complete disappearance of coliform bacteria in the sludge subjected to sonication with ultrasound at a frequency of 25 kHz. The influence of ultrasound on the disintegration of filamentous bacteria present in the activated sludge has also been proven [Butarewicz et al., 2017; Hawrylik 2018].

The results obtained in this study prove the apparent effectiveness of ultrasound in destroying the indicator bacteria present in the treated wastewater. Ultrasonic disintegration can be successfully used in wastewater disinfection processes in municipal wastewater treatment plants.

CONCLUSIONS

1. The conducted experiment showed the effective operation of ultrasound with a frequency of 20 and 40 kHz in the disintegration process of coliform bacteria and fecal coliforms present in treated municipal wastewater.
2. In all tested options of the ultrasonic cleaner operation, a high average degree of reduction of the number of microorganisms was demonstrated, amounting to over 81%, 94%, and 95%, respectively, for coliform bacteria and nearly 92% and 97% for fecal coliforms.
3. The best results in terms of destroying coliform bacteria and fecal coliforms are achieved by ultrasound with a frequency of 40 kHz.
4. Low-frequency ultrasound can be successfully used in the disinfection of municipal wastewater.

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