Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2023.29983

312 (2023) 55–63
November

Analysis of the impact of ultrasonic disintegration on changes in the physico-chemical and biological properties of municipal wastewater

Eliza Hawrylik[a,*], Ewa Szatyłowicz[b]

[a]*Department of Chemistry, Biology and Biotechnology, Faculty of Civil Engineering and Environmental Sciences, Bialystok University of Technology, Wiejska 45E, 15-351 Bialystok, Poland, email: e.hawrylik@pb.edu.pl*
[b]*Department of Technology in Environmental Engineering, Faculty of Civil Engineering and Environmental Sciences, Bialystok University of Technology, Wiejska 45E, 15-351 Bialystok, Poland, email: e.szatylowicz@pb.edu.pl*

Received 10 October 2022; Accepted 2 September 2023

ABSTRACT

Raw municipal wastewater is a reservoir of many physical, chemical and microbiological pollutants. The treatment process carried out in municipal wastewater treatment plants focuses on giving the wastewater physical and chemical properties similar to those of surface waters. It does not take into account the microbiological properties of wastewater. The aim of the conducted research was therefore to determine the impact of ultrasonic disintegration on changes in the physico-chemical and microbiological properties of municipal wastewater. Raw wastewater samples were subjected to ultrasound with a frequency of 26 kHz, and the results of the work allowed to determine the effect of sonication on changes in physico-chemical (pH, biochemical oxygen demand, chemical oxygen demand, total nitrogen, total phosphorus and total suspensions) and microbiological properties (total number of bacteria, coliform, streptococcal faeces, *Clostridium* bacteria) of raw municipal wastewater. An additional aspect was to carry out biotests of raw wastewater samples to determine their acute toxicity using the Microtox system and *Aliivibrio fischeri* luminescent bacteria. The obtained results allowed to conclude that the impact of ultrasounds with a frequency of 26 kHz causes a significant reduction in the number of microorganisms present in raw wastewater – by nearly 93% in relation to the total number of bacteria after 10 min of the sonication process and about 73% of bacteria of the *Clostridium* genus, 98% of coliform, as well as complete reduction of fecal streptococci after 25 min of sonication. In addition, it has been proven that ultrasonic disintegration does not significantly change the physico-chemical properties of raw municipal wastewater or the acute toxicity of the tested wastewater.

Keywords: Ultrasonic disintegration; Municipal wastewater; Physico-chemical properties; Microorganisms; Toxicity

1. Introduction

According to the definition of Polish legislation, wastewater is water introduced into water reservoirs, watercourses or the ground, used for domestic or economic purposes, liquid animal excrement or leachate from landfills [1]. Various types of wastewater go to the treatment plant, that is, domestic, industrial, municipal wastewater, rainwater or infiltration water, and their composition depends on many factors such as the place of their generation or the type of sewage system. Wastewater entering typical municipal treatment plants is a mixture of water used in households, industrial plants and municipal services, as well as water entering the sewage system as a result of leakage from the network [2–4]. The main groups of sewage pollution are: physical (suspensions, color and turbidity), chemical (organic and inorganic substances) and biological (pathogenic bacteria, viruses, parasitic protozoa and other parasites).

* Corresponding author.

Presented at the 15th Scientific Conference on Micropollutants in the Human Environment, 14–16 September 2022, Częstochowa, Poland

1944-3994/1944-3986

The purification process itself is aimed at giving the wastewater physico-chemical properties similar to those of surface water, without taking into account their microbiological properties.

The largest share of raw municipal wastewater is domestic wastewater, the composition of which is determined by the population of the human gut microbiome. According to the literature data, the biocenosis of the human digestive system consists mainly of bacteria from the genera *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria* (8%) and *Actinobacteria* (3%), which dominate the qualitative composition of raw wastewater. The number of pathogenic bacteria detected in wastewater is a minority of the total number of bacteria [5,6]. The occurrence of pathogenic bacteria in raw municipal wastewater is characteristic for individual regions. Sanitary hazard is associated with the possibility of microorganisms contained in sewage entering other environments: soil or waters of the receiver [3]. Unfortunately, despite the rich reservoir of pathogenic microorganisms that pose a threat to human health and life, the microbiological quality of wastewater leaving wastewater treatment plants is not monitored. Wastewater treatment in a classic mechanical–biological treatment plant is associated with the removal of microorganisms in three processes: sorption on suspension particles, feeding by higher organisms, and death caused by competition for food or unfavorable environmental conditions. However, due to the very high initial microorganisms, despite their high level of removal, they are ubiquitous in wastewater leaving treatment plants [7,8]. The problem of the general toxicity of wastewater, caused by the increase in the amount and variety of chemical compounds and their mixtures released into the environment, also seems to be important.

The technique of using ultrasound in wastewater treatment and sewage sludge processing is a method that is gaining more and more popularity. Its primary purpose is disinfection, the effectiveness of which depends on the time and frequency of ultrasound action and their intensity, as well as on the individual characteristics of the species of microorganisms that are deactivated. The ultrasound process is indicated as an alternative in the treatment of wastewater from pharmaceutical residues and their metabolites, and it shows high efficiency of disintegration in relation to many species of bacteria [9–11].

Therefore, it seemed advisable to conduct research on the impact of ultrasonic disintegration on changes in the physico-chemical and microbiological properties of municipal wastewater. Raw wastewater samples were subjected to ultrasound at a frequency of 26 kHz. The results of the work made it possible to determine the effect of sonication on changes in pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen, total phosphorus and total suspended solids in raw municipal wastewater. Moreover, the effect of ultrasonic disintegration in relation to specific groups of microorganisms was determined. Additionally, biotests were carried out on raw wastewater samples before and after the disintegration process in order to determine their acute toxicity using the Microtox system.

The undertaken research topic is important from the point of view of contemporary problems faced by sewage treatment plants. Species diversity of pathogenic organisms present in wastewater and sewage sludge illustrates the undeniable need to introduce a customary disinfection process. The ideal method seems to be the use of ultrasonic waves, which, apart from the disinfecting effect, also have other advantages, such as: no formation of by-products and low requirements as to the quality of the medium subjected to the process. The test results obtained through the use of real samples of raw municipal sewage can be a recommendation for the use of ultrasonic technology in everyday operation of municipal sewage treatment plants.

2. Material and methods

The research was carried out in laboratory conditions. Each time, the raw wastewater intended for analysis was collected from the Municipal Sewage Treatment Plant in Bialystok. According to the data of the sewage treatment plant administrator (Wodociągi Białostockie Sp. z o.o.), the research facility has a capacity of 100,000 m^3/d, and the treatment process is based on the use of a biological method using activated sludge. The experiment was performed by treating the raw wastewater with technique of ultrasound at a frequency of 26 kHz using an ultrasonic homogenizer from Hielscher ultrasonics GmbH (Fig. 1).

Each time the process was carried out at four intervals of ultrasonic wave exposure: 10, 15, 20 and 25 min. Three test series were performed. Fig. 2 shows a diagram of the physico-chemical and microbiological analysis performed.

Each time, 2 L of raw wastewater were subjected to approach of ultrasound, maintaining the same process

Fig. 1. Ultrasonic homogenizer by Hielscher Ultrasonics GmbH with the ultrasound frequency of 26 kHz (Author's photo).

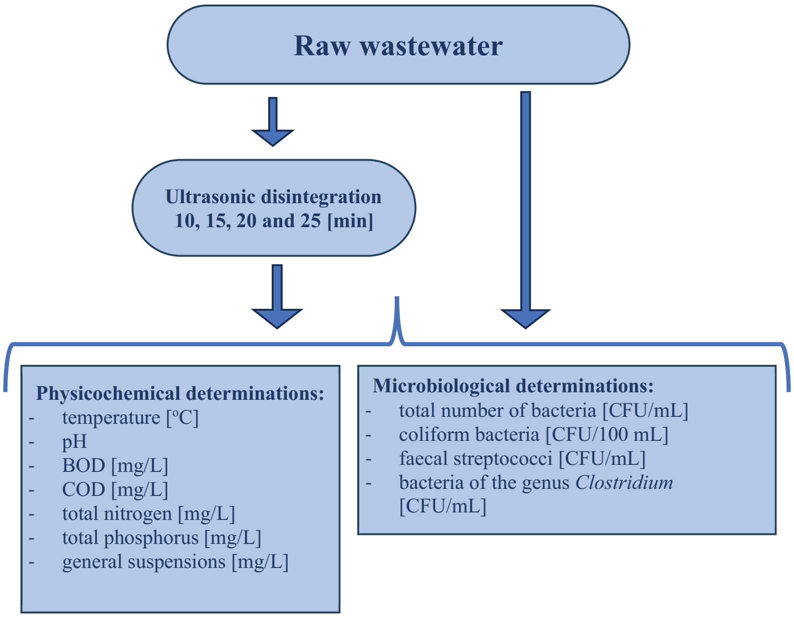

Fig. 2. Diagram of physico-chemical and microbiological analysis performed.

conditions, that is, the maximum amplitude and power of the device. Wastewater samples for analysis were collected before and after the sonication process performed in an appropriate time interval. Physico-chemical and microbiological analyses were performed in accordance with the current methodology.

Physico-chemical determinations were made in the laboratories of the Department of Technology in Environmental Engineering at the Faculty of Civil Engineering and Environmental Sciences of the Bialystok University of Technology.

The collected samples of raw wastewater and wastewater after ultrasonic processes were subject to determinations of the following items in accordance with the current methodology [12–15]. Total phosphorus was determined spectrophotometrically using the ammonium molybdate method according to PN-EN ISO 6878:2006 [16]. Total nitrogen was determined using the spectrophotometric method with the Merck Pharo 300 UV-VIS Spectrophotometer, number 114763. This method is analogous to the DIN 38405-9 method and the PN-EN ISO 11905-1 method [17]. The total suspension was filtered through a quantitative filter, previously weighed, dried and re-weighed, in accordance with the PN-EN 872:2007 standard [18]. pH was measured using the potentiometric method based on the HQD probe with INTELLICAL sensors according to the PN-EN ISO 10523:2012 standard [19]. COD was measured by dichromate spectrophotometry based on a Hach thermoreactor and a Merck Pharo 300 spectrophotometer. Merck cuvette tests according to PN-ISO 15705:2005 were used for the determination [20]. Biochemical oxygen demand (BOD) was determined with the 5-day manometric method based on the OxiTop Standard System, WTW TS 606/2 thermostatic cabinet, in accordance with the PN-EN 1899-2:2002 standard [21].

All microbiological analysis were performed in the microbiological laboratory of the Department of Chemistry, Biology and Biotechnology at the Faculty of Civil Engineering and Environmental Sciences of the Bialystok University of Technology.

The total number of bacteria was determined on the basis of the surface plating method in accordance with the standard PN-EN ISO 6222:2004 [22] and PN-EN ISO 8199:2019-01 [23]. The research used microbiological substrate enriched agar from BTL Sp. z o.o. The total number of bacteria (CFU – colony forming unit) was determined in samples previously made with serial dilutions ranging from 10^{-1} to 10^{-6}. Samples were incubated at 26°C for 96 h. Grown colonies were counted using a JUL colony counter with Flash&Go software, and then converted to the total number of bacteria according to the formula:

$$L = \frac{C}{\left(N_1 + 0.1N_2\right)} \times d \times a \qquad (1)$$

where L – total number of bacteria (CFU/mL), C – sum of colonies on all plates selected for counting, N_1 – number of platelets from the first calculated dilution, N_2 – number of platelets from the second calculated dilution, d – the dilution factor that corresponds to the lowest calculated dilution, a – the seed quantity factor when seeding 0.1 mL $a = 10$.

Determination of the most probable number (NPL) of coliforms was performed using the fermentation-probe method in accordance with PN-EN ISO 9308-2:2014-06 [24] and the 1681 procedure recommended by the EPA [25], with a 5-probe system limited to a 2-probe system. The research used the microbiological medium Eijkman Lactose Medium by BIOCORP Polska Sp. z o.o. The most probable number of coliform bacteria was determined in samples that had previously been serial diluted in the range of 10^{-1}–10^{-6}. The samples were incubated at 37°C (coliform bacteria) for 48 h. Based on the results obtained, the NPL index was read from the tables provided in the standards.

Determination of the number of fecal streptococci (bacteria of the genus *Enterococcus*) was carried out based on the membrane filtration method in accordance with PN-EN ISO 7899-2:2004 [26]. Slanetz and Bartley medium made in accordance with the procedure contained in the above-mentioned standard was used in the study. The number of fecal streptococci in raw wastewater was determined in diluted samples in the range of 10^{-1}–10^{-3}. The samples were incubated at 37°C for 48 h. The grown colonies with a pinkish-bordeaux color were counted and converted to the number of fecal streptococci.

Determination of the number of spore-forming, sulfite-reducing bacteria (bacteria of the genus *Clostridium*) was performed based on the membrane filtration method in accordance with PN-EN 26461-2:2001 [27]. The study used a medium made in-house in accordance with the procedure in the aforementioned standard. The number of bacteria of the genus *Clostridium* was determined in samples diluted from 10^{-1} to 10^{-3}. The samples were incubated at 37°C for 24 h. The grown black-colored colonies were counted and converted to the number of sulfite-reducing spore-forming bacteria.

An additional research aspect that was undertaken in this study was to determine the toxicity of the tested wastewater using the luminescent bacteria *Aliivibrio fischeri*. The study used a Microtox Model 500 luminometer (Strategic Diagnostics Inc., Newark, USA) along with Modern Water's reagents dedicated to this kit, and, as a bioindicator, *Aliivibrio fischeri* bacteria designated by the manufacturer as *Vibrio fischeri* NRRL B-11177. The tests were carried out in accordance with PN-EN ISO 11348-3:2008 [28]. A characteristic feature of the microorganisms used is the ability to luminescence, that is, to produce light under the influence of enzymatic reactions, which decreases in the case of harmful effects of toxins in proportion to their concentration [29]. The system used in the study also consisted of a PC and software for data collection and processing (Microtox Omni 4.1.). In accordance with the manufacturer's recommendations, a liquid-phase test was performed on the samples tested. The result of the test was the half maximal effective concentration value expressed in % (EC_{50}), which was converted to units of acute toxicity. The obtained EC_{50} values after 5 and 15 min were converted to acute toxicity units (TU_a):

$$TU_a = \frac{1}{EC_{50}} \times 100 \tag{2}$$

where TU_a – acute toxicity units, EC_{50} – half maximal effective concentration.

The obtained results were analyzed in relation to three systems allowing to determine the toxicity class of the tested wastewater samples. According to the system proposed by Persoone [30], 5 classes of toxicity were distinguished:

- class 0: $TU_a = 0$ non-toxic sample;
- class 1: $0.4 < TU_a < 1$ no significant toxicity (low acute toxicity);
- class 2: $1 < TU_a < 10$ significant toxicity;
- class 3: $10 < TU_a < 100$ high acute toxicity;
- class 4: $TU_a > 100$ very high toxicity.

The second of the cited systems developed by Sawicki et al. [31] assumed the definition of 4 classes of sample toxicity:

- class 1: $TU_a < 10$ no significant toxic effect;
- class 2: $10 < TU_a < 25$ significant toxic effect – low-toxic sample;
- class 3: $25 < TU_a < 100$ significant toxic effect – toxic sample;
- class 4: $TU_a > 100$ significant toxic effect – highly toxic sample.

The last system used to evaluate the obtained results was the toxicity classification according to the EEC Directive [32] distinguishing the following toxicity classes:

- class 0: $TU_a = 0$ non-toxic sample;
- class 1: $TU_a < 1$ low-toxic sample;
- class 2: $1 < TU_a < 10$ toxic sample;
- class 3: $11 < TU_a < 100$ highly toxic sample;
- class 4: $TU_a > 100$ extremely toxic.

3. Results and discussion

The application of ultrasonic disintegration to wastewater and sludge treatment processes is the subject of many scientific papers. The literature overwhelmingly focuses on sewage sludge. Among the issues addressed are the use of ultrasound-treated sludge in the digestion process resulting in increased biogas production and reduced digested sludge [33,34], or the use of method of ultrasound for sludge conditioning [35,36]. There are also reports directly related to the use of ultrasonic waves in wastewater treatment processes. Maleki et al. and Mahvi et al. [37–39] investigated the possibility of using technique of ultrasound in the treatment of phenolic wastewater. Other scientific works prove the high disintegration efficiency of ultrasonic waves against roundworms and nematodes, as well as against fungi and *Cryptosporidium parvum* cysts present in sewage [40–42]. There are also articles focusing on changes in the amount of organic matter in wastewater after the use of the sonication process [43].

The research carried out in the course of this study was aimed at determining the effect of ultrasound with a frequency of 26 kHz on the change of physico-chemical and microbiological properties of raw municipal wastewater, as well as the assessment of the potential ecotoxicity of this wastewater. Table 1 shows the changes in the physico-chemical parameters of the tested wastewater as a result of the ultrasonic disintegration process depending on its duration.

Before and after the ultrasonic disintegration process, changes in the following wastewater parameters were determined at four time intervals (10, 15, 20 and 25 min): temperature, pH, BOD, COD, total nitrogen, total phosphorus and total suspensions. It was found that increasing the duration of process of ultrasound causes an increase in values for each of the parameters tested except pH. The gradual increase in temperature recorded during the experiment (12.3°C after 25 min of the process) is a natural phenomenon, related to the thermal effect of the interacting ultrasound wave- the absorption of the emitted radiation causes heat to be emitted and the wastewater to heat up. The amount of organic matter in the tested material, which consists of BOD and COD determinations, increased slightly during the whole process (in the case of BOD, an increase of 12.3% was determined in relation to the control sample, while for COD it was nearly 10.0%), which is consistent with literature reports that indicate better removal of organic matter from wastewater at ultrasound frequencies of 130 kHz compared to lower values [43,44]. The action of ultrasonic waves resulting in the formation of a cavitation phenomenon contributed to an increase in the amount of total suspended solids present in the raw wastewater with respect to the control sample (16.7% compared to the initial value). There was also an increase in the content of biogenic compounds, that is, nitrogen and total phosphorus, which was not significant (from 83.2 mg/L before the process to 88.0 after 25 min of disintegration in the case of total nitrogen, and from 8.99 to 11.24 mg/L for total phosphorus). In terms of changes in pH values, a slight decrease in values was observed (from 7.68 to 7.60 after 25 min of sonication), so it can be concluded that approach of ultrasound in this configuration does not significantly affect the reaction of wastewater, which both before and after the process oscillates at the limit of neutral reaction.

Table 2 and Fig. 3 show the effect of ultrasonic disintegration on changes in the microbiological properties of municipal raw wastewater.

The process of low-frequency ultrasound shows a disintegrating effect against the microorganisms included in the analysis. The destruction of microorganisms is visible after 10 min of ultrasonic waves, and also increases its effectiveness along with the extension of the duration of the impact. Ultrasound with a wavelength of 26 kHz shows the lowest effectiveness against bacteria of the genus *Clostridium* (decrease in the number of these microorganisms by 10.71% after 10 min of the sonication process, and 72.40% after 25 min), which is probably related to the species characteristics of these microorganisms and their ability to create spore forms. For coliform bacteria, the removal efficiency is higher, ranging from 46.15% after 10 min of operation, 81.54% after 15 min, through 95.38% after 20 and 98.23% after 25 min of operation. More than 50% of the number of fecal streptococci in relation to the control sample is disintegrated after 10 min of sonication, 25 min of ultrasonic waves gives 100% efficiency in their removal. The disintegration efficiency of the total number of bacteria is high in each time interval – 93.77% after 10 min, over 99% after just 15 min of sonication. The results obtained in the course of

Table 1
Impact of method of ultrasounds on changes in the physico-chemical properties of raw municipal wastewater

Ultrasound – 26 kHz Operation time (min)	Temperature (°C)	pH[a]	Biochemical oxygen demand (mg/L)[b]	Chemical oxygen demand (mg/L)[c]	Total nitrogen (mg/L)[d]	Total phosphorus (mg/L)[e]	General suspension (mg/L)[f]
Before the trial – 0 min	20.0 ± 0.4	7.68 ± 0.08	612.2 ± 6.7	1469.0 ± 25.7	83.2 ± 0.9	8.99 ± 0.32	839.5 ± 11.5
10 min	24.2 ± 0.6	7.64 ± 0.04	654.4 ± 4.8	1,575.1 ± 32.4	84.2 ± 0.8	9.05 ± 0.29	854.4 ± 12.2
15 min	26.1 ± 0.5	7.65 ± 0.09	662.3 ± 5.7	1,598.2 ± 33.4	86.4 ± 1.0	9.14 ± 0.27	926.7 ± 10.9
20 min	29.0 ± 0.8	7.63 ± 0.05	671.5 ± 7.0	1,605.6 ± 36.8	86.9 ± 0.9	10.06 ± 0.33	996.8 ± 13.0
25 min	32.3 ± 0.7	7.60 ± 0.07	686.3 ± 7.9	1,615.8 ± 40.4	88.0 ± 1.1	11.24 ± 0.40	1004.6 ± 14.4

[a]according to the PN-EN ISO 10523:2012 standard;
[b]according to the PN-EN 1899-2:2002 standard;
[c]according to the PN-ISO 15705:2005 standard;
[d]according to the PN-EN ISO 11905-1 standard;
[e]according to the PN-EN ISO 6878:2006 standard;
[f]according to the PN-EN 872:2007 standard.

Table 2
Impact of technique of ultrasonic disintegration on changes in microbiological properties of raw municipal wastewater

Ultrasound – 26 kHz Operation time (min)	Total number of bacteria (CFU/mL)[a]	Coliform bacteria (CFU/100 mL)[b]	Faecal streptococci (CFU/mL)[c]	Bacteria of the genus *Clostridium* (CFU/mL)[d]
Before the trial – 0 min	2.97×10^6	1.3×10^4	2.24×10^2	3.08×10^2
10 min	1.85×10^5	0.7×10^4	1.10×10^2	2.75×10^2
15 min	1.20×10^4	2.4×10^3	0.67×10^2	2.10×10^2
20 min	2.41×10^3	0.6×10^3	0.32×10^2	1.52×10^2
25 min	1.83×10^3	2.3×10^2	0	0.85×10^2

[a]according to the PN-EN ISO 6222:2004 and PN-EN ISO 8199:2019-01 standards;
[b]according to the PN-EN ISO 9308-2:2014-06 standard and the 1681 procedure recommended by the EPA;
[c]according to the PN-EN ISO 7899-2:2004 standard;
[d]according to the PN-EN 26461-2:2001 standard.

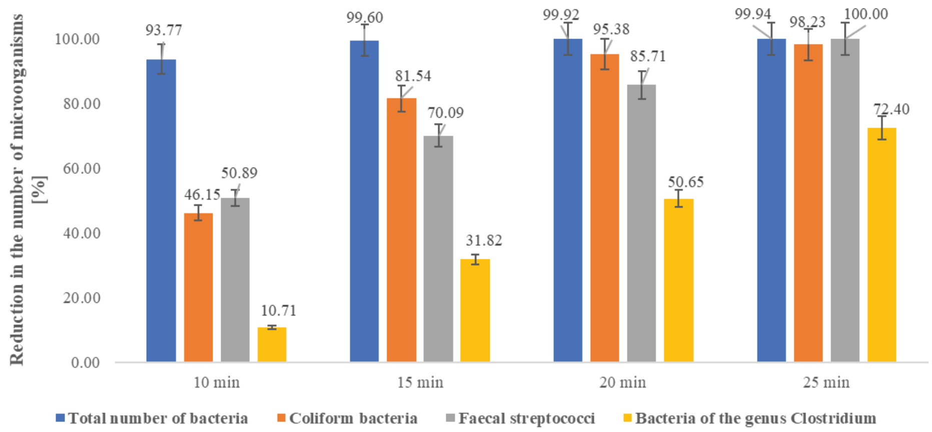

Fig. 3. Percentage reduction of the number of microorganisms in relation to the control sample under the influence of ultrasound technique.

this study are consistent with the reports of scientists. The first references to the use of method of ultrasound for the destruction of microorganisms appeared in the 1980s [45]. At the turn of the 20th century, McClements [46] indicated that the inactivation of microorganisms using technique of ultrasound was more effective when combined with thermal disinfection and the use of chlorine. Currently, the use of ultrasonic waves is considered one of the effective methods of disinfection of wastewater and sewage sludge [47,48]. Low-frequency ultrasound process successfully destroys various types of microorganisms. The literature indicates the effective elimination of bacteria belonging to the *Enterobacteriaceae* family and pathogenic microorganisms of the *Staphylococcus* genus using ultrasound at 25 and 40 kHz, and the possibility of using ultrasound at 22–40 kHz for such species as *Enterococcus faecalis* and *Clostridium perfringens* [49–51]. Data on the disintegration of coliform bacteria are also confirmed – the literature shows high efficiency of destroying *Escherichia coli* bacteria using method of low-frequency ultrasound (in the range of 20–40 kHz) [9,11,52–55].

Table 3 shows the toxicity assessment of the tested wastewater samples evaluated using the Microtox kit.

An additional aspect that the authors of this study wanted to draw attention to is the acute toxicity of raw municipal wastewater and its possible changes under the influence of ultrasonic disintegration. Acute toxicity is considered to be the adverse effects occurring within 24 h of administering harmful substances [56]. The use of the Microtox kit based on biotoxicity tests using living organisms is a widely used technique due to the speed of the tests and the relatively low technical complexity and therefore relatively low cost [57]. The Microtox kit used in the experiments uses the *Aliivibrio fischeri* luminescent bacteria capable of producing light under the influence of enzymatic reactions taking place in the cells. Although, as reported by Widder [29] and Włodarczyk et al. [58] these microorganisms are common in marine waters, their abundance is not high. Therefore, the observation of their natural luminescence is possible in marine organisms with which they live in symbiosis, and their concentration oscillates at the limit of 1,000 cells/mL [59,60]. The biological test based on the inhibition of bioluminescence of bacteria *Aliivibrio fischeri* is one of the most widely used bacteria l tests to assess the toxicity of wastewater [61]. The experiments carried out made it possible to classify raw municipal wastewater as toxic. The analyzed wastewater both before and after the disintegration process qualified to the 3rd class of toxicity according to the Persoone scale and the EC Directive (highly toxic sample), while the scale according to Sawicki treats them somewhat more leniently, ranking them as samples with significant toxic effect, characterized by low toxicity. Based on the results obtained, it can be concluded that raw wastewater contains substances in its composition that cause toxic effects on microorganisms. The use of bioindication methods does not allow for the identification of the types of substances responsible for toxicity, but it indicates the biological effects of chemical substances. [62]. The luminescent bacteria *Aliivibrio fischeri* used in the experiments represented the level of destruents in the trophic chain, however, according to the literature, this can be considered a reliable result, comparable to biotests carried out on organisms with higher trophic levels, for example, *Daphnia magna* [63–66].

4. Practical implications of this study

The increase in the amount of generated wastewater and sewage sludge translates into increasing health risks. The pandemic caused by the spread of the SARS-CoV-2 virus has revealed a clear need to develop and implement methods to prevent the spread of pathogens that would reduce the epidemiological risk of microorganisms entering the environment with wastewater and sewage sludge. The introduction of the usual disinfection process seems to be a solution to the problem. It is not obvious, however, to select the appropriate method, which, in addition to high destructive effectiveness in relation to microorganisms, should additionally be characterized by the lack of formation of toxic by-products and the smallest number of contraindications from the medium subjected to the process. According to literature data [9,11,50,67], the appropriate method seems to be the use of ultrasonic waves, which is directly related to the selection of the scope of research carried out in the article.

Table 3
Acute toxicity of raw municipal wastewater

	EC_{50} after 5 min	TU_a after 5 min	Toxicity according Persoone [30]	Toxicity according Sawicki et al. [31]	Toxicity according EC Directive [32]	EC_{50} after 15 min	TU_a after 15 min	Toxicity according Persoone [30]	Toxicity according Sawicki et al. [31]	Toxicity according EC Directive [32]
Raw municipal wastewater	7.264	13.77	Class 3	Class 2	Class 3	5.147	19.43	Class 3	Class 2	Class 3
Raw municipal wastewater after 25 min of ultrasound treatment	7.995	12.51	Class 3	Class 2	Class 3	5.364	18.64	Class 3	Class 2	Class 3

The results obtained in the course of this work confirm the high effectiveness of low-frequency ultrasonic waves in relation to the disintegration of microorganisms present in raw wastewater coming from municipal sewage treatment plants. This, therefore, sets the initial direction for conducting similar research in relation to other types of media generated in sewage treatment plants (treated wastewater and sewage sludge). The results obtained with the use of real wastewater samples also allow us to believe that there is a real possibility of introducing ultrasonic disinfection in specific facilities after taking into account all technological parameters for specific sewage treatment plants. The fact of examining the influence of ultrasound on changes in the physico-chemical parameters of wastewater subjected to the process is also significant. It has been shown that ultrasonic disintegration does not significantly change the physico-chemical properties of raw municipal sewage. Carrying out the process therefore does not interfere with the usual results achieved during the wastewater treatment process.

The discussed issue certainly requires broader research, taking into account both the changes in the frequency range of the ultrasonic waves, the time of their operation, and the types of wastewater subjected to the process.

5. Conclusions

- Ultrasonic disintegration does not significantly change the physico-chemical properties of raw municipal wastewater.
- Technique of low frequency ultrasound (26 kHz) successfully destroys various types of microorganisms – a 25-min process is able to almost completely reduce coliform bacteria (98.23% reduction) and fecal streptococci (100.00% reduction). The number of bacteria of the genus *Clostridium* is also slightly reduced (72.40% reduction).
- Even the 10 min process of ultrasonic disintegration reduces the total number of bacteria by over 93% (93.74%), achieving nearly 100% efficiency (99.94%) with the maximum tested time interval of 25 min.
- Raw municipal wastewater is of significant acute toxicity. Ultrasonic disintegration did not change the acute toxicity of the tested wastewater.

Acknowledgements

The research was carried out as the part of the work No. WZ/WB-IIŚ/2/2021 and No. W/WB-IIŚ/4/2022, conducted at the Bialystok University of Technology and financed from the research subsidy provided by the minister responsible for science.

References

[1] Announcement of the Marshal of the Sejm of the Republic of Poland of 8 October 2020 on the Publication of the Uniform Text of the Act on Collective Water Supply and Collective Sewage Disposal [Journal of Laws 2020, Item 2028].
[2] Z. Dymaczewski, Sewage Treatment Plant Operator's Guide, Polish Association of Sanitary Engineers and Technicians, Poznan, 2011.
[3] K. Olańczuk-Neyman, B. Quant, Wastewater Disinfection, Publisher "Seidel-Przywecki" Sp. z o.o., Warsaw, 2015.

[4] A. Bolt, E. Burszta-Adamiak, K. Gudelis-Taraszkiewicz, Z. Suligowski, A. Tuszyńska, Sewers. Design, Execution, Operation, Publisher "Seidel-Przywecki" Sp. z o.o., Warsaw, 2015.

[5] D.N. Frank, A.L. St. Amand, R.A. Feldman, E.C. Boedeker, N. Harpaz, N.R. Pace, Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases, Proc. Natl. Acad. Sci. U.S.A., 104 (2007) 13780–13785.

[6] L. Ye, T. Zang, Bacterial communities in different sections of a municipal wastewater treatment plant revealed by 16S rDNA 454 pyrosequencing, Appl. Microbiol. Biotechnol., 97 (2013) 2681–2690.

[7] G. Tchobanoglous, F.L. Burton, H.D. Stensel, Wastewater Engineering: Treatment, Disposal and Reuse, 4th ed., McGraw-Hill Inc., New York, 2003.

[8] Q. Wu, W.-T. Liu, Determination of virus abundance, diversity and distribution in a municipal wastewater treatment plant, Water Res., 41 (2009) 1001–1012.

[9] P. Foladori, B. Laura, A. Gianni, Z. Giulano, Effects of sonication on bacteria viability in wastewater treatment plants evaluated by flow cytometry-fecal indicators, wastewater and activated sludge, Water Res., 41 (2007) 235–243.

[10] Y. Guo, F. Song, Enhancement Efficacy of Ultraviolet and Ultrasonic on Wastewater Disinfection, 2010 4th International Conference on Bioinformatics and Biomedical Engineering, IEEE, Chengdu, China, 2010, pp. 1–4.

[11] A. Butarewicz, Application of Ultrasounds for the Disintegration of Microorganisms in Sewage and Sewage Sludge, Publishing House of the Białystok University of Technology, Bialystok, 2016.

[12] K. Ignatowicz, Metals content chosen for environmental component monitoring in graveyards, Fresenius Environ. Bull., 20 (2011) 270–273.

[13] Ch. Manjushree, M.G. Mostafa, T.K. Biswas, A. Mandal, A. Kumar Saha, Characterization of the effluents from leather processing industries, Environ. Processes, 2 (2015) 173–187.

[14] B. Łozowicka, P. Kaczyński, J. Szabunko, K. Ignatowicz, D. Warentowicz, J. Łozowicki, New rapid analysis of two classes of pesticides in food wastewater by quechers-liquid chromatography/mass spectrometry, J. Ecol. Eng., 17 (2016) 97–10.

[15] M. Dziadel, K. Ignatowicz, Assessment of the quality of wastewater generated during production at a tannery plant, J. Ecol. Eng., 23 (2022) 109–115.

[16] PN-EN ISO 6878:2006, Polish Committee for Standardization, Water Quality. Determination of Phosphorus. Spectrometric Method with Ammonium Molybdate, 2006.

[17] PN-EN ISO 11905-1:2001, Polish Committee for Standardization. Water Quality. Nitrogen Determination, Part 1: Peroxodisulphate Mineralization Method, 2001.

[18] PN-EN 872:2007, Polish Committee for Standardization. Water Quality. Determination of Suspended Solids, Method Using Filtration Through Glass Fiber Filters, 2007.

[19] PN-EN ISO 10523:2012, Polish Committee for Standardization, Water Quality. Determination of pH, 2012.

[20] PN-ISO 15705:2005, Polish Committee for Standardization, Water Quality. Determination of the Chemical Oxygen Demand Index (SP-COD). Minimized Method with the Use of Sealed Tubes, 2005.

[21] PN-EN 1899-2:2002, Polish Committee for Standardization, Water Quality. Determination of Biochemical Oxygen Demand After *n* Days (BOD_n), Part 2: Method for Undiluted Samples, 2002.

[22] PN-EN ISO 6222:2004, Polish Committee for Standardization, Water Quality. Enumeration of Growthable Microrganisms. Determination of the Total Number of Colonies by Plating on Nutrient Agar, 2004.

[23] PN-EN ISO 8199:2019-01, Polish Committee for Standardization, Water Quality. General Requirements and Guidelines for Microbiological Testing by Culture Method, 2019.

[24] PN-EN ISO 9308-2:2014-06, Polish Committee for Standardization, Water Quality. Enumeration of *Escherichia coli* and Coliform Bacteria. Part 2: Most Probable Number Method, 2014.

[25] U.S. EPA, Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013, Revised Edition, U.S. Environmental Protection Agency, Washington, D.C., 2003.

[26] PN-EN ISO 7899-2:2004, Polish Committee for Standardization, Water Quality. Detection and Quantification of Faecal Enterococci. Part 2: Membrane Filtration Method, 2004.

[27] PN-EN 26461-2:2001, Polish Committee for Standardization, Water Quality. Detection and Quantification of Spores of Sulphite Reducing Anaerobes (Clostridia). Part 2: Membrane Filtration Method, 2001.

[28] PN-EN ISO 11348-3:2008, Polish Committee for Standardization, Water Quality. Determination of the Inhibitory Effect of Water Samples on Light Emission by *Vibrio fischeri* (Study on Luminescent Bacteria). Part 3: Method Using Lyophilized Bacteria, 2008.

[29] E.A. Widder, Bioluminescence in the ocean: origins of biological, chemical, and ecological diversity, Science, 328 (2010) 704–708.

[30] I. Mantis, D. Voutsa, C. Samara, Assessment of the environmental hazard from municipal and industrial wastewater treatment sludge by employing chemical and biological methods, Ecotoxicol. Environ. Saf., 62 (2005) 397–407.

[31] J. Sawicki, G. Nałęcz-Jawecki, J. Mankiewicz-Boczek, K. Izydorczyk, B. Sumorok, A. Drobniewska, M. Kaza, Comprehensive Ecotoxicological Analysis of Surface Waters, Department of Environmental Biology, Medical University of Warsaw, Warsaw, 2007.

[32] Directive EWG - ACE 89/BE 2/D3 Finale Report Commission EC.

[33] M. Simonetti, G. Rossi, V. Cabbai, D. Goi, Tests on the effect of ultrasonic treatment on two different activated sludge waste, Environ. Prot. Eng., 40 (2014) 23–34.

[34] C.M. Braguglia, M.C. Gagliano, A. Gallipoli, G. Mannini, The impact of sludge pretreatment for sludge anaerobic digestion: effect of floc structure and microbial population, Bioresour. Technol., 110 (2015) 43–49.

[35] X. Feng, J.C. Deng, Q. Yu, H.L. Li, Physical and chemical characteristics of waste activated sludge treated ultrasonically, Chem. Eng. Process. Process Intensif., 48 (2009) 187–194.

[36] P. Wolski, L. Wolny, I. Zawieja, Ultrasonic processors and drainage of sewage sludge, Rocz. Ochr. Sr., 17 (2015) 450–460.

[37] A. Maleki, A.H. Mahvi, F. Vaezi, R. Nabizadeh, Ultrasonic degradation of phenol and determination of the oxidation by products toxicity, Iran J. Environ. Health Sci. Eng., 2 (2005) 201–206.

[38] A. Maleki, A.H. Mahvi, M. Alimohamadi, A. Ghasri, Advanced oxidation of phenol by ultraviolet irradiation in aqueous system, Pak. J. Biol. Sci., 9 (2006) 2338–2341.

[39] A.H. Mahvi, A. Maleki, M. Alimohamadi, A. Ghasri, Photooxidation of phenol in aqueous solution: toxicity of intermediates, Korean J. Chem. Eng., 24 (2007) 79–82.

[40] A.H. Mahvi, M.H. Dehghani, E.B. Kia, Inactivation of nematodes by ultrasonic, J. Med. Sci., 5 (2005) 75–77.

[41] M.H. Dehghani, A.H. Mahvi, G.R. Jahed, R. Sheikhi, Investigation and evaluation of ultrasound reactor for reduction of fungi from sewage, J. Zhejiang Univ. Sci., B, 8 (2007) 493–97.

[42] M. Olvera, A. Eguía, O. Rodríguez, E. Chong, S.D. Pillai, K. Ilangovan, Inactivation of *Cryptosporidium parvum* oocysts in water using ultrasonic treatment, Bioresour. Technol., 99 (2008) 2046–2049.

[43] E. Naffrechoux, S. Chanoux, J. Suptil, Sonochemical and photochemical oxidation of organic matter, Ultrason. Sonochem., 7 (2000) 255–259.

[44] S. Naseri, F. Vaezi, A.H. Mahvi, R. Nabizadeh, S. Haddadi, Determination of the ultrasonic effectiveness in advanced wastewater treatment, Iran. J. Environ. Health Sci. Eng., 3 (2006) 109–116.

[45] J.A. Ordonez, B. Sanz, P.E. Hernandez, P. Lopez-Lorenzo, A note on the effect of combined ultrasonic and heat treatments on the survival of thermoduric streptococci, J. Appl. Bacteriol., 54 (1984) 175–177.

[46] D.J. McClements, Advances in the application of ultrasound in food analysis and processing, Trends Food Sci. Technol., 6 (1995) 293–299.
[47] G. Matafonova, V. Batoev, Review on low- and high-frequency sonolytic, sonophotolytic and sonophotochemical processes for inactivating pathogenic microorganisms in aqueous media, Water Res., 166 (2019) 115085, doi: 10.1016/j.watres.2019.115085.
[48] M. Vázquez-López, L.E. Amabilis-Sosa, G.E. Moeller-Chávez, A. Roé-Sosa, P. Neumann, G. Vidal, Evaluation of the ultrasound effect on treated municipal wastewater, Environ. Technol., 40 (2019) 3568–3577.
[49] A. Rusin, A. Machnicka, Ultrasonic Cavitation in Excessive Activated Sludge Hygienization, Scientific Works of Central Mining Institute. Mining and Environment, Central Mining Institute, 2011, pp. 73–80.
[50] D. Nowak, The use of ultrasound for decontamination of sewage sludge, Eng. Environ. Prot., 18 (2015) 459–469.
[51] E. Hawrylik, K. Zaręba oraz, A. Butarewicz, The influence of ultrasounds on the survival of microorganisms present in sewage sludge, Environ. Eng. - Young Eye, Sewage Sludge, 31 (2017) 111–123.
[52] J. Bień, L. Stępniak oraz, J. Palutkiewicz, The effectiveness of water disinfection in the ultrasonic field, Environ. Prot., 4 (1995).
[53] S. Pathki, M.S. Kumar, A.V. Vaidya, Solubilization and elimination of coliforms from sewage sludge by sonication, J. Environ. Eng. Sci., 56 (2014) 89–92.
[54] A.H. Mahvi, M.H. Dehghani, M. Yunessian, Ultrasonic technology effectiveness in fecal coliform disinfection of water, Hamdard Medicus, 50 (2007) 156–161.
[55] E. Hawrylik, A. Butarewicz, Influence of low-frequency ultrasound on the disintegration of coliform and fecal coliform bacteria, J. Ecol. Eng., 22 (2021) 192–197.
[56] G. Rosen, A. Osorio-Robayo, I. Rivera-Duarte, D. Lapota, Comparison of bioluminescent dinoflagellate (QwikLite) and bacterial (Microtox) rapid bioassays for the detection of metal and ammonia toxicity, Arch. Environ. Contam. Toxicol., 54 (2008) 606–611.
[57] C.A. Ajuzieogu, L.O. Odokuma, C.B. Chikere, Toxicity assessment of produced water using Microtox Rapid Bioassay, South Asian J. Res. Microbiol., 1 (2018) 1–9.
[58] E. Włodarczyk, L. Wolny, M. Próba, Toxicity analysis of sewage sludge treated with polyelectrolytes using luminescent bacteria, Environ. Prot. Eng., 20 (2017) 447–455.
[59] G. Nałęcz-Jawecki, S. Baran, J. Mankiewicz-Boczek, E. Niemirycz, L. Wolska, J. Knapik, K. Piekarska, M. Bartosiewicz, The first Polish interlaboratory comparison of the luminescent bacteria bioassay with three standard toxicants, Environ. Prot. Eng., 36 (2010) 95–102.
[60] A.A. Salyers, D.D. Whitt, Microbiology. Diversity, Pathogenicity and the Environment, PWN, Warsaw, 2010.
[61] N. Kováts, M. Refaey, B. Varanka, K. Reich, A. Ferincz, A. Ács, Comparison of conventional and *Vibrio fischeri* bioassays for the assessment of municipal wastewater toxicity, Environ. Eng. Manage. J., 11 (2012) 2073–2076.
[62] A. Butarewicz, E. Wrzaszcz, S. Rosochacki, Toxicity of sewage from industrial wastewater treatment plants, J. Ecol. Eng., 20 (2019) 191–199.
[63] A. Baran, M. Tarnawski, Phytotoxkit/Phytotestkit and Microtox® as tools for toxicity assessment of sediments, Ecotoxicol. Environ. Saf., 98 (2013) 19–27.
[64] B. Czech, I. Jośko, P. Oleszczuk, Ecotoxicological evaluation of selected pharmaceuticals to *Vibrio fischeri* and *Daphnia magna* before and after photooxidation process, Ecotoxicol. Environ. Saf., 104 (2014) 247–253.
[65] M. Abbas, M. Adil, S. Ehtisham-ul-Haque, B. Munir, M. Yameen, A. Ghaffar, M. Iqbal, *Vibrio fischeri* bioluminescence inhibition assay for ecotoxicity assessment: a review, Sci. Total Environ., 626 (2018) 1295–1309.
[66] C.A. Ajuzieogu, L.O. Odokuma, Comparison of the sensitivity of *Crassostrea gigas* and *Vibrio fischeri* (Microtox) for toxicity assessment of produced water, J. Adv. Biol. Biotechnol., 17 (2018) 1–10.
[67] A. Kwarciak-Kozłowska, A. Krzywicka, Effect of ultrasonic field to increase the biodegradability of coke processing wastewater, Arch. Waste Manage. Environ. Prot., 17 (2015) 133–142.