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

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\textbf{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, \textit{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 \textit{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 \textit{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.

\textit{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).
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 Białystok. 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 m³/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
Physico-chemical determinations:  
- temperature [°C]  
- pH  
- BOD [mg/L]  
- COD [mg/L]  
- total nitrogen [mg/L]  
- total phosphorus [mg/L]  
- general suspensions [mg/L]  

Microbiological determinations:  
- total number of bacteria [CFU/mL]  
- coliform bacteria [CFU/100 mL]  
- faecal streptococci [CFU/mL]  
- bacteria of the genus Clostridium [CFU/mL]  

The total number of bacteria was determined on the basis of the surface plating method in accordance with the standard 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].

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 second calculated dilution, $a$ – the dilution factor that corresponds to the lowest calculated dilution, $d$ – 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 $10^{-1}$ to $10^{-6}$. 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 $37^\circ C$ for 24 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 $37^\circ C$ 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$_a$):

$$\text{TU}_a = \frac{1}{\text{EC}_{50}} \times 100$$

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: $0.4 < \text{TU}_a < 1$ no significant toxicity (low acute toxicity);
- class 2: $1 < \text{TU}_a < 10$ significant toxicity;
- class 3: $10 < \text{TU}_a < 100$ high acute toxicity;
- class 4: $\text{TU}_a > 100$ 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: $10 < \text{TU}_a < 25$ significant toxic effect – low-toxic sample;
- class 3: $25 < \text{TU}_a < 100$ significant toxic effect – toxic sample;
- class 4: $\text{TU}_a > 100$ 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: $1 < \text{TU}_a < 10$ toxic sample;
- class 3: $11 < \text{TU}_a < 100$ highly toxic sample;
- class 4: $\text{TU}_a > 100$ 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 ($12.3^\circ C$ 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**

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

*according to the PN-EN ISO 10523:2012 standard; 
according to the PN-EN 1899-2:2002 standard; 
according to the PN-ISO 15705:2005 standard; 
according to the PN-EN ISO 11905-1 standard; 
according to the PN-EN ISO 6878:2006 standard; 
according to the PN-EN 872:2007 standard.

**Table 2**

<table>
<thead>
<tr>
<th>Ultrasound – 26 kHz</th>
<th>Total number of bacteria (CFU/mL)</th>
<th>Coliform bacteria (CFU/100 mL)</th>
<th>Faecal streptococci (CFU/mL)</th>
<th>Bacteria of the genus <em>Clostridium</em> (CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before the trial – 0 min</td>
<td>2.97 × 10⁶</td>
<td>1.3 × 10⁴</td>
<td>2.24 × 10²</td>
<td>3.08 × 10²</td>
</tr>
<tr>
<td>10 min</td>
<td>1.85 × 10⁵</td>
<td>0.7 × 10⁴</td>
<td>1.10 × 10²</td>
<td>2.75 × 10²</td>
</tr>
<tr>
<td>15 min</td>
<td>1.20 × 10⁵</td>
<td>0.7 × 10⁴</td>
<td>0.67 × 10²</td>
<td>2.10 × 10²</td>
</tr>
<tr>
<td>20 min</td>
<td>2.41 × 10⁴</td>
<td>0.6 × 10³</td>
<td>0.32 × 10²</td>
<td>1.52 × 10²</td>
</tr>
<tr>
<td>25 min</td>
<td>1.83 × 10⁴</td>
<td>2.3 × 10²</td>
<td>0</td>
<td>0.85 × 10²</td>
</tr>
</tbody>
</table>

*according to the PN-EN ISO 6222:2004 and PN-EN ISO 8199:2019-01 standards; 
according to the PN-EN ISO 9308-2:2014-06 standard and the 1681 procedure recommended by the EPA; 
according to the PN-EN ISO 7899-2:2004 standard; 
according to the PN-EN 26461-2:2001 standard.
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 bio-tests 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.
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.

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