1. INTRODUCTION

Wastewater and sewage sludge is a place of occurrence of many microorganisms, including pathogenic and relatively pathogenic bacteria. They may reach other environments, e.g. receiver waters or soil, thus creating a biological threat. The amount of wastewater discharged in total is constantly increasing. Changes in the amount of total effluent discharged throughout the country over the last decade (2008–2017) are shown in Figure 1. According to Polish law, the Act of June 7, 2001 on the collective water supply and collective sewage disposal (Journal of Laws of 2018, item 1152, as amended) [4] regulates a number of issues related to the proper functioning of water and sewage companies. In the field of wastewater treatment, it imposes on these enterprises the obligation to ensure proper functioning of wastewater treatment plants operated by enterprises and what is inseparably connected with it – the obligation to properly treat the wastewater.

The required effect of wastewater treatment, called
also the degree of purification or efficiency of the wastewater treatment plant, results from the pollution and properties of wastewater brought to the treatment plant as well as the conditions that should be met by wastewater discharged to the receiver.

Quality of treated wastewater should be in accordance with the requirements set out in the Ordinance of the Minister of the Environment of 18 November 2014 on conditions to be met when introducing the sewage into waters or soil [5], and on substances particularly harmful to the aquatic environment (Dz. U. 2014, item 1800). Only in the case of wastewater intended for agricultural use, this regulation imposes an obligation to determine whether there are Salmonella and intestinal parasitic eggs belonging to Ascaris sp., Trichuris sp., Toxocara sp. in the wastewater. For sewers inserted into waters or the earth, no sanitary requirements are specified.

2. SANITARY QUALITY OF TREATED WASTEWATER

Wastewater is defined as all runoff of rainwater, as well as industrial or municipal wastewater or their connections carried by water. The type and amount of sewage generated depends both on the number of population and negative effects of human activities: household, recreation and industry. All mentioned factors influence drainage patterns as well as chemical and biological status of treated wastewater [6].

Wastewater leaving to wastewater treatment plant is regarded as treated wastewater. Treated wastewater contains a number of organic and inorganic substances. They may also contain potentially toxic elements such as: As, Cd, Cr, Cu, Pb, Hg, Zn. Their low concentrations can have an effect at the phytotoxic level, without creating a risk to humans. However, from the point of a human health view, the most worrying are pathogenic microorganisms and macroorganisms, especially in the agricultural use of sewage [7]. The classic wastewater treatment processes ensure a high, up to 99%, reduction in the bacteria count. Despite the high efficiency of removing bacteria from wastewaters, they still contain, among others, the coliform bacteria ranging from 10^4 to 10^6/100 ml [8].

Due to the legal regulations in force in Poland, treated sewage is examined for microbiological or parasitological purposes only if it is intended for agricultural use. As a rule, municipal wastewater treatment plants do not conduct microbiological tests on the quality of sewage discharged to consumers.

A typical example is the Białystok Wastewater Treatment Plant (BOS), which is the largest facility of this type in north-eastern Poland. During the year, the treatment plant receives about 15 500 000 m³ of municipal sewage and 204 000 m³ of industrial sewage. Wastewater treatment processes are based on the conventional activated sludge method and are divided into technological nodes: mechanical and biological [9]. As shown by studies of raw sewage and
treated sewage carried out by Butarewicz [2], the sanitary properties of raw sewage did not differ from the average values characteristic for municipal sewage. The average effectiveness of the total number of bacteria removal during the wastewater treatment process ranged from 87–94%, and the number of the coli group bacteria decreased by 93.3–97.7% respectively [2].

Sanitary safety of treated wastewater will not be ensured without prior disinfection. Wastewater discharged to receivers will always be a source of pathogenic organisms transmission to the environment, which cannot be eliminated in the process of their purification [2, 10, 11].

Numerous methods used to destroy microorganisms in various ways affect the vegetative cells and spore forms. Otherwise, viruses or bacteria and fungi, and parasitic protists (protozoa) and worms react in a different way to the disinfection process. Classical disinfection of sewage and by-products that arise during purification processes can be carried out by physical and chemical methods [10]. One of these methods is ultrasonic disintegration.

3. METHODOLOGY OF LABORATORY TESTS

The aim of the experiments was to determine the effect of low-frequency ultrasound (20 and 40 kHz) on the survival of Escherichia coli bacteria present in ultrapure water, that was treated as the equivalent of purified wastewater. The use of only two ultrasound values in the experiment results from the limitations of the equipment at the disposal of the research laboratory. The reason for the application of ultrapure water resulted from previous microbiological tests indicating a small number of E. coli bacteria in treated wastewater, with the additional presence of other bacteria species that could adversely affect the outcome of the experiment [2].

The study of the influence of low-frequency ultrasound on the disintegration of microorganisms contained in ultrapure water was carried out at the turn of January and February 2019 in the laboratory of the Department of Chemistry, Biology and Biotechnology of the Faculty of Civil and Environmental Engineering at Białystok University of Technology.

The Polsonic ultrasonic washers for ultrasound generation of 20 and 40 kHz were used for disintegration. The studies used reference bacterial species from the ATCC collection (American Type Culture Collection) – E.coli bacteria from the ATCC ® 11775 ™ collection were used.

In the first stage of the research, E. coli was cultured on the broth substrate to grow the bacteria. Samples were incubated in an incubator at 37°C for 24 hours. After incubation of bacteria, up to 3 dm³ ultrapure water was added to 30 cm³ of bouillon bacterial culture and placed in the Polsonic ultrasonic washer, which produced ultrasound at a frequency of 20 kHz. The tests were carried out in a continuous and pulsation mode of the device operation. Analogically, tests were carried out in a second washer that generated ultrasound at 40 kHz in a continuous mode. Samples were sonicated for a maximum of 20 minutes. Prior to the sonication, the number of bacteria in the reference sample (not subjected to ultrasound) was determined. For this purpose, 1 cm³ of the test medium

[312x694]E.coli

bacteria from the

[312x682]ATCC ® 11775 ™ collection were used.

[312x667]In the first stage of the research, E. coli was cultured on the broth substrate to grow the bacteria. Samples were incubated in an incubator at 37°C for 24 hours. After incubation of bacteria, up to 3 dm³ ultrapure water was added to 30 cm³ of bouillon bacterial culture and placed in the Polsonic ultrasonic washer, which produced ultrasound at a frequency of 20 kHz. The tests were carried out in a continuous and pulsation mode of the device operation. Analogically, tests were carried out in a second washer that generated ultrasound at 40 kHz in a continuous mode. Samples were sonicated for a maximum of 20 minutes. Prior to the sonication, the number of bacteria in the reference sample (not subjected to ultrasound) was determined. For this purpose, 1 cm³ of the test medium was taken and serial dilutions in the range from 10⁻¹ to 10⁻⁶ were prepared, transferring the collected volume into tubes containing 9 cm³ of physiological saline. The test sample was then sonicated. After 3, 5, 7, 10, 15, 20 minutes, 1 cm³ of the mixture was taken and then dilutions identical to those of the reference sample were made.

In a further stage of the research, samples were collected for agar plates ranging from 10⁻¹ to 10⁻⁶. To determine the number of bacteria, the plates were incubated in an incubator at 37°C for 24 hours. After incubation, the number of colony forming units (CFUs) grown on the plates was determined. Only plates with 10 to 150 colonies were considered. On the basis of the obtained results, average values of cfu/cm³ were calculated.

In the experiments conducted, the number of bacteria in the reference sample as well as in samples subjected to sonication, was calculated based on the formula:

\[ N = \frac{A}{R} \]

where:

N – number of cfu in 1 cm³,
A – number of colonies grown on the plate,
R – sample dilution.
4. RESEARCH RESULTS AND DISCUSSION

The diversity of microorganisms contained in treated wastewater is quite significant, and the greatest threat is associated with the occurrence of, among others, pathogenic bacteria. *Escherichia coli* rods are one of the basic indicators of sanitary quality of water, sewage or sewage sludge, hence the choice of this type of microorganisms for the research was not accidental [12].

Table 1 shows changes in the count of *Escherichia coli* bacteria in ultrapure water subjected to ultrasound at 20 and 40 kHz as the temperature recorded during the process increases, while in Figure 2, the percent changes in the number of microorganisms depending on the sonication time are presented.

Based on the obtained results, a significant reduction in the number of *Escherichia coli* bacteria inoculated in ultrapure water was found.

Already after 3 minutes of ultrasound sonication at the frequency of 20 kHz in the pulse mode of operation, a drop in the number of bacteria by as much as 92.66% with a slight increase in temperature - about 2°C, was recorded. Along with the prolongation of the ultrasonic process, the number of microorganisms gradually decreased reaching the efficiency of their destruction of 95.91%. The temperature range during the measurements was low reaching a maximum of 29°C, with the initial value of 21°C.

Operation of ultrasounds with a frequency of 20 kHz in a continuous mode of the device operation resulted in similar effects as in the pulsed operation of the ultrasonic cleaner. After 3 minutes of the process, the number of microorganisms decreased by 19.25%, while after 5 minutes it was 81.24% and after 20 minutes – 96.21%. During the whole process, the temperature increased by 6°C (21–27°C).

<table>
<thead>
<tr>
<th>Time of sonication [min]</th>
<th>20 kHz – continuous work</th>
<th>20 kHz – pulsating work</th>
<th>40 kHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.61*10⁷</td>
<td>21</td>
<td>2.52*10⁷</td>
</tr>
<tr>
<td>3</td>
<td>1.30*10⁷</td>
<td>23</td>
<td>1.85*10⁶</td>
</tr>
<tr>
<td>5</td>
<td>3.02*10⁶</td>
<td>24</td>
<td>1.48*10⁶</td>
</tr>
<tr>
<td>7</td>
<td>1.66*10⁶</td>
<td>25</td>
<td>1.40*10⁶</td>
</tr>
<tr>
<td>10</td>
<td>1.43*10⁶</td>
<td>26</td>
<td>1.33*10⁶</td>
</tr>
<tr>
<td>15</td>
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<td>1.17*10⁶</td>
</tr>
<tr>
<td>20</td>
<td>6.10*10⁴</td>
<td>29</td>
<td>1.03*10⁶</td>
</tr>
</tbody>
</table>

Figure 2. Percentage changes in the number of *Escherichia coli* treated with ultrasound.
The work of the ultrasonic cleaner at the frequency of wave generation at the level of 40 kHz did not cause such a clear growth inhibition of microorganisms present in ultrapure water. After 5 minutes of the sonication process, there was a decrease of 40.17%, after 15 minutes – 54.39%, and after 20 minutes, the number of bacteria decreased by 68.62% compared to the reference sample. The temperature during the process increased by 7°C, reaching a maximum of 28°C.

The overall temperature increase during all tested variants of the sonication process was small, therefore the temperature achieved in the experiment did not have a major impact on the microbial destruction process.

Research on ultrasonic disintegration is carried out in various scientific centres, but they mainly concern the sewage sludge. A significant part of the work deals with the issues of sonication and subsequent use of sewage sludge in the fermentation process. Sludge subjected to ultrasonic disintegration is more susceptible to fermentation, which results in: increased production of biogas, which is a carrier of renewable energy, and a reduction in the amount of digested sludge [13, 14]. Ultrasonic waves are also used for conditioning sewage sludge and their biochemical stabilization [15, 16].

Ultrasonic disintegration also improves the sanitary quality of sewage sludge. Rusin and Machnicka [17] pointed out the action of ultrasound at the frequency of 25 and 40 kHz for the elimination of bacteria belonging to the Enterobacteriaceae family and pathogenic microorganisms of Staphylococcus genus. Nowak [18] emphasized the possibility of using ultrasound at 22 kHz frequency for such species as Enterococcus faecalis and Clostridium perfringens. The decrease in the number of microorganisms of Enterococcus faecalis was also achieved by Hawrylik et al. [19] at an ultrasound frequency of 40 kHz. Butarewicz [2] pointed to the effective action of low-frequency ultrasound on selected species of indicator bacteria (Escherichia coli, Enterococcus faecalis, Salmonella enteritidis and Bacillus subtilis) present in the sewage sludge. Disintegration of filamentous bacteria in the sludge was found by Butarewicz et al. [20]. These reports have been confirmed by Hawrylik [21].

Unfortunately, the small number of items raises the problem of ultrasonic destruction of bacteria present in wastewater. Similar, as in this work, results were obtained by Bień [22], who applied E. coli bacteria to water. Operation of ultrasounds at the frequency of 21 kHz resulted in the effectiveness of destroying these microorganisms in the range of 0 to 90%. Foladori et al. [23] showed high sensitivity of E. coli bacteria to ultrasound sonication at 20 kHz. Butarewicz [2] pointed to a reduction in the number of E. coli rods in treated wastewater. Only 45-minute interaction of ultrasounds at the frequency of 40 kHz resulted in a decrease in the number of microorganisms by 90%, with simultaneous increase in temperature from 19.6 to 45°C. Better results were obtained as a result of ultrasounds at 20 kHz – after 5 minutes of the device operation, a decrease of over 96% was recorded [2].

Results presented here show the effectiveness of low-frequency ultrasound on the destruction of microorganisms present in ultrapure water. The achieved effect of ultrasonic hygienization is associated with the cavitation process, which are increasingly used in the disposal of microorganisms [24]. The use of ultrasounds to disintegrate bacteria contained in wastewater can contribute to the improvement of sanitary safety of the receiver’s water.

5. CONCLUSIONS

1. Operation of ultrasounds at 20 kHz resulted in more than 90% drop in the number of Escherichia coli bacilli after 3 minutes of the sonication process in the pulse mode of the ultrasound washer operation. Similar effect was obtained after 10 minutes with continuous device operation.
2. Ultrasound at 40 kHz resulted in a reduction in the number of microorganisms at a level close to 70% after 20-minute exposure time.
3. Better results of reducing the number of E. coli in water/wastewater can be achieved due to the effect of ultrasounds at 20 kHz.
4. Low-frequency ultrasound should be used for disintegrate Escherichia coli bacteria contained in treated wastewater.

ACKNOWLEDGMENT

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REFERENCES


[4] Ustawa z dnia 7 czerwca 2001 r. o zbiorowym zaopatrzeniu w wodę i zbiorowym odprowadzaniu ścieków (Law of 7 June 2001 on collective water supply and collective sewage disposal) (Dz.U. 2018, poz. 1152 tj.).

[5] Rozporządzenie Ministra Środowiska z dnia 18 listopada 2014 r. w sprawie warunków, jakie należy spełnić przy wprowadzaniu ścieków do wód lub do ziemi, oraz w sprawie substancji szczególnie szkodliwych dla środowiska wodnego (Regulation of the Minister of the Environment of 18 November 2014 on conditions to be met when introducing sewage into waters or into the ground, and on substances particularly harmful to the aquatic environment) (Dz.U. 2014, poz. 1800).


