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Influence of Ultrasonic Field Parameters on the Biochemical Activity of Leachates from the Composting Process

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Abstract: Reuse of leachates by recycling them for composting is part of the circular economy. However, directly returning compost leachates to the mixture might increase the concentration of contaminants in the stabilized mass. The application of the ultrasonic modification of leachate was aimed at increasing the activity of microorganisms and the availability of nutrients, and reducing the number of pathogenic species. The purpose of the work was to determine the impact of variable ultrasonic (time and amplitude) field parameters on the biochemical properties, and number of selected groups of microorganisms, of leachates from a composting process. The tests used short sonication times of 15, 30, 60, 90, and 120 s and vibration amplitudes of 15.25, 30.5, and 46 µm. The assessment was made on the basis of changes in numbers of microbial communities (mesophilic, thermophilic, *Escherichia coli*, *Salmonella* spp. and fungi) and enzymatic activity (dehydrogenases-DHA), as well as respiratory activity (AR). Based on the conducted research, it was found that the leachate sonication time of 60 s and amplitude 30.5 µm were the most effective. The above parameters were considered borderline, above which there were no significant differences in the values of the analyzed indicators.

Keywords: leachates; ultrasounds; dehydrogenases; respiratory activity; microbial communities; mesophilic; thermophilic; *Escherichia coli*; *Salmonella* spp.; fungi

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1. Introduction

The ultrasonic field has been successfully used in many fields for many years: the medicine, food, paper, chemical, and pharmaceutical industries, and many biotechnological processes [1]. The emission of ultrasonic waves in liquids induces numerous physical, physicochemical, chemical and biological processes. According to Grosser et al. [2], the advantages of the sonication process include: improvement in the biodegradability of recalcitrant organic pollutants, especially in the case of mature leachate; the possibility, in terms of technology, of using the ultrasound field prior to or post-treatment; the absence of the need to use chemical reagents; the occurrence of a number of biochemical and thermal processes (including sonication) as a result of the effect of the ultrasonic field (including sonication), leading to consequences for pollution degradation; unlike the case of other methods, it does not lead to an increase in turbidity nor an increase in the content of suspended solids in the effluent; contribution to a significant increase in the SCOD value [2]. It should also be noted [3] that the leachate treatment methods currently used in technological facilities, including air stripping, coagulation, flocculation, and settling, are costly and energy-consuming, and the essence of their operation is based on the introduction of chemical reagents into the environment. However, as a result of using other cleaning methods, such as reverse osmosis or active carbon adsorption, we observe the redirection of pollutants from one primary medium, i.e., leachate, to biomass, or sludge.

Ultrasonic energy is a common and efficient disintegration technology for solving issues associated with removing toxic and hazardous organic compounds [4]. The active

action of an ultrasound leads to irreversible macroscopic changes in the medium and secondary phenomena of a physical and chemical nature occur, such as dispersion, ultrasonic coagulation, oxidation, and depolymerization [5]. Mechanical phenomena also take place under the influence of the ultrasonic field. They lead to the destruction of the structure of the medium as a result of particle vibrations, the intensity of which depends on the frequency and amplitude of the ultrasonic waves, which are caused by the varying pressures of the ultrasonic wave [6].

Depending on the applied ultrasonic wave intensity, the type and scope of biological changes occurring in microoganisms is fundamentally different. These include:

- Low intensity from 0 to 1 W/cm^2 (acceleration of physiological processes in microorganisms);
- Average intensity from 1 to 3 W/cm^2 (acceleration of physiological processes in microorganisms; changes in cell morphology may also occur);
- High intensity from 3 to 10 W/cm^2 and more (irreversible cell damage, death).

Nuengmatcha et al. [7] found that increasing the power of an ultrasonic field causes greater turbulence in the solution, which, in turn, increases the action of reactive radicals [7]. Changes in the medium and cells caused by the low or medium intensity of the ultrasonic field are generally reversible, i.e., after the emission is discontinued, after a shorter or longer time, the initial state is restored. On the other hand, an ultrasonic field with a sufficiently high intensity can be a bactericidal factor [2]. The decisive role in the mechanism of a bactericidal effect is played by the phenomenon of ultrasonic cavitation [8–10]. As Xie et al. [11] reports, a low-intensity ultrasound significantly affects biological materials, i.e., affecting a change in stimulating enzyme activity, cell growth, and biosynthesis [11]. The effectiveness of ultrasonic waves on microorganisms, in addition to intensity, also depends on the number of bacterial cells, the type and shape of the bacteria examined, as well as on their morphological structure and age. The effect of the ultrasonic field on microorganisms also depends on its intensity: The use of low intensity can increase the biochemical activity of microorganisms in the sample. A gradual increase in the intensity of sonication can first inactivate microorganisms and then lead to cell destruction. Higher susceptibility to the action of the ultrasonic field is shown by the rod-shaped rods of larger dimensions than small ones and spherical ones [12,13]. In addition, microbial cells may be inactivated by using an ultrasonic field with a lower intensity than required for their destruction [14]. Based on the research conducted so far on the effect of ultrasound on individual strains of bacteria, it has been found that cells are easily damaged, including *Salmonella typhimurium*, *Lactobacillus casei*, *Proteus vulgaris*, *Clostridium velchii*, *Pseudomonas fluorescens*, and *Staphylococcus aureus*. The bacteria resistant to ultrasound were: *Sarcina lutea*, *Micrococcus lysodeiticus*, *Acetobacter suboxydans* and yeast—*Saccharomyces cervisiae* [14]. *E. coli* bacteria show medium resistance to ultrasonic waves; however, at high concentrations the bactericidal effect is negligible [14].

The application of biological stimulation through specifically selected and prepared microorganisms and the preparation of a compost leachate with an ultrasound field may have an important impact on the intensification and improvement of the effectiveness of the composting process [15]. Ultrasound allows the duration of unit processes to be significantly shortened compared to other known techniques. However, one of the main problems discouraging operators from using ultrasound for pre-conditioning is the high operating and investment costs of ultrasound devices [6].

The idea of the conducted research was to confirm the possibility of using leachate as a biopreparation for composting. The limit of the ultrasonic field parameters for which an increase in biochemical activity and the inactivation of microorganisms in the leachate from the composting process was determined. It is assumed that the recirculation of sonicated effluents taken from composting can reduce composting time, and compost stability and maturity. Leaks collected during composting during the thermophilic phase are colonized by bacteria, actinomycetes and fungi present in the logarithmic growth phase. Leachate sonication is intended to increase the biochemical activity of microorganisms

in the sample. The dynamics of processes taking place during composting can also be determined by analyzing microorganism communities and using tests for measuring the respiratory activity, and dehydrogenase activity, of microorganisms in leachate. DHA is caused by a wide group of endocellular enzymes, which are present in all living cells and are essential in catalyzing the biological oxidation of organic compounds [16]. According to Barrena et al. [17], in the case of the composting process, DHA can be used as a method of assessing the biological activity of the thermophilic and mesophilic stages. Moreover, a correlation was observed between DHA and the static respiration index during the maturation mesophilic stage [17]. In addition, a correlation can be noted between DHA and some operational and biochemical parameters of the composting process, such as temperature, nitrogen content or other enzymatic activities [18]. Determination of DHA in the effluents gives a picture of the biochemical activity of the microorganisms that are in the sample. The most popular common laboratory procedure used for DHA determination is the method developed by [19], which uses triphenyltetrazolium chloride—the TTC test.

The aim of the research was to determine whether ultrasound preparation affects the microorganisms and their general abundance, as well as enzymatic activity (dehydrogenases). The measurement of the DHA of microorganisms and AR was used to control the biochemical activity in the leachates. The effectiveness of the composting process depends on the condition of the microorganisms present in the compost, which, thanks to the enzymes present in the cells, are responsible for the biochemical transformation. A novelty in the presented work is the assessment of the impact of the ultrasonic field on the biochemical activity in leachate. Research to date has focused on assessing the impact of sonication on physicochemical parameters in leachate.

2. Materials and Methods

2.1. Characteristics of the Substrate

The research substrate was the leachate from the composting process carried out in laboratory conditions coming from the bioreactor for composting. The process of composting was carried out in the bioreactor with a capacity of 45 L. The bioreactor was equipped with a temperature-monitoring system, process gases and a suction and pressure pump with a capacity of 60 L/h to maintain an adequate degree of aeration. The upper part of the reactor contained an easy-to-remove cover that allowed easy loading of the load and material sampling at any time. The air was supplied from the bottom of the reactor by means of an aeration pump, and its flow was regulated smoothly by means of a flow regulator. Temperature measurement took place at three points, using thermocouples placed inside the bioreactor. The temperature values were recorded by 3 sensors located at a distance of 10 cm from the double-perforated platen. The weight of the mixture for composting was 10 kg. The mixture of bioreactor feed contained sewage sludge 35%, green wastes 45%, sawdust 10% and organic fraction of municipal waste 10% (OFMSW). The sewage sludge used in the study was dewatering digested sludge (35%), collected from a regional waste-water treatment plant (Silesian region, Poland). The organic fraction of municipal solid waste (10%) (OFMSW) was also used. It is a fraction of the permeate (<80 mm) that was established with the sifting of mixed municipal waste from a city of over 200,000 residents. Sawdust (BA—bulking agent) 10% and green wastes (GW) 45% in the form of grass clippings were used as co-substrates for composting experiments.

The leachates were collected on day 7 of the process. Table 1 shows the physicochemical characteristics of the leachate from the composting process. Table 2 shows microbiological characteristic of the leachates. A research diagram is shown in Figure 1. Selected samples were subjected to analysis and technological research on the day of collection, according to the methodology in Sections 2.2 and 2.3. All determinations were made using a three-point repetition.

Table 1. Characteristics of the substrate-leachate from the composting process.

Index	Unit	Value
Dry solids (DS)	(g/L)	8.9 ± 0.1
Volatile solids (VS)	(g/L)	3.2 ± 0.1
pH	-	7.69 ± 0.2
Total Kjeldahl nitrogen (TKN)	(mg N/L)	1015 ± 42.0
Carbon content	(% DS)	24.88 ± 1.2
Nitrogen content	(%DS)	0.94 ± 0.01
Dissolved organic carbon	(mg C/L)	1870 ± 25%
Dissolved total Kjeldahl nitrogen	(mg N/L)	895 ± 12
Dissolved ammonium nitrogen	(mg N—NH_4^+/L)	786.2 ± 20

Table 2. Characteristics of microbiological analysis for leachates.

	Units	Value
Mesophilic	CFU/cm^3	$185 \times 10^4 \pm 15$
Thermophilic		$380 \times 10^4 \pm 8$
Fungi		$10 \times 10^4 \pm 2$
E. coli	cm^3	10^{-6}
Salmonella spp.		0

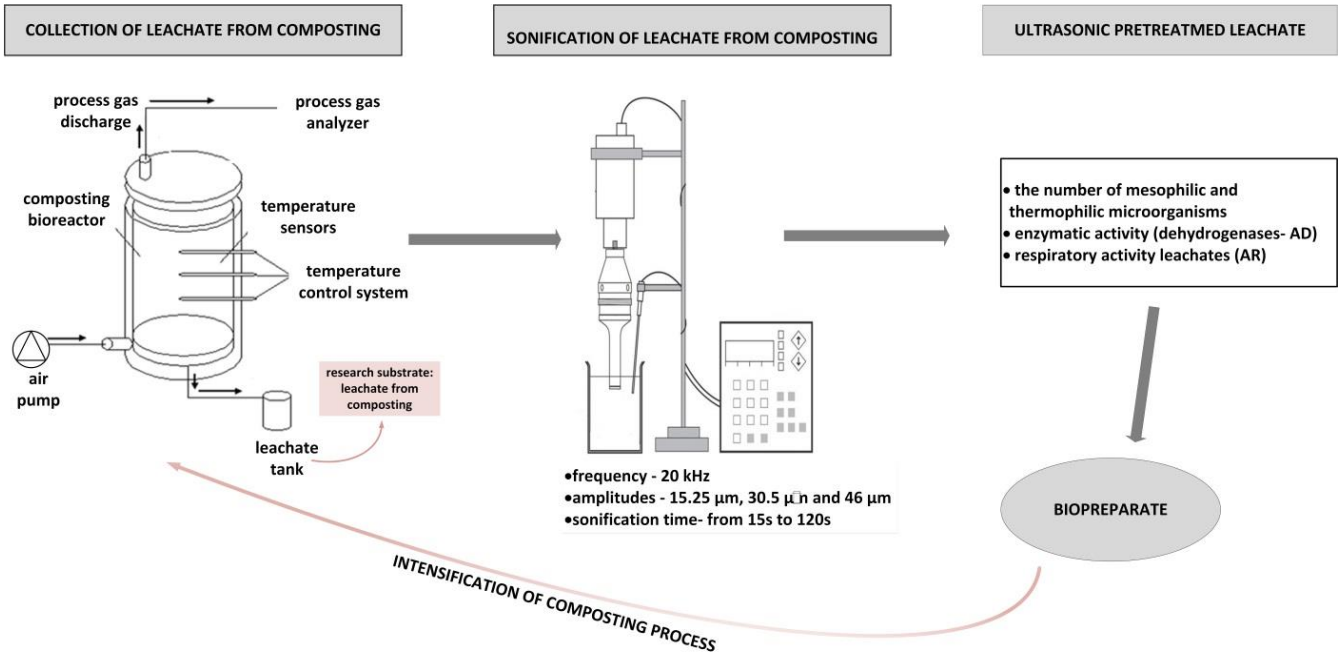

Figure 1. Test diagram.

2.2. Physicochemical Analysis

The following analytical, chemical, and physical designations were used in the course of conducted tests:

- Dry solids (DS) and volatile solids (VS) determined in accordance with PN–EN 12880 [20]
- The pH was determined using the potentiometric method (pH measurements were conducted using a 59002-00 pH meter by ColePalmer);
- Total Kjeldahl nitrogen (TKN), nitrogen content, dissolved total Kjeldahl nitrogen, and dissolved ammonium nitrogen were determined using the titration method according to standard methods (APHA, 1999) [21];

- Carbon content and dissolved organic carbon were determined using a TOC 10 C Analyzer PX by Kiper, with an AS 40 autosampler by Dione.

All analyses were triplicated. The results are presented in the tables and figures as arithmetic means.

2.3. Microbiological Analysis

The research methodology involved performing qualitative and numbers analyses for the presence of mesophilic, thermophilic, Escherichia coli, Salmonella spp. and fungi microorganisms. The microorganisms were determined using selective media specific for the test group (Merck KGaA, Darmstadt, Germany) and confirmed by molecular analysis EMA- qPCR [22,23]. Results are presented as means with standard deviation.

2.4. Determination of Dehydrogenase (DHA) Activity of Microorganisms in Leachates

DHA is determined by the TTC test, which uses triphenyltetrazolium chloride (TTC) that is catalysed by reduction dehydrogenases to triphenyl formazan (TF). TF is red. The intensity of the red colour of the test sample is directly proportional to the original amount of dehydrogenases in the control sample (leachate without sonication) [24,25]. Tris-HCl buffer was added to all samples, quickly warmed to 37 °C, and then TTC and glucose solution was added to all tubes except for the blank, to which distilled water was added. Sulphite was used as a deoxidising agent. All samples were mixed well and re-placed in a water bath. Then they were incubated in the dark at 37 °C. After 60 min, the reaction was stopped by adding methyl alcohol to a volume of 25 cm^3. The samples were filtered through a qualitative filter into volumetric flasks and made up to 50 cm^3 with methyl alcohol. The absorbances of the stained samples were determined spectrophotometrically at a wavelength of 490 nm assuming the concentration of the produced TF as the measure of the dehydrogenase activity. Three replicates were made for each sample tested. Enzyme activity is expressed in ugTF/g 20 h.

2.5. Determination of Respiratory Activity (AR) of Microorganisms in Leachates

AR of microorganisms in the leachate was determined using the test of determining the rate of oxygen consumption after its earlier oxygenation. During the measurement, the sample was mixed, and the consumption of oxygen by microorganisms was read after the selected measurement time. The aerobic activity of microorganisms was measured in a 100 cm^3 cylinder-sealed, glass-shaped cylinder equipped with an oxygen probe connected to an oxygen meter. The temperature during measurement of respiratory activity was 20 °C. The test sample was then saturated with oxygen while stirring intensively. A sealed vessel with an oxygen probe in it to ensure thorough mixing was set on a magnetic stirrer (Figure 2). After a certain time, oxygen consumption was read. All determinations were carried out in triplicate. The results of respiratory activity in the test sample were given as unit oxygen consumption in mg O_2/L. The measuring time was 15 min and the oxygen consumption was measured every 1 min. The results were calculated according to the Formula (1) [26]:

$$AR = \frac{\Delta O_2}{\Delta T} = mg/\left(dm^3 \cdot min\right)O_2 \quad (1)$$

where:

ΔO_2—difference between the highest and the lowest oxygen concentration.
ΔT—time difference.

Figure 2. Apparatus for determining the respiratory activity of leachate microorganisms (1—oxygen meter; 2—oxygen probe; 3—magnetic stirrer).

2.6. Sonicated Leachates

The Sonics VC 750 disintegrator was used to sonicate the leachate. The ultrasonic disintegrator generated an ultrasonic wave with a vibration frequency of f = 20 kHz. For the needs of the research, the times of 15, 30, 60, 90, 120 s and vibration amplitudes of 15.25 µm, 30.5 µm, 46 µm were used as variables of sonication. The volume of the sonicated samples was 0.250 L (250 cm^3) in a vessel with a diameter of 5 cm. Sonication energy was used to calculate the acoustic power (2) followed by the acoustic wave intensity, using the Formula (3) [27]:

$$N = E_s / t_s \ [W] \quad (2)$$

$$I = N/S \ [W/cm^3] \quad (3)$$

where:

N—acoustic power (W).
E_s—sonification energy (J).
t_s—sonification time (s).
I—acoustic wave intensity (W).
S—area of the surface that the wave passes through (cm^3).

3. Results and Discussion

3.1. Ultrasound of the Leachate—Selection of Amplitude and Sonication Time

Changes in the acoustic energy value depending on the applied sonication times and oscillation amplitudes are shown in Table 3. According to the source literature [27–29], the application of an ultrasonic technique requires optimizing the operating parameters, such as frequency, oscillation amplitude, wave intensity, acoustic energy and interaction duration. The capacity and geometry of the tank where the process takes place are also an important element of optimizing sonication conditions.

Table 3. Change in energy (E_s) introduced into the sample depending on the applied amplitude and sonication time.

UD Amplitude	t = 15 s	t = 30 s	t = 60 s	t = 90 s	t = 120 s
A = 15.25 µm	E_s = 450 ± 15 J	E_s = 990 ± 25 J	E_s = 1860 ± 30 J	E_s = 2700 ± 20 J	E_s = 3970 ± 30 J
A = 30.5 µm	E_s = 510 ± 10 J	E_s = 1050 ± 20 J	E_s = 2160 ± 24 J	E_s = 3330 ± 22 J	E_s = 4680 ± 24 J
A = 46.0 µm	E_s = 540 ± 12 J	E_s = 1110 ± 14 J	E_s = 2340 ± 14 J	E_s = 3600 ± 23 J	E_s = 4800 ± 25 J

The sonication process is also described through its intensity. This reliable parameter determines the amount of energy carried by an acoustic wave and reaching the medium surface, perpendicular to the wave propagation direction [30,31]. It is assumed that, in order for cavitation to occur in a liquid medium, an ultrasonic wave intensity must reach at least 1.0 W/cm^2. Table 4 lists the acoustic waves intensities depending on the applied sonication time and amplitude.

Table 4. Change in the acoustic wave intensity (I) of the sample depending on the applied amplitude and sonication time.

UD Amplitude	t = 15 s	t = 30 s	t = 60 s	t = 90 s	t = 120 s
A = 15.25 µm	I = 1.09 ± 0.05 W/cm^2	I = 1.2 ± 0.1 W/cm^2	I = 1.13 ± 0.04 W/cm^2	I = 1.09 ± 0.1 W/cm^2	I = 1.2 ± 0.1 W/cm^2
A = 30.5 µm	I = 1.25 ± 0.02 W/cm^2	I = 1.29 ± 0.08 W/cm^2	I = 1.32 ± 0.06 W/cm^2	I = 1.36 ± 0.1 W/cm^2	I = 1.43 ± 0.1 W/cm^2
A = 46.0 µm	I = 1.32 ± 0.07 W/cm^2	I = 1.36 ± 0.05 W/cm^2	I = 1.43 ± 0.1 W/cm^2	I = 1.47 ± 0.07 W/cm^2	I = 1.47 ± 0.05 W/cm^2

On the basis of the obtained test results concerning the value of the ultrasonic wave intensity, it was found that, in the case of propagation of the ultrasonic wave with selected values of the amplitude of the US field vibrations, i.e., 15.25; 30.5 and 46.0 µm, the recorded value of the wave intensity exceeded 1 W/cm^2, the value recognized in the literature [32,33] as the threshold value above which the cavitation phenomenon is initiated in the modified medium.

3.2. Results of Microbiological Analysis

Microbiological analysis in the leachate showed the absence of *Salmonella* bacteria and the presence of bacteria from the *E. coli* group. *Sallmolell* spp. was not found in the leachate collected for testing. Bacteria from the genus *Salmonella* spp. in the composting process usually come from sewage sludge. The sludge added to composting was digested and accounted for 35% by weight of the total mixture. Composting sewage sludge is a process that has a positive effect on the elimination of pathogenic bacteria (e.g., *Salmonella* spp.) This is confirmed by the results obtained by Wolna-Maruwka et al. (2009); studies in which the elimination of bacteria from the genus Salmonella was noted in the process of composting sewage sludge already at a temperature of 50–58.8 °C. Drains were collected after the thermophilic phase of the process, which could cause the elimination of the studied group of microorganisms. In addition, in seasonally digestate sewage sludge, there are also decreasing numbers of *Salmonella* spp. bacteria, which could also have contributed to their lack in leachate [26].

In the collected effluent, the occurrence of mesophilic and thermophilic microorganisms was noted, whereas a small number of fungi were noted. The waste was dominated by thermophilic microorganisms. In addition, the presence of coliforms was reported, which negatively affects their use as a biopreparation for composting. The increase in their numbers was recorded at 15 s and 30 s and the amplitude of 15.25 µm, increasing the amplitude of vibrations resulting in a decrease in the number of all examined groups. The obtained

results of microbiological analysis are presented in Figures 3–5 and Table 5. The number of mesophilic microorganisms for the amplitude 15.25 was comparable, regardless of the increasing sonication time. For the amplitude of 30.5 µm, a gradual increase in the analyzed group was observed with the increase in the sonication time, the highest number was obtained at 90 s. Further sonication did not affect the increase in the number of mesophilic microorganisms. For the amplitude of 46 µm, the highest number was recorded for the time of 15 s, and then a gradual decrease in the number of mesophils was observed with increasing sonication time. In the case of mesophilic microorganisms, the greatest number was recorded for the time of 90 s and the amplitude of 30.5 µm (Figure 3). The number of thermophilic microorganisms for the amplitude 15.25 was comparable regardless of the increasing sonication time. For the amplitude of 30.5 µm, the highest abundance was recorded for the time of 30 s and it was maintained for the remaining times used. Further sonication did not affect the increase in the number of thermophilic microorganisms. For the amplitude of 46 um, the number of thermophilic microorganisms was at a similar level for the sonification times: 15, 30, and 60 s; further sonication caused a decrease in the number of the studied group of microorganisms (Figure 4).

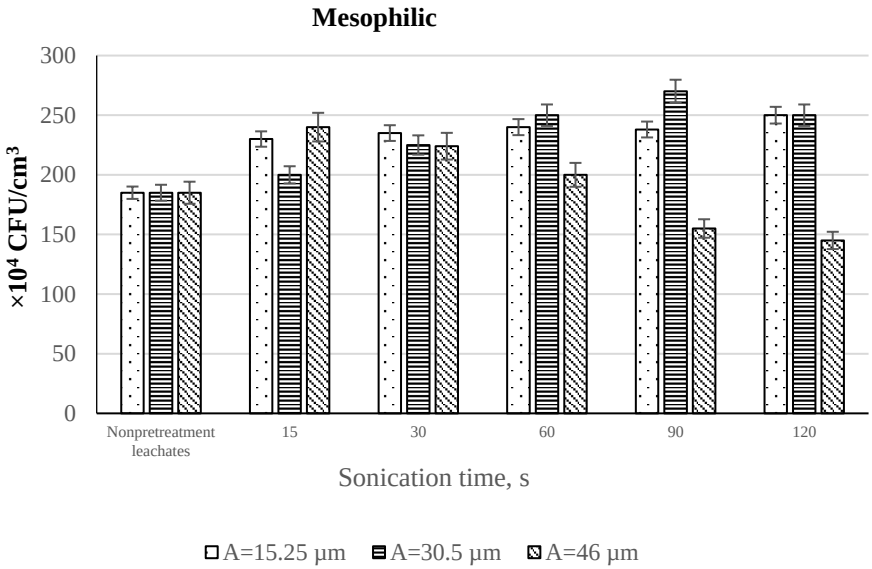

Figure 3. Results of mesophilic analysis for leachates.

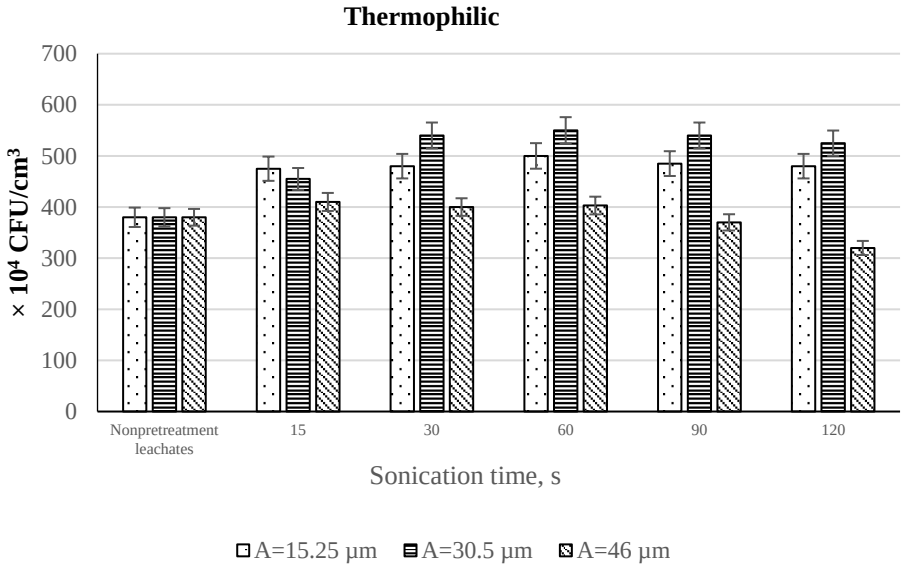

Figure 4. Results of thermophilic analysis for leachates.

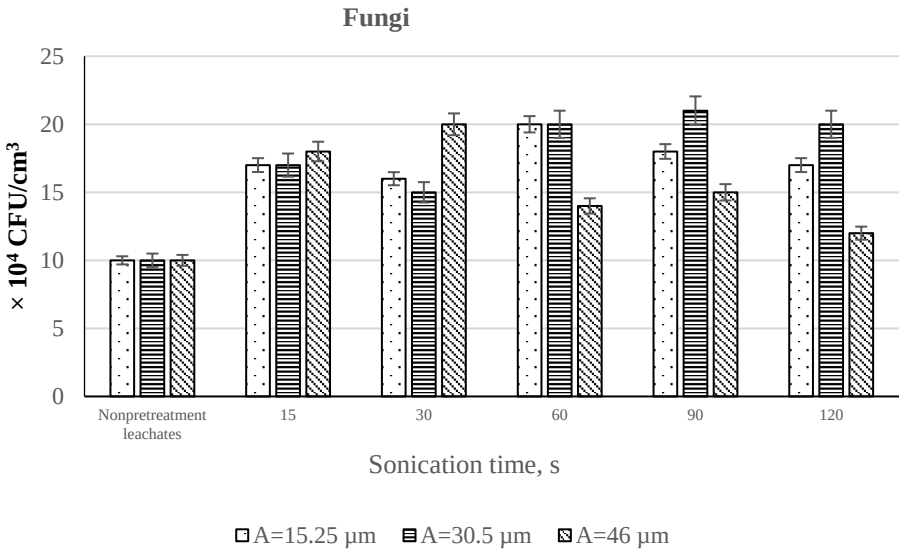

Figure 5. Results of fungi analysis for leachates.

Table 5. Results of *E. coli* (cm^3) analysis for leachates.

	UD = 15.25 µm	UD = 30.5 µm	UD = 46 µm
15 s	10^{-5}	10^{-4}	10^{-4}
30 s	10^{-5}	10^{-3}	10^{-3}
60 s	10^{-4}	10^{-3}	10^{-2}
90 s	10^{-4}	10^{-2}	10^{-2}
120 s	10^{-3}	10^{-2}	10^{-2}

The greatest number of fungi for the amplitude of 15.25 was recorded for the time of 60 s, followed by a gradual decrease in the number of the group in question. For the amplitude of 30.5 µm, the highest number was recorded for the time of 90 s. However, for the amplitude of 46 µm, a gradual increase in the number of fungi was observed until 30 s, followed by a gradual decrease in the number of microorganisms up to 120 s.

3.3. Results of Dehydrogenase (DHA) Activity of Microorganisms in Leachates

Enzyme activity is a sensitive indicator used to evaluate and monitor the composting process. In the research of Barren et al. (2008), maximum DHA values (0.54 mg TPF dry weight 1 h 1) were observed at the thermophilic end stage or at the beginning of the mesophilic stage (days 20 to 30) [16]. It is likely that, at the end of active composting, the maximum DHA levels corresponded to high biological activity, which gradually decreased during the maturation stage [34–36]. Nevertheless, DHA is a parameter that allows you to track the evolution of the biological activity of the composting process, because it correlates well with the temperature of the profile in the reactor.

The main factor negatively affecting the dehydrogenase activity (DHA) in composts and, thus, in the effluents, is the increase in the pH level in the substrate, which is often associated with the increase in temperature and the start of the thermophilic phase of the process [37,38]. The effluents were collected for testing on day 7 of the process, i.e., after the thermophilic phase had begun; hence the low level of enzyme activity. According to Wolna-Maruwka, a rapid decrease in the level of dehydrogenase activity (DHA) was noted along with the start of the thermophilic phase [39].

The level of dehydrogenase activity (DHA) for all leachate combinations was low. Analysing changes in the level of dehydrogenase activity (DHA) in the effluents under the influence of variable ultrasonic field parameters (Figure 6), it was found that the highest level of enzymatic activity occurred in the effluents with an amplitude of 15.25 µm and 60 s

and 90 s. For the amplitude 15.25 µm, a gradual increase in DHA was observed with the increase in the sonication time to 90 s, followed by a decrease comparable to the value at 60 s. A value of 30.5 µm, the highest value, was recorded for the time of 60 s. In the case of the amplitude of 46 µm, the highest value of DHA had already been obtained at the time of 15 s; further sonication caused a gradual decrease in the analyzed parameter (Figure 6).

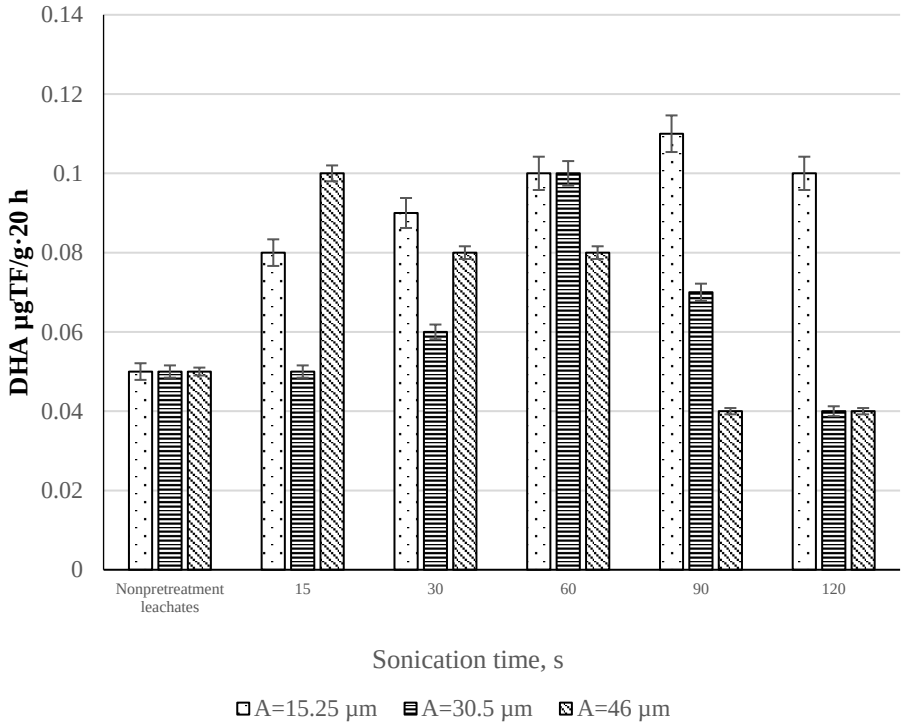

Figure 6. Results of DHA analysis for leachates.

However, as Benitez et al. observed, the improvement in sludge activity by ultrasound took 4 h after irradiation to reach the peak level, when an increase above 50% of DHA was achieved by ultrasonic irradiation [23]. Low levels of dehydrogenases have been reported in leachate collected after the thermophilic phase, and in the future it is planned to analyze the parameter in leachate from other composting phases. In addition, it is also planned to modify the method in terms of response time. The introduced changes and additional analyses will allow for a more detailed analysis of the level of dehydrogenase activity in the leachate from composting. In the presented studies, it was also found that the dynamics of changes in the dehydrogenase activity (DHA) was similar to changes in the number of mesophilic and thermophilic bacteria (Figures 3–5).

According to Tiquia, dehydrogenase activity makes it possible to trace the physiological state of microorganisms [36]. In the research conducted by Piotrowska-Cyplik et al., there was no statistically significant relationship between the total number of meso- and thermophilic bacterial cells and the activity of dehydrogenases in the compost [40].

3.4. Results of Respiratory Activity (AR) Activity of Microorganisms in Leachates

Resurfacing of leachate influenced the increase in the respiratory activity of microorganisms found in the drips. The concentration of O_2 [$mgO_2/L \cdot min$], irrespective of the amplitude and time of sonication, was more than 47% higher compared to the control (Figure 7). The highest AR value was obtained for 30.5 µm vibration amplitude and 60 s time.

In the works on composting [28,40], a correlation was found between DHA activity and respiratory activity. A similar relationship was obtained in the leachate conditioned by the ultrasonic field: the highest DH and AR activity were observed for the amplitude of 30.5 µm and times 60 s.

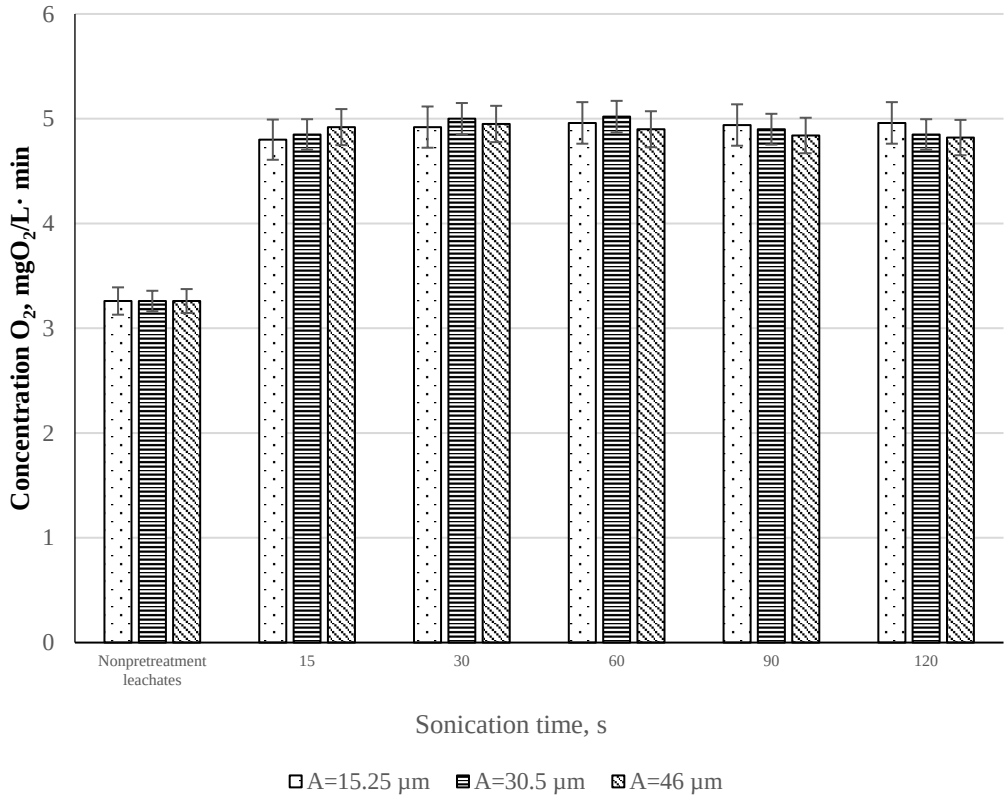

Figure 7. Oxygen concentration in the leachates after 15 min at various sonication times.

4. Conclusions

This study was undertaken to evaluate the effectiveness of the ultrasonic field with variable parameters (i.e., ultrasonic field intensity, vibration amplitude, sonication time) on biochemical properties and the number of selected groups of microorganisms in the leachate from the composting process. The noted increase in the activity of microorganisms and the availability of nutrients, as well as the decrease in the number of pathogenic species, may constitute the basis for the use of sonified leachate as biopreparations in the composting process. The implementation of the proposed technological solution may significantly affect an increase in the susceptibility of organic matter to biodegradation, which closely relates to the idea of sustainable development in the context of reducing environmental pollution. However, the introduction of such a solution on a technical scale would require a careful analysis of the amount of leachate generated, because their quantity and quality vary seasonally. Economic analysis of such an investment and solution would require more data on the composting process itself, for technological and installation solutions. The research carried out was only to check whether the use of leachate as a "biopreparation" is possible at all. When it comes to improving composting efficiency, studies should be carried out on the composting process with the recycling of sonicated leachate, to check how it affects the course of the process and the quality of the obtained compost. The limitations of the application of ultrasound at the moment must be supported by strong evidence, to use such solutions on a real scale.

Based on the research, the following conclusions were drawn:

1. The obtained values of the tested indicators, i.e., dehydrogenase activity (DHA) and respiratory activity (AR) of microorganisms in leachate modified with an ultrasonic field, indicate the potential possibility of using prepared leachates as biopreparations that influence the intensification of the composting process.
2. The overtone of compost leachates influenced the increase in the number of mesophilic and thermophilic microorganisms for the amplitude of 30.5 µm in relation to the control sample.

3. Sonicated leachate from composting resulted in a decrease in the number of bacteria from the *E. coli* group, a gradual decrease was observed along with an increase in the amplitude and the time of the leaching of leachate.
4. A similar dynamic of changes in dehydrogenase activity (DHA) to changes in mesophilic and thermophilic bacteria, was observed. The level of dehydrogenase activity (DHA) for all the leachate combinations was low, which was related to the collection of the leachate for the thermophilic phase, in which a decline in AD is usually observed.
5. The hypersecretion of leachate positively influenced the respiratory activity of microorganisms found in the drips. In the case of sonicated effluents, regardless of the time and amplitude of conditioning, respiratory activity was more than 47% higher than in the control sample.

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