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Comparison of microbial activity of selected biopreparations and leachates for composting

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ABSTRACT

The aim of this study was to compare the effectiveness of selected commercial biopreparations (Radivit, DBC Plus L, DBC Plus R5) and leachates, prepared using with the usage of ultrasounds with low frequencies and short exposure times. The comparison was made based on the qualitative and quantitative analysis of microbial communities and enzymatic activity, and, in the case of sonicated leachates, based on respiratory activity. It was found based on the results of enzymatic analysis that among the tested commercial biopreparations, Radivit may be the most useful for intensification of the composting process. Sonication of leachates resulted in an increase in the number of microorganisms and, to a small extent, in higher enzymatic activity, and respiratory activity. Furthermore, it was found that the leachates sonication time of 15 s was the most effective. The stated value of the sonification time was considered to be borderline, above which no significant changes in the value of the analyzed indicators were recorded. In the case of mesophilic bacteria in the leachate after 15 s of sonication, an increase in the number of 50 CFU/cm^3 (colony forming units) was obtained in relation to the unsaturated leachate. For thermophilic bacteria, an increase of almost 200 CFU/cm^3 compared to the control sample was noted. In the case of respiratory activity, a 47% increase was obtained after sonication of the leachate for 15 s. Lengthening of the sonication time did not increase the leachate respiratory activity.

Keywords: Biopreparations; Leachates; Microbial activity; Enzymatic activity; Composting

1. Introduction

The classic course of the composting process occurs with the participation of autochthonous microflora. However, the composted mass may contain substances resistant to biodegradation, for example, keratin waste. Microorganisms that naturally inhabit waste may have the ability to decompose hardly degradable substances, but this is a very time-consuming and inefficient process. In this case, it is required to enrich the microflora of compost with selected microbial strains [1]. Biopreparations are formed by purposefully selected and prepared microbial strains and/or selected enzymes. Biopreparations, also known as effective microorganisms (EM) or bacterial strains, are carefully selected microbial strains with a specific composition of species and quantitative proportions. This means that an active component of biopreparations is microflora formed by bacteria, fungi or protozoa, and nematodes [2]. The group of biopreparations is also distinguished by vaccines, which are used especially in plant protection and in the protection and use of the forest environment. The choice of right strains of microorganisms determines the effects of the biological decomposition of organic substances and the speed of the biodegradation process [3]. The main purpose of using biopreparations is the decomposition of long-chain carbon compounds by specific enzymatic activity of microorganisms

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or facilitating the proliferation of other species in the suspension. In addition, it is important that the biopreparation have an effect on the distribution of pollutants of different origins [4]. Biopreparations are non-toxic to humans, animals, and plants. They are characterized by the safety of use having the National Institute of Hygiene certificate, and according to the European Federation of Biotechnology, they belong to the first class of so-called "Microorganisms that have never been described as responsible for human disease and pose no threat to the environment" [5–7]. They do not cause corrosion of metals and do not damage ceramics and plastics. Selected strains are safe for the environment because they are close to those present in nature. Furthermore, this also means that they contain only non-pathogenic microorganisms [5]. The use of biological preparations ensures favorable conditions for the biodegradation process. Some examples include:

- elimination or reduction of chemical cleaning methods, immediate beginning of the decomposition processes,
- reducing the time of biostabilization and increasing its effectiveness,
- liquidation or reduction of odors in the process of biodegradation,
- process simplicity, safety, speed, and low implementation costs,
- improvement of the nitrification process, adsorption of gases that lead to corrosion of devices,
- higher reduction of fat, protein, and carbohydrates in relation to the natural microflora,
- reduction of the content of heavy metals presents in the polluted environment due to the ability of their collection by propagated microorganisms [3,6–8].

It should be added that selective action represents the strength of biopreparations. Some preparations, however, are sensitive to external factors and are characterized by low durability. Due to the fact that microorganisms are active components of bacterial preparations, their metabolic activity may be limited by the effect of environmental parameters such as low or too high pH values, temperatures, presence of significant contents of heavy metals, etc. Regardless of the advantages resulting from the use of preparations, the effectiveness of bacterial strains is undoubtedly lower than that of chemical agents [3,5].

Biopreparations are used in the following area [8,9]:

- Storage of waste—activation and acceleration of decomposition processes, biochemical removal of unpleasant odors;
- Bioremediation of land—removal of pollution from soil contaminated with hydrocarbons, assimilation of nitrogen, and phosphate—carious complex;
- Agriculture—plant growth activation, bioorganic fertilizer;
- Purification of waters, lakes, ponds—eutrophication, pollution, control of algae development, reduction of sediments, degradation of agricultural, and industrial wastewater polluting water;
- Sewage treatment plant—degradation of industrial and municipal sewage, pumping stations, activation and reduction of sediments, removal of animal fats, and odor control;
- House and garden—ecological cleaning agents, lawn growth activator, and activator of green waste decomposition;
- Food industry—decomposition of fats, proteins, starch, and odor control;
- Paper industry—enzymatic activity affecting the decomposition of cellulose, lignin, and starch;
- Fuel and energy industry—a biodegradable sorbent, decomposition of hydrocarbon, and aliphatic compounds;
- Chemical industry—enzymatic activity, decomposing anionic, nonionic, cationic surfactants, and other detergents;
- Electrical machinery industry—microorganisms contained in the preparation compete with the harmful effects of sulfur-reducing bacteria and quickly convert sulfur to non-aggressive sulfate;
- Leather industry—degradation of phenols, cresols, benzene, xylenes, and their derivatives;
- Mining and iron and steel industry—decomposition of cyanide derivatives, degrades long carbon chains, aromatic compounds, and halogens;
- Animal husbandry—slurry treatment, removal of unpleasant odors;
- Fish farming—distribution of manure and food debris, intensification of aquaculture.

Intensification of the composting process can be carried out by inoculating the compost mixture with biopreparations containing selected communities of microorganisms that enable and accelerate the degradation of hardly decomposing wastes such as cellulose or lignin. The advantage of biopreparations is shortening of the duration of the entire composting process. The possibility of using leachates as biopreparations for composting is a promising technological solution. Ultrasonic preparation of leachates from the thermophilic phase settled by bacteria, actinomycetes, and fungi occurring in the logarithmic growth phase, followed by their recirculation, is aimed to improve process efficiency. The ultrasonic conditioning of biopreparations results in a reduction of the total duration of the process time and improved stability and maturity of the compost.

The aim of the study was to compare the effectiveness of selected commercial biopreparations and leachates, designed to intensify the composting process. The comparison was made based on the qualitative and quantitative analysis of microbial communities and enzymatic activity, and, in the case of sonicated leachates, based on respiratory activity.

2. Experimental part

2.1. Substrates

The research substrates were commercial biopreparations and leachates released from the reactor during the process, and prepared using the ultrasounds at low frequencies and short exposure times. Commercial biopreparations before the analysis were activated in accordance with the manufacturer's recommendation (dilution of the recommended amount in tap water about 2 h before the analysis), and the following tests were used: Radivit composted by Neudorff; biopreparation DBC Plus type L and biopreparation DBC Plus type R5. Biopreparat Radivit contains live microorganisms (bacteria and fungi) that accelerate composting process. The product is perfectly suitable for composting of all types of waste. Bioprepatrat DBC Plus type

L occurs in the form of a powder, contains air-dried, and lyophilised bacteria of the genus: *Bacillus* sp., *Artherobacter* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Enterobacter* sp., and surfactants, buffers, and enzymes that significantly accelerate the removal of fatty deposits. It is intended for cleaning of lighter fractions and crude refined petroleum products, and used for the purification of fry catchers and sewage collectors from animal and vegetable fats. Biopreparat DBC Plus Type R5 is manufactured in the form of powder, and finds a wide range of applications in the support of wastewater treatment, and is effective in all types of wastewater treatment plants. The active ingredient of the preparation is microflora created by various strains of microorganisms. It is used for the treatment of sewage contaminated with petroleum derivatives and for cleaning the ground or places of transhipment and transport of multi-molecule hydrocarbons. This biopreparation supports the degradation of phenol and its derivatives, aromatic hydrocarbons.

The leachates was sampled from the composting process conducted in the laboratory environment in the bioreactor for composting. The process of composting was carried out in the two 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 contains an easy-to-remove cover that allows easy loading of the load and material sampling at any time. The air is supplied from the bottom of the reactor by means of an aeration pump, and its flow is regulated smoothly by means of a flow regulator. Temperature measurement takes place at three points using thermocouples placed inside the bioreactor. The temperature values are recorded by three sensors located at a distance of 10 cm from the double perforated platen. The mixtures for the composting process were prepared by adding constant quantities by weight of raw matter co-substrates and adding suitable doses of biopreparation and inoculation of the mixture with the leachate from the process of composting. The weight of the mixture for composting was 10 kg. The mixture of bioreactor feed contained 35% of sewage sludge, 45% of grass, 10% of wood chips, and 10% of organic fraction of municipal waste. The leachates were collected after the thermophilic composting process (after the seventh day of the composting process), and their volume was about 1,100 ml from one bioreactor. The highest temperature of 55°C during composting was recorded on the fourth and fifth days of the process, followed by a decrease to about 33°C on the sixth day and 30°C on the seventh day, remaining at this level until the end of the process, that is, until 28 d. The leachate for research was from a single uptake and they were analyzed before sonication.

2.2. Microbiological analysis

The test substrates were subjected to microbiological analysis. Furthermore, enzymatic activity, and respiratory activity were determined for the leachates. The microbiological analysis included qualitative and quantitative analyses of the presence of mesophilic, thermophilic, *Escherichia coli*, *Salmonella* spp., and fungi microorganisms [10]. The analysis consisted in the preparation of a series of dilutions from the tested substrates from 10^{-1} to 10^{-8}, from which 1 mL was collected and cultured on a selective liquid or solid support. After the appropriate incubation time, the grown colonies of microorganisms were counted, and the result was expressed in colony forming units—CFU/cm^3. For mesophilic microorganisms, the incubation temperature and time were 37°C/24 h, whereas for thermophilic 55°C/24 h; the media used: ordinary agar with the addition of broth. The Fig. 1 shows a schematic diagram of the Koch plate method used to determine the size of the studied groups of microorganisms.

E. coli titer was determined using Ejkman's liquid medium (lactose with bromocresol purple) by the fermentation-tube method. Sample dilutions of 10^{-1}–10^{-7} were used for the study. 1 cm^3 of each dilution was plated on Ejkman's liquid medium and incubated 48 h at 37°C. The change from violet to yellow (acidification of the medium due to fermentation of lactose) and the appearance of sediment at the bottom of the tubes were accepted as positive results. Confirmatory tests were performed on Endo solids. The culture of all positive and uncertain samples was transplanted from Ejkman to Endo medium by surface culture and incubated at 37°C for 24 h. The presence of typical, dark red colonies with metallic gloss was assumed as positive. *Salmonella* spp. Bacilli were detected on Salmonella-Shigella (SS) selective agar. Incubation was carried out at 37°C for 48 h. Fungi were determined on Saboroud's solid medium, incubated at 28°C for 4 d.

2.3. Analysis of enzymatic activity

In commercial biopreparations after activation and in leachates, lignolytic activity (laccases, peroxidases), general activity of saccharose saccharification enzymes (FP-ases) were determined. The activity of cellulolytic enzymes was determined using the methods recommended by the International Commission of Biotechnology [12]. These methods consist in measuring the amount of reducing sugars released during the enzyme solution on the substrate. For the determination of FP-aza as substrate, 50 mg of Whatman No 1 paper was used. The amount of enzyme that releases 1 μmol of reducing sugars (in glucose excretion) was used for 1 min (pH 4, temperature 50°C). Laccase activity was determined by the modified Leonowicz and Grzywnowicz's method [13] using syringaldazine as a substrate. Enzyme activity was expressed in μmol, obtained as a result of lacquer treatment on the substrate under the assay conditions (temperature 27°C, pH 5.0).

2.4. Determination of respiratory activity of microorganisms in leachates

In commercial biopreparations after activation and in leachates, lignolytic activity Respiratory activity of leachates was determined based on the rate of oxygen consumption. Measurements were made in non-sonicated samples (control sample) and in samples subjected to sonification. A glass bottle with a sealed stopper with capacity of 100 cm^3 in which the oxygen probe was mounted was used to determine respiratory activity. All samples were oxygenated before the measurement by intense stirring with a magnetic

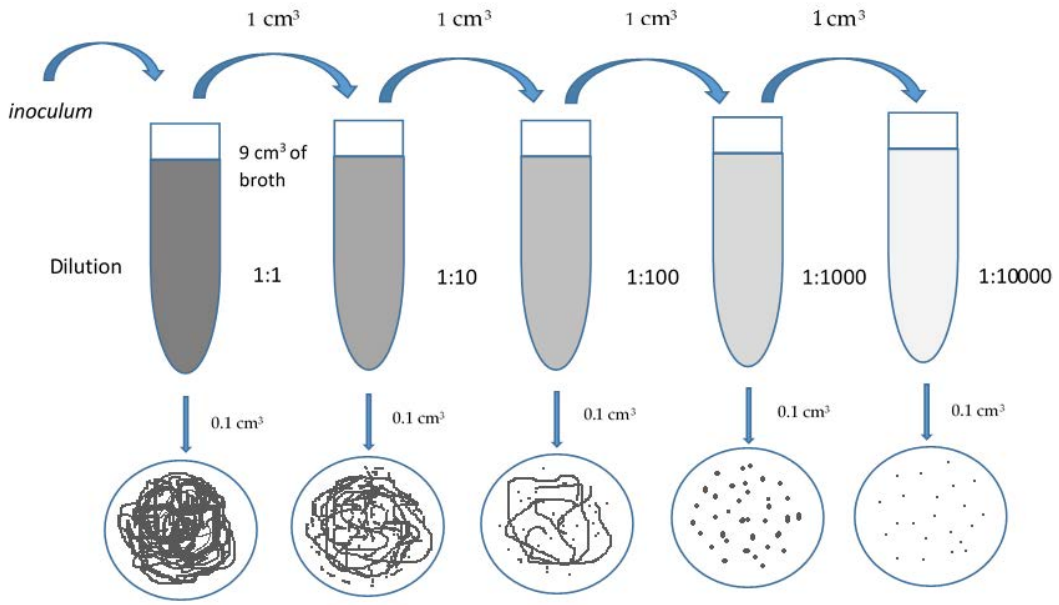

Fig. 1. Diagram of the Koch method [11].

stirrer [14]. The temperature during measurement of respiratory activity was 20°C. All determinations were conducted in triplicate. The results of respiratory activity (AR) in the test sample were given as unit oxygen consumption in mg/dm³ min. The results were calculated according to the Eq. (1) [15]:

$$AR = \frac{\Delta O_2}{\Delta T}[\text{mg}/(\text{dm}^3 \cdot \text{min})\text{O}_2] \quad (1)$$

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

2.5. Sonicated leachates

The Sonics VC 750 disintegrator was used to sonicate the leachates. The ultrasonic disintegrator generated an ultrasonic wave with a vibration frequency of f = 20 kHz. For the purposes of the research, times of 5, 10, 15, 20, 30 s and vibration amplitude of 13.42 μm were used as sonication variables (lowest possible on the device used). The volume of sonicated leachates was 200 mL.

Changes in the acoustic energy value are presented in the graph above as a dependence on the applied times of sonication and vibration amplitudes of 13.42 μm. With this range of variables in the sonication process, the acoustic energy applied to the sludge sample was different and characteristic for the sonication time (Fig. 2).

According to the literature data [16,17], the application of ultrasonic technique requires the optimization of operating parameters such as frequency, vibration amplitude, wave intensity, acoustic energy, and duration of interaction. The volume and geometry of the tank in which the process takes place is also an important element in optimizing the conditions of sonication.

3. Results and discussion

3.1. Results of microbiological analysis

The microbiological tests confirmed the absence of *E. coli* and *Salmonella* species in these three commercial biopreparations. In the leachates, the microbiological analysis showed the absence of *Salmonella* species bacteria and the presence of *E. coli* bacteria. Presence of mesophilic and thermophilic microorganisms was found in the collected leachates, whereas no fungi were found. Furthermore, the presence of fungi was found in the biopreparations: DBC Plus type L and type R5, which are classic bacterial preparations. The results of microbiological analysis for biopreparations and leachates are shown in Tables 1 and 2.

In the studied biopreparations there were differences in the number of mesophilic and thermophilic microorganisms. The most dominant group of microorganisms in preparations *A*, *B*, and *C* are mesophilic microorganisms. Their high concentration was found to be 11,750 × 10^{-4}, 13,570 × 10^{-4}, and 11,200 × 10^{-4} CFU/cm³. In preparations *B* and *C*, no fungi were observed. The highest number of mesophilic and thermophilic microorganisms for biopreparation DBC Plus type L was found.

Table 2 presents microbiological analysis for leachates subjected to sonication. The waste was dominated by thermophilic microorganisms. Furthermore, the presence of coliforms found to have negative effect on their use as a biopreparation for composting. An increased the number of determined microorganisms was observed. A gradual increase in their numbers was recorded until 15 s.

The choice of right strains of microorganisms determines the effects of the biological decomposition of organic substances in the preparation and the speed of the biodegradation process [3]. The main purpose of using biopreparations is the decomposition of long-chain carbon compounds by specific enzymatic activity of microorganisms or facilitating

Table 1
Results of microbiological analysis for individual biopreparations

Type of microorganisms	Biopreparations		
	A	*B*	*C*
Mesophiles,·10^{-4} CFU/cm^3	1175 ± 8	1357 ± 136	1120 ± 86
Thermophiles,·10^{-4} CFU/cm^3	121 ± 2	127 ± 13	56 ± 18
Fungi, 10^{-4} CFU/cm^3	40 ± 0.5	–	–
E. coli, cm^3	0		
Salmonella spp., cm^3	0		

(*A*, Radivit; *B*, DBC Plus type L; *C*, DBC Plus type R5)

Table 2
Results of microbiological analysis for leachates

Type of microorganisms	Leachates	UD *t* = 5 s	UD *t* = 10 s	UD *t* = 15 s	UD *t* = 20 s	UD *t* = 30 s
Mesophiles,·10^{-4} CFU/cm^3	149 ± 3	160 ± 5	195 ± 4	225 ± 6	230 ± 9	225 ± 8
Thermophiles,·10^{-4} CFU/cm^3	277 ± 12	300 ± 11	410 ± 8	460 ± 10	470 ± 14	460 ± 11
Fungi, 10^{-4} CFU/cm^3	5 ± 0.5	15 ± 1.5	14 ± 2	15 ± 2.4	15 ± 1.8	15 ± 1.3
E. coli, cm^3	10^{-6}	10^{-6}	10^{-6}	10^{-5}	10^{-5}	10^{-5}
Salmonella spp., cm^3	0	0	0	0	0	0

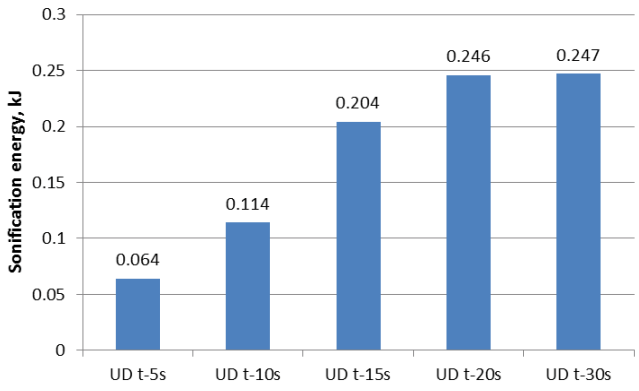

Fig. 2. Changes in acoustic energy versus sonication time for the amplitude of the ultrasonic wave.

proliferation of other species in the suspension. In addition, it is important that the biopreparation includes the widest range of pollutants in different conditions [4].

3.2. Results of enzymatic activity

Enzyme activity is a sensitive indicator used for evaluation and monitoring of the bioremediation process [18–21]. Enzymes that have been found useful for monitoring hydrocarbon removal include soil dehydrogenases, catalases, and ureases [22–25]. In the studies of the effect of remediation strategies on biological activity of oil-contaminated soil, dehydrogenase activity was the most sensitive biological indicator [26]. The use of biopreparations represents a promising strategy for minimizing the use of chemical substances, and their positive effect is confirmed in every range of use of active microorganisms.

Table 3
Results of enzymatic activity analysis for individual biopreparations

Enzymatic activity	Biopreparations		
	A	*B*	*C*
Laccase μmol · min^{-1} · mg^{-1} protein	0.1	0.0	0.0
FP-ases mUFP · min^{-1}	12	0.0	0.0

(*A*, Radivit; *B*, DBC Plus type L; *C*, DBC Plus type R5)

The results of the analysis of enzymatic activity in commercial biopreparations and leachates are presented in Tables 3 and 4. In the biopreparations DBC Plus L and R5, the activity of enzymes was below the detection threshold, which is likely to have been caused by the lack of fungi, microorganisms that are able to hydrolyse lignin and cellulose. In the case of leachates, the activity of laccase was constant, regardless of the sonication time, while a small increase in activity was found for FP-ases after applying the ultrasounds.

According to Nowak [27] low intensity waves (about 20 kHz) can accelerate cell metabolism by improving the penetration of various substrates through cell membranes and increase the rate of substrate transfer to the active enzyme center.

The quantity and type of microorganisms of the medium subjected to sonication has a decisive influence on the final effect of the process. Activation of microorganisms with different properties, structure, and morphology proceeds to varying degrees. Bacteria characterized by spherical shape and small size show greater resistance to ultrasound than large bacteria in the shape of rods. A group of gram-positive bacteria shows greater resistance to ultrasound waves than gram-negative bacteria. Gram-negative *Escherichia coli* cells are characterized by high sensitivity to the sonication process and at low ultrasonic energy the degradation of these cells is

Table 4
Results of analysis of enzymatic activity for leachates

Enzymatic activity	Leachates	UD t = 5 s	UD t = 10 s	UD t = 15 s	UD t = 20 s	UD t = 30 s
Laccase μmol min^{-1} mg^{-1} protein	0.10	0.10	0.11	0.11	0.11	0.11
FP-ases mUFP · min^{-1}	5	6	8	7	7	7

noted. Resistance to the ultrasound field using high energy ultrasound was exhibited by gram-positive *Enterococcus faecalis* cells [28].

3.3. Results of respiratory activity of microorganisms in leachates

Respiratory activity was determined only for leachates. Respiratory activity (AO) was more than 47% higher in the case of sonication leachates. No significant differences were found based on the obtained results after the time of 15 s. This indicates that this time was sufficient to achieve maximum respiratory activity for the parameters tested. Fig. 3 shows the obtained total O_2 concentration after 15 min. Furthermore, Fig. 4 presents the temporal changes in the AO for leachates without modification and after sonication.

For the assumed linear relationship describing changes in oxygen concentration as a function of sonification time for the studied research series, a high value of the linear determinant R^2 in the 0.718 – 0.875 range was obtained. Small ultrasounds with low wave value influence the increase of the rate of biochemical transformations involving microorganisms and change in the morphology of microbial cells. Changes in the medium and cells caused by low and medium concentration of the ultrasound field are reversible. At high intensity of ultrasound, irreversible damage to the cells of microorganisms occurs. This is related to the formation and collapse (annihilation) of the cavitation bubble, and thus with the simultaneous increase in pressure. Therefore, it is believed that the use of an ultrasonic field of appropriate intensity may be a factor influencing the activation of microorganisms or showing a bactericidal effect [29].

Numerous studies have shown that the addition of biological biopreparation has a positive effect on the biodegradation of organic substances contained in waste in the composting process. Biopreparation consisted of saprophytic lipolytic, proteolytic, and cellulolytic bacteria. Its addition caused the activation of the biodegradation process to occur quickly, whereas the decomposition process was accelerated and the unpleasant odours, which usually accompany the processes of decomposition, were removed. Furthermore, an improvement in hygienization was observed as no bacteria of the genus *Salmonella*, *Shigiella*, *E. coli bacilli*, and other potentially pathogenic bacteria were found [3]. The use of biopreparations has been widely described in the literature, for example, the biological protection of plants (reducing the spread of pathogens and pests) in order to increase the productivity of crops, improve the microbiological condition of the soil, modify the physical or chemical properties of the soil, and improve yields [30–33]. Studies of the impact of biopreparations on the reduction of energy consumption and CO_2 emissions in shallow and deep soil tillage also confirmed their positive effect [34].

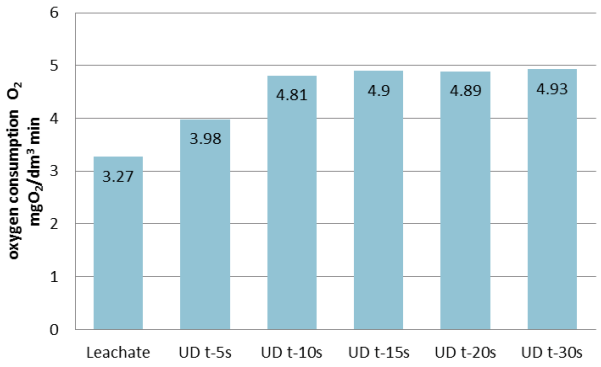

Fig. 3. Total oxygen consumption in the leachates after 15 min at various sonication times.

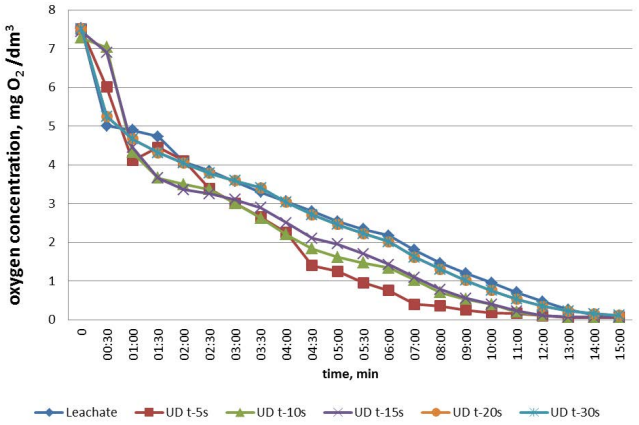

Fig. 4. Oxygen concentration in the leachates after 15 min at various sonication times.

4. Conclusions

It was found based on the results of enzymatic analysis that among the tested commercial biopreparations, Radivit may be the most useful for intensification of the composting process. In other biopreparations the undetectable activity of enzymes determined the rejection of their use for composting. Treatment of leachates with ultrasounds resulted in an increase in the number of microorganisms and, to a small extent, in higher enzymatic activity and respiratory activity. Furthermore, it was found that the most efficient leachates sonication times were 15 s. This is the limiting value of the sonification time, above which no changes were observed in the performed determinations. Due to the presence of pathogenic species, the use of leachates to intensify the composting

process may affect the safety of using them as biopreparations and may cause secondary contamination of the compost.

Acknowledgments

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