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MICROBIOLOGY OF POST-FERMENTATION LEACHATE

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ABSTRACT: Using post-fermentation leachate as fertilizer is a good alternative and solution to the problem of waste at wastewater treatment plants and contains the principles of sustainable development. Leachate from anaerobic treatment of sewage sludge contains valuable elements such as phosphorus, nitrogen and potassium, which can improve soil properties. The production of liquid fertilizer minimizes the negative impact of leachate on the main biological stream in the WWTP and improves the energy efficiency of the entire wastewater treatment system. This transfers into the operating costs of the facility. When thinking about the agricultural use of digestion leachate and its introduction into the environment, a very important issue is its microbiological contamination. The purpose of the conducted research was to determine the microbiological quality of digestion leachates from municipal wastewater treatment plants and to determine the parameters of their hygienization. The number of bacteria in raw leachate is indicative of a sanitary risk in the case they are used in agriculture as fertiliser. Heating the leachate at 60°C for 15 minutes produces an effect comparable to that achieved by heating the leachate to 70°C. The sonication process itself did not affect the better temperature effect. Heating the leachate for 20 minutes after prior sonication for 20 minutes does not result in the complete elimination of microflora.

KEYWORDS: post-fermentation leachate, sanitary risk, hygienization, microbiological stability

Introduction

The wastewater treatment technologies used generate a lot of by-products. It is estimated that about 90-95% of what is put into the anaerobic digestion (AD) process is a nutrient-rich byproduct (Möller & Müller, 2012). Treatment of leachate after anaerobic stabilization is expensive and often challenging due to large changes in its quantity and composition over time. However, leachate is more microbiologically stable and richer in nitrogen compounds than untreated organic waste (Pivato et al., 2016).

They are characterized by high concentrations of total nitrogen occurring mainly in the form of ammonium nitrogen, organic matter expressed in COD, or total suspension. In addition, the organic matter present in the leachate is not susceptible to further decomposition (MÖller & Müller, 2012; Obarska-Pempkowiak et al., 2009). Returning post-fermentation leachate to the mainstream of WWTP has an adverse effect on sedimentation and biological efficiency of wastewater treatment. This is due to the irregular inflow of leachates, which are characterized by a high variability of the pollutant load. Although the volume of leachates usually does not exceed 2.5% of the amount of all wastewater, the load of biogenic compounds in liquors may even reach 30% of the load of raw wastewater (Masłoń & Tomaszek, 2007). Nitrogen in the leachates is mainly in the form of ammonium, which means that when recycled to the biological part, it can increase oxygen consumption and the need for organic carbon. This may cause an increase in the demand for energy used to oxygenate the activated sludge reactors and an increase in the concentration of nitrogen and phosphorus in the treated wastewater. Thus, reducing the concentration of nutrients in the post-fermentation leachate is necessary before they are directed to the mainstream of the wastewater treatment plant. The production of liquid fertilizer minimises the negative impact of leachate on the main biological stream in the WWTP and improves the energy efficiency of the entire wastewater treatment system. This transfers into the operating costs of the facility.

The topic of post-technological waters is often overlooked in the literature. The quality of these waters varies greatly. Currently, wastewater treatment plant operators are not aware of the problems generated by leachate during wastewater treatment plant operations. Most of the research carried out on sludge was aimed at finding new solutions in the aspect of sludge management rather than studying the impact of post-technological waters on the operation of wastewater treatment plants or creating an alternative investment using post-technological waters (Klaczyński, 2016).

Nowadays, there is a growing interest in the possibilities of managing waste such as sewage sludge, animal waste, ash, or distillery waste in agriculture (Lamolinara et al., 2022; Jóźwiak et al., 2021). Also, the use of leachate as fertilizer is a good alternative and solution to the problem of this waste at wastewater treatment plants. Leachate from the anaerobic treatment of sewage sludge contains valuable elements such as phosphorus, nitrogen, and potassium, which can improve soil properties and, therefore, can be used to produce fertilizer products. Despite the existence of guidelines for the safe reuse of wastewater in agriculture, the presence of emerging contaminants and sometimes the impracticality of irrigating crops located far from wastewater treatment plants necessitates alternative options that allow the recovery of valuable nutrients such as phosphorus (González-Morales et al., 2021). Improper use of post-fermentation, with too long a retention time in the soil without use by crops, can result in the movement of nutrients and microorganisms into groundwater (Formowitz et al., 2012; Sampat et al., 2019; Grzyb et al., 2015).

However, manure may contain pathogens and/or components that compromise food safety, limiting its direct use as a fertiliser. The solution lies in processes such as recycling and recovery of manure. For this purpose, anaerobic digestion (AD) and composting are used to stabilize manure so that it can be safely used for land application. AD, which stabilizes organic waste and reduces pathogens and odours, is a process that converts organic waste into methane-rich biogas and post-fermentation (DG). Biogas is used for energy, while DG is usually used as fertiliserin agriculture (Kowaĉić et al., 2021).

Despite the awareness of the microbiological risk associated with the use of this type of waste as a fertiliser, there is no information in the literature on the microbiological quality of digestion leachate. Theoretically, a properly conducted methane fermentation process preceded by hygienization or sterilization of waste should result in the elimination of pathogenic microorganisms (Olesienkiewicz & Grudziński, 2017). When thinking about the use of post-fermentation leachate and its intro-

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duction into the environment, a very important issue is its sanitary condition. As a product formed from wastewater, post-fermentation can contain many microorganisms. The largest proportion of microorganisms in wastewater are viruses, of which there are about 140 types, and the number of their particles exceeds the number of bacterial cells five times. The most important viruses are adenovirus, calicivirus, hepatitis E virus, picornavirus (including enterovirus, poliovirus, hepatitis A virus), and rotavirus. Pathogenic bacteria of aquatic origin include *Campylobacter jejuni, Campylobacter coli*, pathogenic strains of *Escherichia coli, Salmonella spp.* (including *S.typhi* and *S.paratyphi* species), *Shigella spp., Vibrio cholerae, Yersinia enterocolitica, Legionella spp., Pseudomonas aeruginosa, Aeromonas spp.* Parasite eggs (*Ascaris, Trichuris,* and *Toxocara*) can also be a problem (Grudziński et al., 2015).

The presence of such diverse microflora can be a sanitary threat to the environment. The basis for their safe use is to carry out a hygienization process.

The purpose of the conducted research was to determine the microbiological quality of post-fermentation leachate from a municipal wastewater treatment plant and to determine the parameters of its hygienization. Since hygienization processes do not result in the complete elimination of microorganisms from the material, one must reckon with microbiological instability caused by the restoration of microflora in stored digestion leachates (Sahlström, 2003).

Research methods

1. Material and its initial preparation

Mesophilic post-fermentation leachates obtained from the Municipal Wastewater Treatment Plant in Rzeszow were used for the study. Sample preparation consisted of percolating the leachate obtained from the wastewater treatment plant through a sterile filter with a mesh diameter of 0.5 mm.

2. The course of the experiment

- Evaluation of the effectiveness of the hygienization process

  The pretreated leachate was hygienized using temperature and/or ultrasound. For each test variant, the volume of hygienized leachates was 300 ml. For each of the two research variants, microbiological determinations were performed three times.
  - First variant. Studies of the effect of temperature on the hygienization process of leachates were conducted using a temperature magnetic stirrer. The leachate was heated to temperatures of 60, 70, and 80°C, and then maintained for 15 and 30 minutes. During heating, the leachates were continuously stirred at a speed of 30 rpm. Determinations to check the effectiveness of hygienization were tested just after the temperature was reached and after 15 and 30 minutes of temperature maintenance.
  - Second variant. An ultrasonic generator was used to study the effect of sonication on the sanitary condition of leachates. The leachates were sonicated for 10, 15, and 20 minutes at an ultrasonic intensity of 20 kHz and an amplitude of 30 µm. After each sonication variant, the leachates were also heated to 70 and 80°C, which was maintained for 15 minutes. During heating, the leachates were continuously agitated at 30 rpm. Determinations to check the effectiveness of leachate hygienization were tested after the specified sonication times, just after the temperature was reached, and after it was maintained for 15 minutes.
- Study of biological stability of leachate after hygienization process

  The pretreated digestion leachate was hygienized using temperature. For each test variant, the volume of hygienized leachates was 900 ml. The leachates, after the hygienization processes, were selected for microbiological stability studies. They were heated at 60, 70, and 80°C for 15 minutes.

  Hygienized leachate of 0.5 L volume was stored in sterile containers with a volume of 1L placed in a greenhouse at a constant temperature of 20°C. Tests to control their sanitary condition were conducted immediately after hygienization and after the first and fifth days after hygienization.

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3. Methodology of microbiological determinations

The effectiveness of the digestion leachate hygienization processes was evaluated based on selected microbiological quality indicators (Table 1).

Table 1. Indicators of microbiological quality of the studied post-fermentation leachate

1.	Number of psychrophilic bacteria on the A-enriched agar and R2R agar media	Culture method by standard PN-EN ISO 6222:2004
2.	Number of mesophilic bacteria on the A-enriched agar and R2R agar media	Culture method by standard PN-EN ISO 6222:2004
3.	Number of coli *Escherichia coli* bacteria	Culture method on chromogenic medium
4.	Presence of *Salmonella*	Culture method and biochemical tests by standard PN-EN ISO 6579-1:2017-04
5.	The number of eggs of intestinal parasites	Detection and quantification – by standard PN-Z-19005:2018-10
6.	Luminometry determination of ATP concentration *	PROMEGA protocol for 20/20 luminometer

*at work given as the RLU value – general and extracellular.

Results and discussion

1. The effectiveness of the hygienization process

The microbial counts in the tested leachates are significantly lower than in raw wastewater and comparable to the counts reported for wastewater after the biological treatment process (Łagód et al., 2006). In the raw leachate, *Salmonella* bacteria were found, and the absence of live eggs of intestinal parasites has been reported.

The leachate filtration process will not reduce microbial counts and RLU values (Figures 1-3).

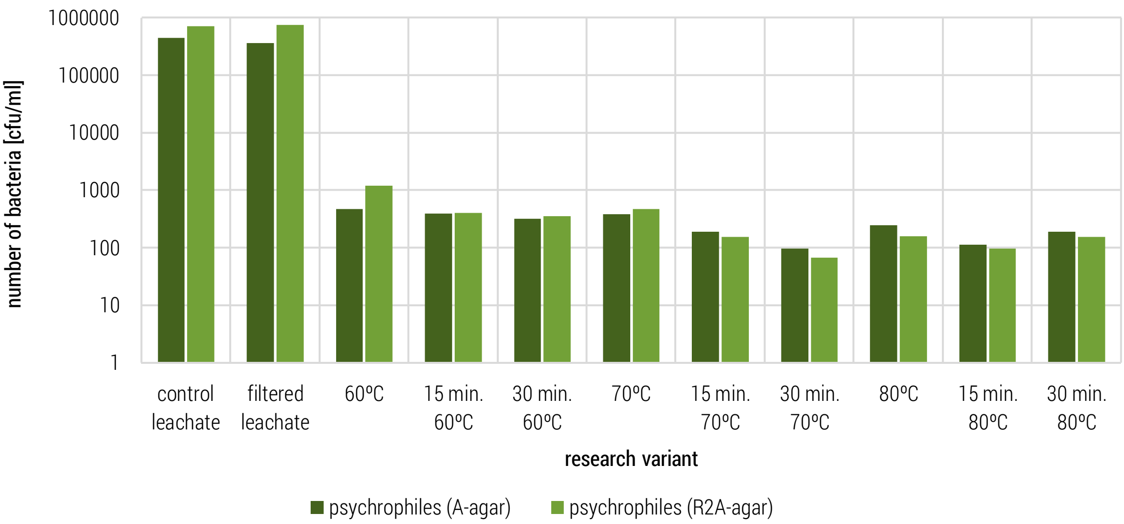

Figure 1. Number of psychrophilic bacteria in digestion leachate subjected to filtration and heating

The process of heating the leachate, regardless of the temperature used, resulted in a reduction in the number of both psychrophilic and mesophilic bacteria. The absence of *coliforms* and *Escherichia coli* (except for leachate heated to 60˚C) and *Salmonella* bacteria was also observed. The results indicate that the leachate heated to 60˚C was not fully hygienized. At 70˚C, RLU values were obtained that were about 10 times lower compared to RLU values in the raw leachate (Figure 3). Several deter-

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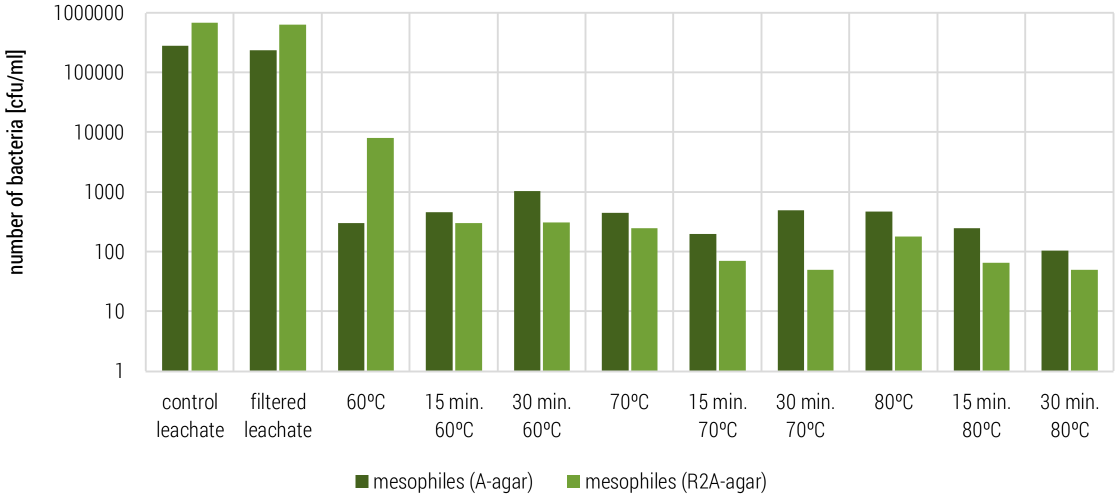

Figure 2. Number of mesophilic bacteria in post-fermentation leachate subjected to filtration and heating

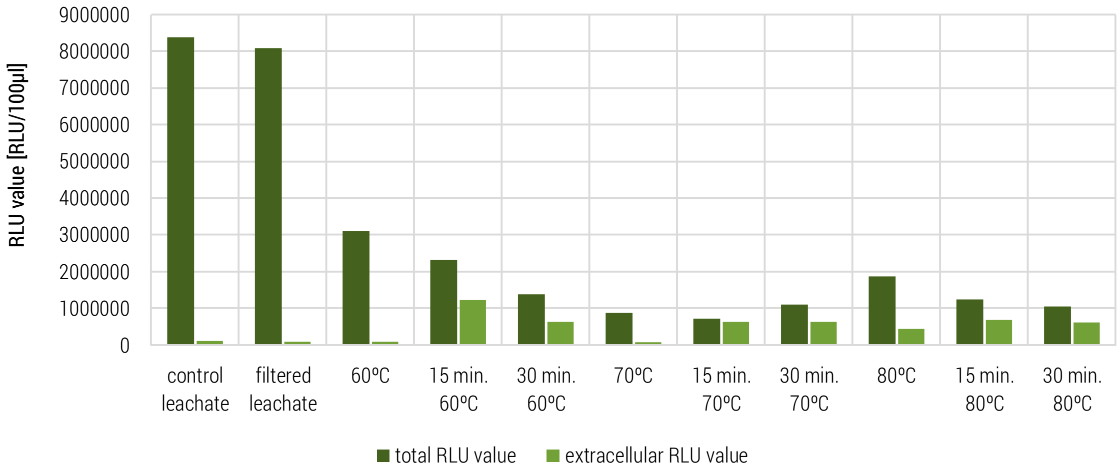

Figure 3. RLU value in post-fermentation leachate subjected to filtration and heating

minations of RLU values in heated leachate maintained at 80˚C for 2 hours showed no reduction in the value of this indicator. The reason for this was the existence of an interfering factor in the determination. Luminometric determination of ATP concentration is an assay sensitive to external factors (Hansena et al., 2019).

The literature reports that the R2A agar medium stimulates the growth of a greater number of bacteria than the reference medium (A-enriched agar). The results obtained do not always confirm this thesis. On several occasions, an inverse relationship was obtained, which may be due to the fact that the R2A agar medium is designed to determine the number of microorganisms in poor environments. In contrast, the leachates studied are rich in biogenic compounds (Daniłowicz et al., 2016; Urbanowska et al., 2019).

The sonication process alone did not affect the reduction of psychrophilic and mesophilic bacteria (Figure 4). With the time of sonication, the effect of reducing *coliforms* and *E. coli* was obtained. The increase in the number of mesophilic and psychrophilic bacteria determined by the culture method after the sonication process can be explained by the fact that ultrasound causes the flocculent suspension of microorganisms to disintegrate. In the culture method, better colony growth from single cells is evident.

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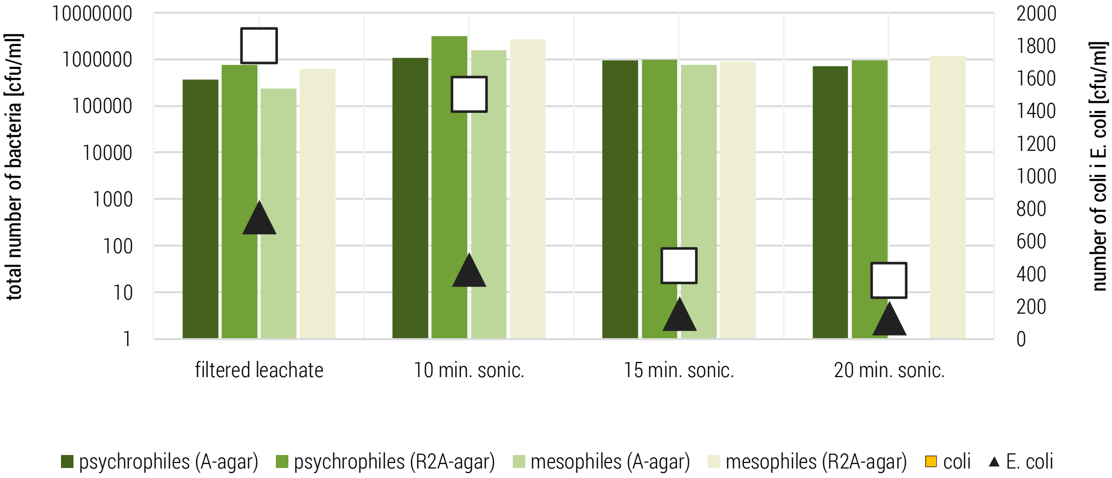

Figure 4. The number of bacteria in the tested post-fermentation leachate subjected to sonication

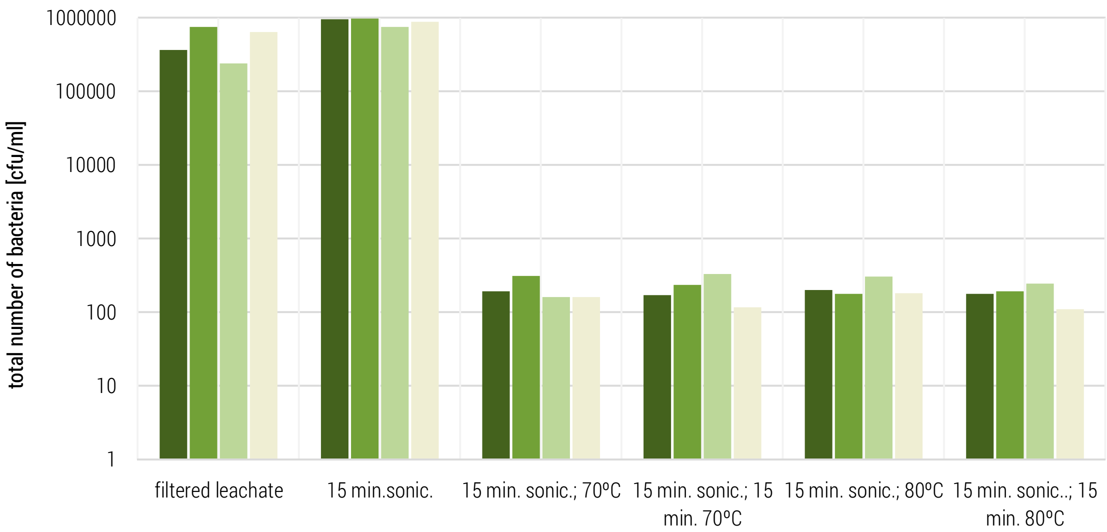

Figure 5. Number of mesophilic and psychrophilic bacteria in post-fermentation leachate subjected to sonication and temperature

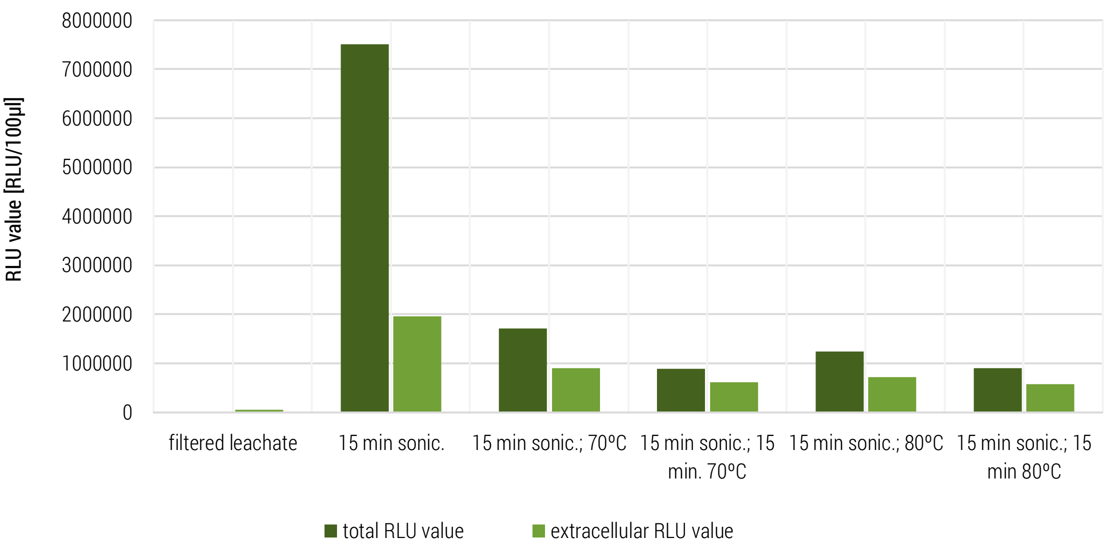

Figure 6. ATP value in the post-fermentation leachate subjected to sonication and temperature

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Heating the leachate at both 70˚C and 80˚C preceded by a sonication process resulted in the elimination of most psychrophilic and mesophilic bacteria, as well as total *coliforms, E. coli*, and *Salmonella* bacteria. Viable psychrophilic bacteria and mesophilic bacteria remain in the leachate after hygienization under these conditions. This is evidenced by the number of bacteria determined by the culture method and the RLU value (Figures 5 and 6). Viable psychrophilic and mesophilic bacteria in the leachate can form the basis for microflora restoration.

Before agricultural use of a waste product such as post-fermentation, the conditions of the Regulation of the Minister of the Climate and Environment of January 20, 2015, on recovery by method R10 (Rozporządzenie, 2015) must be met. This document, with regard to post-fermentation, refers us to the provisions of subsequent laws and the resulting regulations. The legal act regulating the permissible content of microorganisms in the post-fermentation is European Union Commission Regulation No. 142/2011 of February 25, 2011, implementing Regulation (EC) No. 1069/2009 of the European Parliament and of the Council laying down (among other things) health rules concerning animal by-products not intended for human consumption (Regulation, 2011). Regulation 3. of Annex V sets standards for fermentation residues: the number of *Escherichia coli* or *Enterococcaceae* (*enterococci* – a family of faecal streptococci) bacteria in the five samples taken and in only one sample can exceed the value of 1000 in 1 g, but cannot exceed 5000 in 1 g. As a result, the risk of *E. coli* or *Salmonella* leaking into the field is reduced. HACCP in biogas plants and control of microbiological indicators are supposed to minimize this risk even further. Finding *Salmonella* bacteria or live ATT eggs in the post-fermentation pulp and exceeding the counts of *E.coli*, *Enterobacteriaceae* or *Enterococaceae* is a problem that is not easily addressed (Walczak & Donderski, 2007).

Thermal treatment of liquid from mechanical dewatering of sewage sludge from sewage treatment plants leads to complete hygienization, resulting in a fully sanitary-safe liquid fertilizer preparation. The resulting preparation is devoid of pathogenic bacteria and intestinal parasite eggs. Compared to other natural fertilizers or waste fertilizing products, the use of the liquid formulation will not cause any risk to human or animal health or life. The contained organic compounds are more easily absorbed by plants as a result of the action of ultrasonic field and heat treatment than when using natural organic fertilisers. However, when producing this type of fertiliser, it is necessary to take into account the emission and energy-consumption aspects of the sanitation process (Piwowar & Dzikuć, 2020).

2. Microbiological stability of hygienized leachate

Studies of thermal hygienization of post-fermentation leachates indicate an increase in the total number of psychrophilic and mesophilic bacteria and RLU values after this process (Figure 7-9). *Coliform* bacteria were not recorded in the hygienized leachate. At 60ºC, there is a resurgence of coliform bacteria after just the first day of leachate storage. No *coliform* growth was observed in leachates hygienized at 70˚C and 80˚C. The biological instability of digestion leachates is due to the presence of biogenic compounds in them, mainly inorganic nitrogen, phosphorus, and organic carbon. In addition, the fertilizer prepared in this way lacks factors that limit the growth of microflora. The potential use of hygienized leachate is associated with sanitary risks (Wolska & Mołczan, 2015).

The results of the total number of both mesophilic and psychrophilic bacteria and the number of RLUs indicate that the temperature-hygienized leachates are not biologically stable (Figure 7-9). Microflora recovery was noted after the first day of heating, and on day 5 the total number of bacteria is almost 10 times higher than on the day of hygienization.

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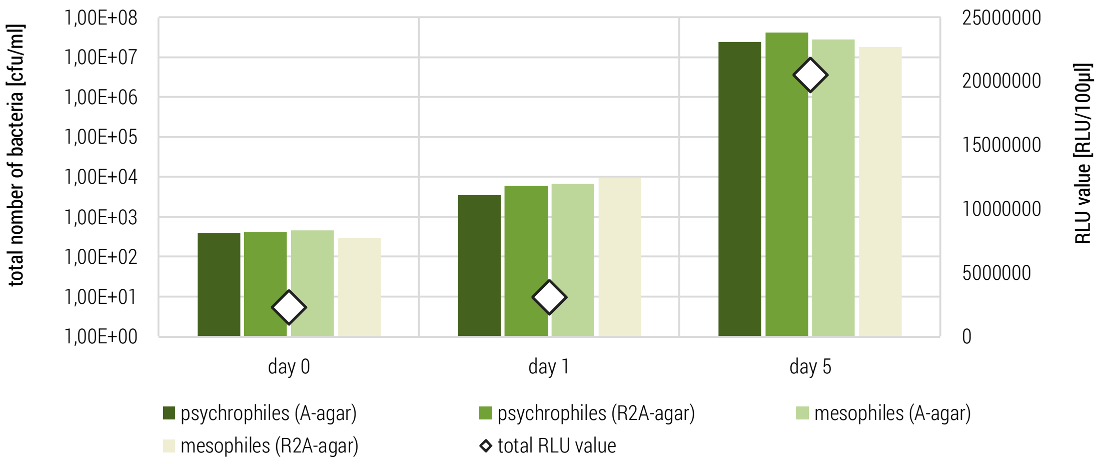

Figure 7. Changes in the total number of bacteria and RLU values in post-fermentation leachate after thermal hygienization at 60°C

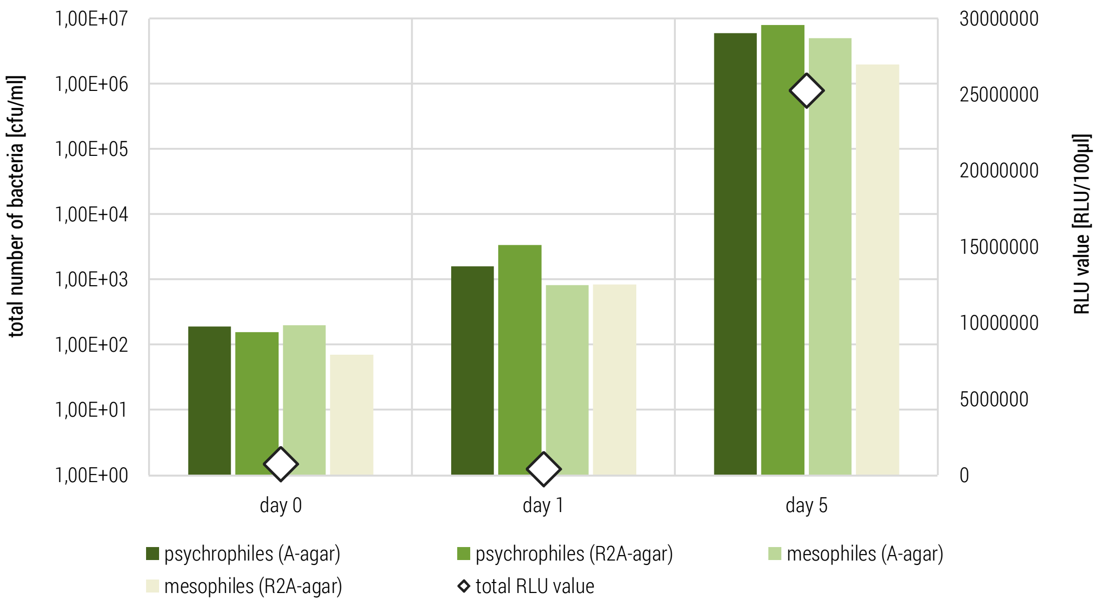

Figure 8. Changes in the total number of bacteria and RLU values in post-fermentation leachate after thermal hygienization at 70°C

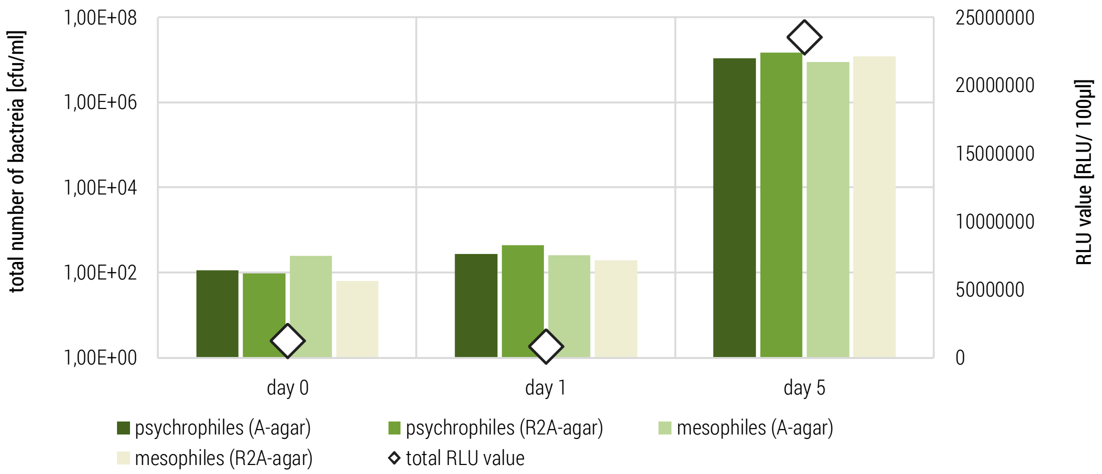

Figure 9. Changes in the total number of bacteria and RLU values in post-fermentation leachate after thermal hygienization at 80°C

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Reproduction of *coliform* and *Escherichia coli* populations was observed on day 1 after heating the leachate at 60°C for 15 minutes. When higher temperatures (70 and 80°C) were applied, no *coliforms* and *E. coli* were detected until day 5 (Table 2).

Table 2. Changes in the number of *coli* and *Escherichia coli* bacteria in hygienization leachate

Researchvariant	*coli* bacteria [cfu/100ml]	E*scherichia coli* [cfu/100 ml]
Raw leachateday 0	1 800	600
60 ºC day 0	0	0
60 ºC day 1	1	0
60 ºC day 5	300	10
70 ºC day 0	0	0
70 ºC day 1	0	0
70 ºC day 5	0	0
80 ºC day 0	0	0
80 ºC day 1	0	0
80 ºC day 5	0	0

In the future, it is planned to conduct research on the quality and microbiological stability of leachates after thermophilic fermentation.

Conclusion

The primary use of the post-fermentation, including its liquid fraction, is as a fertiliser. The production of liquid fertilizer minimises the negative impact of leachate on the main biological stream in the WWTP and improves the energy efficiency of the entire wastewater treatment system. Post-fermentation leachates must be sanitary and safe in order to be used. Heating the leachate to 60˚C and maintaining such temperature for 15 minutes made it possible to achieve a hygienization effect comparable to that achieved by heating the leachate to 70ºC alone. The sonication process caused the disintegration of the flocculent suspension of the leachate, resulting in an increase in the number of microorganisms determined by the culture method. In contrast, the sonication process alone did not have a hygieniceffect on the leachate. Heating the leachate for 30 minutes at 80˚C, after sonication for 15 minutes, did not result in the complete elimination of microflora. Further heating of the leachate is economically unjustified and does not result in the complete elimination of microflora.

Checking the stability of hygienized post-fermentation leachate was aimed at indicating whether hygienized leachate can be stored (e.g. in sales packages). The tests performed showed very rapid recovery of the bacterial microflora of both raw and hygienized leachates. The number of microorganisms after 5 days of storing the leachates at 20°C is many times higher than the number of microorganisms in leachates in which no hygienization was carried out. In the context of using such leachates as a fertiliser, it is suggested that they be used immediately after the hygienization process. No *coliform* bacteria were detected in the stored leachates, but the number of microorganisms is increasing enough to pose a sanitary threat. There is no information in the literature regarding the microbiological quality of post-fermentation leachate. However, there are studies indicating a high content of organic compounds and nutrients (Urbanowaka et al., 2019). Their presence is the basis for the restoration of microflora in leachates.

The contribution of the authors

Conception, J.Z., M.Z. and A.M.; literature, J.Z., M.Z. and A.M.; review, J.Z., M.Z. and A.D.; acquisition of data, J.Z., M.Z. and A.D.; analysis and interpretation of data, J.Z., M.Z. and A.D

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MIKROBIOLOGIA ODCIEKÓW POFERMENTACYJNYCH

STRESZCZENIE: Wykorzystanie odcieków pofermentacyjnych jako nawozu jest dobrą alternatywą i rozwiązaniem problemu odpadów na oczyszczalniach ścieków i wpisuje się w idee zrównoważonego rozwoju. Odcieki z beztlenowego oczyszczania osadów ściekowych zawierają cenne pierwiastki, takie jak fosfor, azot i potas, które mogą poprawiać właściwości gleby. Produkcja nawozów płynnych minimalizuje negatywny wpływ odcieków na główny strumień biologiczny w Oczyszczalni oraz poprawia efektywność energetyczną całego systemu oczyszczania ścieków. Przekłada się to na koszty eksploatacji obiektu. Myśląc o rolniczym wykorzystaniu odcieku pofermentacyjnego i wprowadzaniu go do środowiska bardzo istotną kwestią jest jego zanieczyszczenie mikrobiologiczne. Celem przeprowadzonych badań było określenie jakości mikrobiologicznej odcieków pofermentacyjnych z komunalnych oczyszczalni ścieków oraz określenie parametrów ich higienizacji. Liczba bakterii w odciekach surowych wskazuje na zagrożenie sanitarne w przypadku stosowania ich w rolnictwie jako nawóz. Ogrzewanie odcieku w 60°C przez 15 minut daje efekt porównywalny z efektem ogrzewania odcieku do 70°C. Sam proces sonikacji nie wpłynął na lepszy efekt temperaturowy. Podgrzewanie odcieku przez 20 minut po uprzedniej sonikacji przez 20 minut nie powoduje całkowitej eliminacji mikroflory.

SŁOWA KLUCZOWE: odcieki pofermentacyjne, zagrożenie sanitarne, higienizacja, stabilność mikrobiologiczna