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Microbiological Evaluation of Biodegradation Processes of Solid Waste in Reclaimed Landfills

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Abstract: One of the biggest environmental challenges facing modern society is waste management. The most common method of waste disposal is landfilling of municipal solid waste. The application of advanced microbiological techniques to the study of landfill ecosystems is of critical importance, given the increasing concerns about greenhouse gas emissions and regulatory controls. This study investigated the impact of probiotic and formation water application on biodegradation processes of solid waste in reclaimed landfills. The main ecological groups of soil microorganisms were determined during the experiment. For evaluating the direction of soil microbial processes, the following indexes were calculated: the index of mineralization-immobilization, the oligotrophy index, and the pedotrophy index. Microbiological evaluation of soil samples from the landfill revealed the positive effects of probiotic and associated formation water. The best results in microbial activity were observed upon their application. The analysis of coefficients confirmed that the probiotic and formation water application enhances the availability of nutrients in the soil of reclaimed landfills, benefiting various ecological and trophic groups of microorganisms and fostering favorable conditions for soil microbial communities. Thus, the application of probiotic and formation water could be an innovative biological approach to accelerating waste biodegradation and supporting soil restoration in reclaimed landfills.

Keywords: Landfill, waste biodegradation, probiotic, associated formation water, soil microorganisms

1. Introduction

Waste disposal is one of the major environmental challenges faced by contemporary society. The landfilling of solid waste is associated with several adverse environmental impacts, including depletion of land resources, soil contamination, leaching of hazardous substances into groundwater, and the emission of methane into the atmosphere. Globally, more than 2 billion tons of solid waste are produced yearly, with a minimum of 33% not being disposed of in an ecologically safe way. In many countries, municipal solid waste is disposed of in landfills; however, in numerous cases, improper disposal practices pose a serious threat to the environment (Golubović et al. 2023, Pysarenko et al. 2024). The predominant waste management technique is the use of municipal solid waste dumps, which are a major contributor to human-caused methane release globally. Microbial activity in decomposing waste plays a critical role in the generation release of greenhouse gases (Wang et al. 2017).

Climate change is predicted to impact methane emissions from both natural environments and human-impacted soils, such as landfills (Nazaries et al. 2013), through changes in soil temperature and moisture, as well as associated feedback mechanisms. Previous research (Spokas et al. 2015) suggests that dump management strategies, including changes in topsoil cover depth and structure, could be adjusted to improve methanotrophic activity over an extended period of the annual cycle in response to temporal variations in soil moisture and temperature. The naturally existing methanotrophic community in soils for landfill cover has sufficient diversity to recover rapidly from environmental fluctuations. While current landfill management typically doesn't include direct regulation of methanotrophic populations in topsoil, such approaches could become more widespread in the coming decades as climate change progresses and a deeper understanding of temporal population dynamics emerges (Meyer-Dombard et al. 2020).

Landfills serve as a widely utilized waste management strategy. The landfill microbiome plays a central role in waste degradation within these ecosystems. Various syntrophic microorganisms facilitate substrate metabolism and co-metabolism, driving the bioprocessing of waste in municipal landfills. This results in the partial breakdown of complex organic substrates into simpler polymeric compounds, followed by their complete mineralization into inorganic salts, water, and gases – notably methane. The decomposition of these substrates is governed by the dynamic interaction and succession of bacterial, archaeal, and fungal communities, which are influenced by the biotic and abiotic conditions within the landfill. These microbial activities facilitate hydrolytic, fermentative, acetogenic, and methanogenic pathways, thereby contributing to the overall biodegradation of waste materials. (García de Cortázar et al. 2002, Sekhohola-Dlamini et al. 2020).

Understanding the landfill's microbial ecology is crucial for optimizing waste management and harnessing microbial communities for biotechnological applications. Researchers Zuriash et al. (2024) demonstrated that the dominant metabolic processes include the biodegradation of xenobiotic, as well as the metabolism of terpenoids and polyketides. Additionally, landfill environments harbor metabolically versatile microorganisms, antibiotic resistance genes, mobile genetic elements, and pathogenic bacteria. Research performed by Wang et al. (2017) has shown that microbial abundance is lowest in the surface soil (0-3 cm), while it declines from the uppermost layer of accumulated waste to the central layer (30-90 cm) and then increases from the central layer to the lowest layer (90-150 cm). The microbial analysis identified *Firmicutes, Proteobacteria, and Bacteroidetes* as the dominant phyla, while *Halanaerobium, Methylohalobius, Syntrophomonas, Fastidiosipila* and *Spirochaeta* were the predominant genera. Methylohalobius were found to be more abundant in the top soil layers compared to the deposited waste. At the same time, *Syntrophomonas* and *Fastidiosipila*, which influence methane production, were more abundant in the middle to bottom layers (90-150 cm) of the stored waste. Correlation analysis revealed that microbial diversity within the landfill was most closely associated with the organic matter content of the deposited waste.

The control of secondary pollution and the rapid stabilization of municipal solid waste landfills are closely linked to the dynamics of bacterial communities. Research on the spatial variation in bacterial community structure and associated environmental factors (Naying et al. 2022) has demonstrated that landfill depth significantly influences pH (15.3%), soil organic matter content (16.1%), total nitrogen content (4.9%), and total phosphorus content (4.2%). As depth increases, total N and total P levels in landfill soil samples exhibit a declining trend, whereas organic matter content follows the opposite pattern. The microbial diversity is highest in the middle layers of landfill soil. The dominant bacterial phyla identified are *Firmicutes* and *Proteobacteria*. At the genus level, there is a bacterial succession from *Psychorobacter* and *Pseudomonas* to *Clostridium* and *Savagea*. Variations in pH, soil organic matter content, and total nitrogen and phosphorus concentrations with increasing depth suggest that the succession of bacterial communities is primarily driven by factors influenced by spatial and temporal factors, as well as the specific characteristics of municipal solid waste in different types of landfills.

Statistical analysis of the methanogen and bacterial surveys indicates that depth, landfill ages, C_{total}, P_{total}, pH, and moisture contribute to community variation, with pH and P_{total} identified as the primary controlling factors. These results confirm that a wide range of environmental conditions influence or co-influence microbial diversity within landfill ecosystems. Huang et al. (2003) and Laloui-Carpentier et al. (2006) showed that the dominant methanogenic groups identified in landfills worldwide belong to the genera Methanosarcina, Methanosaeta, Methanoculleus, and Methanofollis, irrespective of the initial material composition, sampling methodology, and extraction technique. Furthermore, the statistical comparison reveals significant differences between bacterial and archaeal communities in leachate and soil waste, suggesting that the different microbial populations are preferentially supported in leachate compared to solid waste (Staley et al. 2018).

Research conducted by Margaryan et al. (2023) has demonstrated the biodegradation potential of polymer materials, highlighting that the duration of biodegradation is influenced by environmental factors such as temperature, humidity, and specific growth conditions. Not all fungal species that colonize polymer materials function as true degraders capable of utilizing the material or its component as an energy source. Instead, the degradation process results from the complex interactions of multiple micro-mycetes rather than a single dominant species. Mycological analysis of soil samples led to the isolation and identification of 17 species of microscopic fungi belonging to the genera *Aspergillus, Alternaria, Fusarium, Cladosporium, Mucor, Rhizopus*, and *Penicillium*. In particular, the majority of these species belong to the *Ascomycota* phylum, while the *Mucoromycota* phylum is represented by four species.

Applying innovative microbiological approaches to the examination of landfill ecosystems is critically essential in light of the mounting concern over greenhouse gas emissions and regulatory controls, the heightened occurrence of chemicals of emerging concern in the waste stream, and the capacity of waste streams to serve as a resource (Meyer-Dombard et al. 2020).

Previous studies have demonstrated the effectiveness of probiotic application in accelerating the humification process and improving soil microbial community (Pysarenko et al. 2021), in restoring contaminated soil (Pysarenko et al. 2022b, Pysarenko et al. 2023a). Research by Pysarenko et al. (2022a, 2023b) has shown the potential of formation water in the remediation of technogenic contaminated land. Thus, it can be concluded that investigating the application of probiotic for landfill remediation is a promising direction but requires further study. In particular, the impact of probiotic on microbiological degradation processes in landfills should be thoroughly examined.

The objective of the study was to investigate the impact of probiotic and formation water application on biodegradation processes of solid waste in a reclaimed landfill.

2. Material and Methods

Field studies were conducted at a reclaimed landfill of Poltava City (Ukraine) during 2021-2024 (in April-May and in October). Probiotics substance and associated formation water (AFW) were applied to the experimental plots on the landfill (0.7-1.0 m. depth). Soil samples were collected using a pedological probe following ISO 18400-206:2018. The control variant was a sample without any substance application. The main probiotic cultures used in the experiment were bacteria of the genus *Bacillus* (trade name "Svieco-Agrobiotic-01"). Associated formation water is produced alongside oil, consisting of inorganic and organic components, as well as mineral compounds.

The experiment includes the following variants:
Variant 1 – control sampling without the application of any substance.
Variant 2 – sampling with probiotic application (1.0 l/t).
Variant 3 – sampling with associated formation water application (10.0 l/t).
Variant 4 – sampling with probiotic (1.0 l/t) +associated formation water application (10.0 l/t).

Microbiological analysis of selected samples was conducted on the 30th day after the substance was applied. The main microbiological indicators were determined during the experiment: total number of bacteria, number of *Ammonifying* bacteria, number of *Amololytic* microorganisms, number of *Pedotrophic* microorganisms, number of *Oligotrophic* microorganisms, *Actinomycetes*.

The identification of ecological and trophic groups of soil microorganisms was conducted by inoculating serial dilution of soil suspension onto selective nutrient media. Specifically, *Ammonifying* bacteria were cultured on meat peptone agar; *Amololytic* bacteria – on starch-ammonia agar, *Pedotrophic* microorganisms – on soil agar; *Oligotrophic* microorganisms – on nutrient-deprived agar. Following inoculation, the cultures were incubated at 28°C for 5 to 14 days, depending on the dynamics of each microbial group. Microbial abundance was assessed using the thermostatic weight method and the count of colony-forming units (CFU). To evaluate the direction of soil microbial processes, the following indexes were calculated: index of mineralization-immobilization (IMI), oligotrophy index (IO), and pedotrophy index (IP) (Andreiuk et al. 2001).

3. Results and Discussion

Microplastics could create a distinct biological niche for specific microbial communities, providing a service that facilitates microbial colonization and persistence (Chen et al. 2024). Microorganisms play a significant role in the degradation of microplastics, employing polymeric carbon as an inactive carbon source (SOC).

Previous studies performed by Shah et al. (2008), Russell et al. (2011), and Zafar et al. (2013) have shown that both bacterial and fungal species are involved in the degradation of microplastics. Furthermore, certain bacteria and fungi have been identified that are capable of utilizing microplastics as a carbon source in various environments, including soil (Mohan et al. 2016) and compost (Jeon et al. 2013), highlighting their potential role in biodegradation and recovery of plastics. The degradation process involves the breakdown of polymer chains into micromolecular, water-soluble intermediates that can be taken up by a microbial cell and further metabolized through specific biochemical pathways (Gewert et al. 2015). The final degradation products are subsequently released into the environment. These include CO_2, H_2O, and CH_4 (Tokiwa et al. 2009).

To assess the completion of the microbiological destruction process of solid waste, it is necessary to study the structure of microbiological indicators at each stage of microbial succession. The microbiological characteristics of the selected soil samples from the reclaimed landfill are presented in Table 1.

Table 1. Abundance of main groups of soil microorganisms, million CFU per 1 gram of dry soil (average values, 2021-2024).

Variant	Total number of bacteria	*Ammonifying* bacteria	*Amololytic* bacteria	*Pedotrophic*	*Oligotrophic*	*Actinomycetes*
Var 1	405±0.05	11.4±0.40	9.4±0.09	2.7±0.13	10.7±0.04	0.110±0.01
Var 2	505±0.09	13.6±0.14	8.0±0.17	7.0±0.11	10.1±0.30	0.571±0.01
Var 3	980±0.03	95.0±0.15	7.1±0.03	98.1±0.45	9.1±0.20	19.3±0.03
Var 4	4100±0.09	103.8±0.70	6.9±0.35	118.1±0.33	9.5±0.25	53.5±0.015

Research results of structure of microbial communities in the reclaimed landfill confirm that probiotic application increases total number of microorganisms by 1.2 times (Var 2); AFW application – increases by 2.5 times (Var 3); probiotic+ AFW application – increases by more than 10 times (Var 4). This indicates a synergistic effect of the action of probiotic and AFW. Considering this, the experiment showed favorable conditions for the development of microorganisms and the presence of nutrients and, consequently, the incompleteness of waste biodegradation process, which is accelerated by the additional of AFW and probiotic. The application of probiotic affects the number of *Pedotrophic* microorganisms. The number of *Pedotrophic* microorganisms in Var 2 increased by 2.5 times compared to control. However, the application of AFW showed a more stimulating effect on increasing the number of *Pedotrophic* microorganisms: by 36 times with AFW application and by 43 times with AFW+probiotic application. It also confirms the enhancing effect of the combined application of probiotic and AFW. The results of the study of *Oligotrophic* microorganisms did not show a significant effect of the application of studied substances on their abundance. The number of *Oligotrophic* microorganisms is slightly reduced (by 0.5-11.2%) with probiotic application and AFW. Therefore, no pronounced after-effects of probiotics and AFW application on *Oligotrophic* microorganisms were found. The activity of actinomycetes is associated with the degradation of the organic fraction of waste. In the control variant, the lowest number of *Actinomycetes* was observed, indicating a slowdown in biodestruction processes. The results of the experiments with variants 2, 3, and 4 showed an increase in the number of *Actinomycetes*. Thus, the application of AFW causes the number of *Actinomycetes* to increase by more than 100 times, and complex application of probiotic and AFW causes the number of *Actinomycetes* to increase by almost 500 times. This indicates a sufficient amount of nutrients for the development of this group of microorganisms. The result is an accelerated decomposition of the organic fraction of waste. Microbiological studies have demonstrated the impact of probiotic and AFW applications on the abundance of *Ammonifying* bacteria. Compared to the control, their population increased by 1.2 times with the probiotic application, by 8.3 times with the AFW application and by 9.1 times with the probiotic + AFW application. These findings indicate that the combined application of the studied substance has a positive influence on the development of the *Ammonifying* bacteria cenosis. The study of *Amylolytic* microorganisms' abundance is crucial for understanding microbiological processes in landfill soils, as these microorganisms utilize mineral nitrogen for their metabolic activity. The findings indicate that the application of probiotic results to the 1.1 times reduction in the number of *Amylolytic* microorganisms, while the combined application of probiotic+AFW leads to 1.4 times reduction, compared to the control.

Based on the studied indicators of microorganism's abundance, the coefficient was calculated (Fig. 1) to describe the intensity and direction of biological processes occurring in the reclaimed landfill.

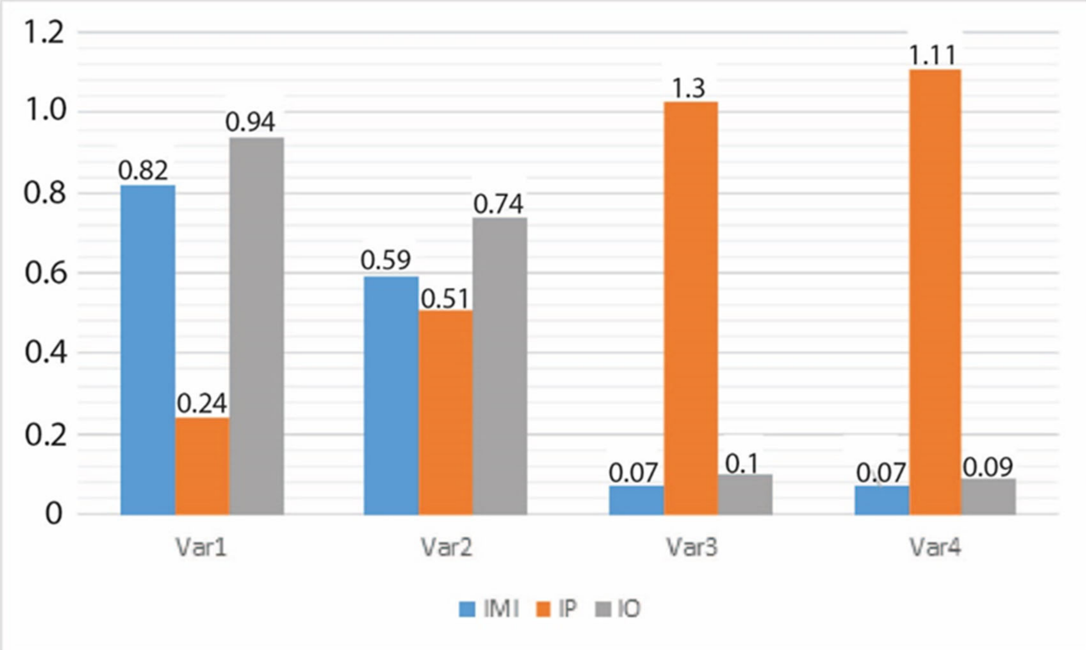

Fig. 1. The values of IMI, IP, and IO coefficients in soil samples of reclaimed landfill

The mineralization-immobilization coefficient characterizes the processes of organic matter decomposition and synthesis. In all studied variants, IMI was <1, indicating favorable conditions for the development of soil microorganisms. In the control variant, IMI was 0.8, suggesting that organic matter decomposition predominated over synthesis. In the variant with AFW and AFW+probiotic application, IMI was 0.07, indicating an increase in the availability of nutrients for microorganisms and a decrease in the rate of organic matter decomposition. The pedotrophy coefficient reflects the intensity of soil organic matter decomposition. The control sample and the probiotic-treated variant, IP < 1, indicated a low level of humus restoration. In the variant with AFW application IP was more than 1, corresponding to an increased intensity of SOM decomposition. The highest pedotrophy coefficient value (IP = 1.1) was recorded in the variants with application probiotic and AFW, indicating an enhancement of the humification process due to the increased availability of nutrients of microorganisms. The highest oligotrophic coefficient (IO) values were observed in the control variant, compared to other variants with probiotic and MSW treatment. The lowest value of oligotrophic coefficient (IO = 0.07-0.09) was recorded in the combined application of studied substances, indicating the creation of favorable conditions for soil microorganism development.

4. Conclusion

The application of advanced microbiological techniques in studying landfill ecosystems is critically important. The study of the main groups of microorganisms in soil samples from the landfill revealed the positive effects of probiotic and associated formation water. The best results in microbial activity were observed with their application. As a result, the total number of bacteria increased by 1.2-47.7 times, *Ammonifying* bacteria by 1.2-9 times, *Pedotrophic* microorganisms by 2.5-47.7 times, and *Actinomycetes* by more than 5-100 times. The analysis of coefficients (IMI, IP, IO) confirmed that the probiotic and AFW application enhances the availability of nutrients in the soil of reclaimed landfills, benefiting various ecological and trophic groups of microorganisms and fostering favorable conditions for soil microbial communities. Thus, the application of probiotic at a dose of 1 l/t and AFW at a dose of 10 l/t represents an innovative biological approach to accelerating waste biodegradation and supporting soil restoration in reclaimed landfills for their future use.

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