Journal of Infrastructure, Policy and Development 2024, 8(13), 7880.
https://doi.org/10.24294/jipd7880

Article

Microorganisms of solid waste as an opportunity for waste disposal and increasing environmental sustainability in the south of Kazakhstan

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CITATION

Akhmet A, Karimova S, Zhumadullayeva A, et al. (2024). Microorganisms of solid waste as an opportunity for waste disposal and increasing environmental sustainability in the south of Kazakhstan. Journal of Infrastructure, Policy and Development. 8(13): 7880. https://doi.org/10.24294/jipd7880

ARTICLE INFO

Received: 9 July2024
Accepted: 16 August2024
Available online: 12 November 2024

Abstract: The study is devoted to the problem of processing the organic waste that is generated as a result of paper, textiles and other industries production as well as food waste. The growth of economic activity in Kazakhstan has resulted in a significant challenge with regard to industrial waste management. The accumulation of waste on the territory of the country has reached 31.72 billion tonnes, comprising approximately 2.5 billion tonnes of hazardous waste, 50 million tonnes of phosphorus-containing waste, over 2.5 million tonnes of lead-zinc waste and more than 120 million tonnes of solid domestic waste. The study object was the Shymkent-Kokys polygons. According to the research carried out, it was determined that the titer of microorganisms of the studied groups is 1–10 CFU/g in the soils selected around the garbage in the area of the Shymkent landfill. The titer of microorganisms in the soil horizons was high at a depth of 20–30 cm and the titer were 10^9 cells/mL. The structure of the soil microbiome obtained around the Shymkent Waste Landfill consists of actinomycetes, *micromycetes*, heterotrophic bacteria, nitrifying, nitrogen-fixing bacteria, enterobacteria, as well as algae and protozoa. It was found that strains KPA1, KPA2 *Pseudomonas* sp. strains KPA3, KPA4, KPA5 *Bacillus* sp. isolated from the soils of the Shymkent-Kokys landfill are able to recycle domestic waste with a high content of cellulose and organic substances up to 95%–97%. The findings can be used to develop more effective organic cellulosic waste management strategies and improve the environmental sustainability of various industries.

Keywords: solid domestic waste; organic waste; *Pseudomonas* sp.; *Bacillus* sp.; cellulose activity

1. Introduction

A significant part of Kazakhstan population lives in the zone of harmful production factors direct influence, the main of which are the emissions of air pollutants. Due to the industrialization and urbanization rapid growth, the increase in the amount of waste consumed by the population is now becoming a serious problem throughout the world and a major problem for mankind. In Kazakhstan, which is currently experiencing a recovery in economic activity, the problem of municipal solid waste management is particularly acute. Currently, Kazakhstan has accumulated more than 31.72 billion tons (t) of waste, including about 120 million tons of solid domestic waste and about 2.5 billion tons of hazardous waste, 50 million posphorus containing waste, more than 2.5 million tons lead-zinc waste (Issayeva et al., 2020; Ministry of Ecology, Geology and Natural Resources of the Republic of Kazakhstan, 2021).

It is known that, along with increase of municipal solid waste volume, it also has serious implications for the environment (Ferronato et al., 2019), in African countries domestic waste and uncontrolled urban management are the main problems of the country, according to Naghel et al. (2022) especially in the Middle East and North Africa countries, in article Kebaili (Kebaili et al., 2022; Naghel et al., 2022) in its study conducted in Algiers, where there is an increase in domestic waste flow combined with a demographic surge. One of the most commonly dumped municipal solid waste is plastic bags and plastic items. These materials decompose over a long period of time with a decomposition period of up to 300 years or more. If not recycled, plastic waste can quickly cover much of the Earth's crust. To prevent this from happening, technologies have been developed to replace plastic waste with biological substances without compromising the environment. In many countries, waste separation and sampling packages and municipal solid waste are not available. At the same time, we can say that the industry for processing the recycled materials is not sufficiently developed. Existing municipal solid waste landfills are, in most cases, common landfills, often operating without proper permits, without complying with health and environmental regulations. There is little accounting for municipal solid waste accumulation. There is no impact monitoring of municipal solid waste landfills on the environment. This situation therefore calls for drastic measures to be taken to optimize all phases of municipal solid waste management (Yoada et al., 2014).

Overall, the development of effective methods for recycling solid domestic waste and celluloseis a key area of work to reduce the environmental impact of waste and ensure sustainable development of society. Implementation of biological recycling methods reduces the environmental load, ensures production of useful products and is an important step towards creating a closed resource cycle. New innovation in organic waste management involves development of new inoculum consortia that include bacterial species with high multiplication rates. Selecting a specific consortium with high scalability potential and introducing genetically modified organisms, *Pseudomonas strains*, is a proven potential and legitimate solution for large-scale organic waste management (Dubeyet al., 2023; Mihai, 2017; Rana et al., 2022; Skariyachanet al., 2022). Decomposition of organic solid waste using black fly larvae *Hermetiaillucens* L. was used in the work by authors Priscilia et al. (2020). Bioprocessing efficiency is high for any industrial waste. Bioprocessing of solid domestic waste, followed by bioresources, is one of countless sustainable energy options. According to literature and worldwide scientific studies, biosolutions from various microorganism impurities, such as *Aspergillus niger*, have been used in composting waste. *A. oryzae, Glyocladium* sp., *Trichoderma* sp., *Streptomyces griseus*, *S. globisporius*, *S. viridosporus*, *Bacillus cereus*, *B. subtilis* has had a positive effect (Selivanovskaya, 2009). As evidenced by the authors' studies, the use of *Lactobacillus sp.*, *B. azotofixans*, *B. megaterium*, *B. mucilaginosus*, *Trichoderma koningii*, *Streptomyces cellulosae* and *micromycetes* the causative agents of white rot have been demonstrated to be an effective method for accelerating degradation rates and stabilizing composted products (Chenebault et al., 2022). In addition, an integrated bioremediation approach using *Clostridium acetobutylicum* and

Enterobacter aerogenes (Ebrahimian et al., 2022; Saravanan et al., 2022) was applied for solid domestic waste biodegradation.

As the large concentration of landfills is becoming a serious environmental problem in today's world, we have conducted a study of the landfills' microflora, where solid domestic waste is accumulated and there is release of microorganisms, active in the decomposition of plastic substances.

The study of the microbial structure of landfill soil presents opportunities for discovering the microbial strains that are able to induce biodegradation, particularly of polyethylene, plastics, in domestic solid waste. A number of foreign studies show that *Bacillus*, related representatives of *Pseudomonas*, *Aspergillus tubengensis* can degrade polyethylene, plastic products, some studies indicate that the larvae of *Galleria mellonella* butterfly overcame 92 milligrams of polyethylene in 12 hours. Therefore, one of the main study objectives was to explore the soil microbiome of landfills and dumping grounds in the residential building yards of Shymkent, the study was aimed at examining the species composition of landfill soil microflora and determining the possibility of organic waste decomposition by microorganisms isolated from landfill soil through cellulolytic activity.

2. Methods and materials

Microorganisms isolated from the soil of the territory "Shymkent-Kokys polygons" located in Shymkent were used as the research object. Shymkent city landfill is located 50 kilometers from the city zone (42.182787597958956 n.l., 69.48165957487062 e.l.) (**Figure 1**). Soil samples were collected from 4 zones of Shymkent landfill in accordance with State standard requirements.

Figure 1. Map of the Shymkent-Kokys landfill.

Soil samples were collected for microbiological studies from 4 zones of the Shymkent landfill of Shymkent city in accordance with requirements of SST 17.4.4.02-2017. Sampling locations are marked as A1, A2, A3, A4. A1—Shymkent

—centre of the landfill, A2—Shymkent—around the landfill, A3—Point near the landfill, A4—Shymkent—landfill point after the route. The research was conducted in autumn 2023.

The elemental composition was determined by the method of atomic adsorption analysis on the spectrometer AAnalyst 800 (Perkin-Elmer) (STS R ISO 7523-2016).

Soil sampling was carried out aseptically using special samplers in accordance with methodological recommendations from depths of 10-50 cm. Soil sampling was carried out according to SST 17.4.4.02-2017. Nature conservation. Soils. Samples were taken from 4 points: points A1; A2., A3, A4.

2.1. Microbiological studies

Chapek's medium was used for the cultivation of *micromycetes*, which consisted of g/L of distilled water: sucrose-30.0 or glucose-20.0, $NaNO_3$-2.0; K_2HPO_4-1.0; $MgSO_4 \times 7H_2O$-0.5; KCl-0,5; $FeO_4 \times 7H_2O$-0.1.

Winogradskii I and II phase medium for nitrifying bacteria. The medium was made up of g/L of water, glucose-20.0; K_2HPO_4-1.0; $MgSO_4 \times 7H_2O$-0.5; $CaCO_3$-20.0; yeast extract-10.0, and 1.0 mL of microelement solution.

GRM nutrient medium was used for the cultivation of heterotrophic bacteria. The composition of the medium was as follows: pancreatic hydrolysate of fish meal-12.0 g; enzymatic peptone-12.0 g; NaCl-6.0 g, and microbiological agar 10.0 ±2.0 g.

Hutchinson's medium was used for cellulose-splitting microorganisms, composition (g/L) of distilled water: Pieces of filter paper (10g/L), $NaNO_3$-2.5; $FeCl_3$-0.01; K_2HPO_4-1.0; $MgSO_4 \times 7H_2O$-0.3; NaCl-0.1; $CaCl_2 \times 4H_2O$-0.1; pH to 7.2–7.3; by adding 20% Na_2CO_3 solution. The medium sterilized at 0.5 atm for 20–30 min.

Continuous aeration was part of the cultivation process. The scale of the brand 'Scout-Pro' was used for preparing the nutrient medium, and for sterilization, the autoclave of the brand SPGA-100-I-HH was used. Microorganisms were cultivated at 25 ℃ for a period of 7 days.

Taxonomic studies:

The taxonomic analysis of microorganisms was carried out using the "Determinant of Bacteria by Berge's B. and the determinant Sutton (Hoult et al., 1997; Sutton et al., 2001) The morphology of *micromycetes* was determined by colonies on Petri dishes. Colonies were characterized by their shape, cross-section, edges, texture, color, and pigment diffusion on agar.

The cellulolytic activity of microorganisms was determined in accordance with the methodology proposed by Netrusov (2005).

2.2. Genetic and molecular analysis of *micromycetes*

The analysis was performed in the laboratory of chemical and molecular-genetic methods of research and analysis of 'Scientific and Production Centre of Microbiology and Virology Centre' LLP (Almaty) using Next generation sequencing (NGS) on Illumina MiSeq sequencer.

3–7 days old strains of fungi were used. Mycelium was frozen at −20 ℃. It was then ground with a pestle in a 1.5 mL Eppendorff tube. DNA was isolated using PureLinkTM Microbiome DNA Purification Kit (Invitrogene, USA).

Variable V3 and V4 regions of the 16S rRNA gene were amplified using universal primers with the addition of Illumina adaptors, forward primer: 95′-CGTCGGGGCAGCAGCGTCAGATGTGTGTGTATAAGAGACAGACAGCCTACGGGGGNGGGWGCA G-3′ and 5′-GTCTCTCGTGGGGCTCGGAGATGTGTGTGTATAAGAGACAGGACAGGACTACHVGG GTATCTAATCC-3′ reverse primer.

The reaction mixture was composed of the following components: A total of 2.5 μL of DNA matrix, 5 μL of each primer at a concentration of 1 μM, and 12.5 μL of KAPA HiFi HotStartReadyMix (KAPA Biosystems, USA) were combined. Polymerase chain reaction (PCR) amplification was conducted on an Eppendorf MastercyclerProS thermocycler (Germany) using the following programme: The reaction mixture was subjected to an initial denaturation at 95 ℃ for 3 min, followed by 25 cycles of denaturation at 95 ℃ for 30 s, annealing at 55℃ for 30 s, and extension at 72℃ for 30 s. The final cycle was extended to 5 min at 72 ℃ for the completion of the extension phase. The PCR product was purified using an AgencourtAMPure PCR purification kit (Beckman Coulter Inc., USA). Subsequently, Nextera XT Index primer adapters (Illumina Inc., USA) were introduced to each sample. The concentration and size of the PCR product were determined through detection in an agarose gel and on an Agilent 2100 Bioanalyser (Agilent, Germany) using the Agilent DNA 1000 Kit. Each sample was adjusted to a 4 nm concentration and pooled. The sequencing was performed on an Illumina MiSeq instrument (USA) using the MiSeq® Reagent Kit v3 600 cycles (Illumina, USA), in accordance with the manufacturer's recommendations.

Subsequent analysis of the data was conducted using MiSeq® Reporter Software (Illumina). Identification of the microorganisms was achieved through analysis of the V3 and V4 regions of the 16S rRNA gene of bacteria in the International Greengenes database (http://greengenes.lbl.gov/).

The concentration of the DNA was determined in ng/μL using a Qubit 2.0 fluorimeter.

Statistical analysis of the results. Mathematical and statistical processing of the obtained data was carried out using the method of variation analysis using the Microsoft Office Excel software package (Schabenberger and Pierce, 2002).

3. Results and discussion

3.1. Number of microorganisms

Microbial titres were 1–10 CFU/g in soil samples collected around the landfill. Microbial titres in the deep soil horizons were 10^9 cL/mL A1, A2–A3 titres were in the samples. High indicators of microorganisms were found in all soil samples at a depth of 10–20 cm and 20–30 cm, which indicates that optimal conditions for microorganisms, oxygen balance and sufficient moisture, gas-air, biogenic elements exchange regime are favourable on horizontal soil plains (**Table 1**). There was a small number of microorganisms on the soil surface especially 0–5 cm, that was

explained by bactericidal action of sunlight and drying factor of wind erosion. Microorganisms' absence or rarity at 0–5 cm depth is assumed to be due to a whole complex of physico-chemical factors that are not favourable for microflora development. Microorganism composition in the soil varies by season: they are most common in late spring and early summer and autumn, and least common in winter. Microbial populations of soils in the areas of waste accumulation consisted of actinomycetes, *micromycetes*, heterotrophic bacteria, nitrifying, nitrogen-fixing bacteria, enterobacteria, as well as algae, protozoa.

Table 1. Microbiological composition around "Shymkent-Kokys polygons" LLP.

No.	Sampling areas	Microbial titre, CFU cL/mL			
		Micromycetes	**Heterotrophic bacteria**	**Nitrifying bacteria**	**nitrogen-fixing**
1	A1				
	0–10 sm	$(2.3 \pm 0.2) \times 10^3$	$(2.5 \pm 0.2) \times 10^2$	$(1.8 \pm 0.2) \times 10^2$	-
	10–20 sm	$(5.7 \pm 0.5) \times 10^6$	$(5.7 \pm 0.5) \times 10^5$	$(5.7 \pm 0.5) \times 10^5$	$(5.7 \pm 0.5) \times 10^5$
	20–30 sm	$(7.3 \pm 0.7) \times 10^7$	$(5.7 \pm 0.5) \times 10^6$	$(6.8 \pm 0.6) \times 10^9$	$(3.3 \pm 0.3) \times 10^4$
	30–40 sm	$(6.7 \pm 0.6) \times 10^4$	$(4.4 \pm 0.4) \times 10^3$	$(6.5 \pm 0.6) \times 10^3$	$(2.3 \pm 0.2) \times 10^2$
2	A2				
	0–10 sm	$(2.3 \pm 0.2) \times 10^3$	$(2.3 \pm 0.2) \times 10^3$	$(2.3 \pm 0.2) \times 10^3$	$(2.3 \pm 0.2) \times 10^3$
	10–20 sm	$(6.5 \pm 0.6) \times 10^6$	$(5.7 \pm 0.5) \times 10^7$	$(5.2 \pm 0.5) \times 10^5$	$(4.5 \pm 0.5) \times 10^4$
	20–30 sm	$(7.1 \pm 0.7) \times 10^9$	$(8.3 \pm 0.8) \times 10^9$	$(6.3 \pm 0.6) \times 10^6$	$(7.3 \pm 0.7) \times 10^7$
	30–40 sm	$(5.3 \pm 0.5) \times 10^3$	$(3.7 \pm 0.3) \times 10^4$	$(1.9 \pm 0.1) \times 10^2$	$(6.5 \pm 0.6) \times 10^8$
3	A3				
	0–10 sm	$(2.3 \pm 0.2) \times 10^3$	$(2.3 \pm 0.2) \times 10^3$	$(2.3 \pm 0.2) \times 10^3$	$(2.3 \pm 0.2) \times 10^3$
	10–20 sm	$(5.7 \pm 0.5) \times 10^6$	$(5.7 \pm 0.5) \times 10^7$	$(6.3 \pm 0.6) \times 10^8$	$(5.7 \pm 0.5) \times 10^5$
	20–30 sm	$(7.9 \pm 0.7) \times 10^9$	$(7.3 \pm 0.7) \times 10^9$	$(4.7 \pm 0.4) \times 10^7$	$(7.4 \pm 0.7) \times 10^7$
	30–40 sm	$(3.7 \pm 0.3) \times 10^2$	$(3.3 \pm 0.3) \times 10^2$	$(3.5 \pm 0.3) \times 10^2$	$(4.2 \pm 0.3) \times 10^3$
4	A4				
	0–10 sm	$(2.3 \pm 0.2) \times 10^3$	$(2.3 \pm 0.2) \times 10^3$	$(2.3 \pm 0.2) \times 10^3$	-
	10–20 sm	$(5.6 \pm 0.5) \times 10^6$	$(5.6 \pm 0.5) \times 10^7$	$(5.8 \pm 0.5) \times 10^5$	$(2.3 \pm 0.2) \times 10^3$
	20–30 sm	$(7.7 \pm 0.7) \times 10^9$	$(7.3 \pm 0.7) \times 10^9$	$(7.9 \pm 0.7) \times 10^7$	$(4.5 \pm 0.4) \times 10^8$
	30–40 sm	$(1.6 \pm 0.1) \times 10^5$	$(1.2 \pm 0.1) \times 10^3$	$(1.7 \pm 0.1) \times 10^3$	$(1.3 \pm 0.1) \times 10^2$

-: undetected.

The microbial population of the soil of "Shymkent-Kokys polygons" consisted of actinomycetes, *micromycetes*, heterotrophic bacteria, nitrifying, nitrogenfixing bacteria. The taxonomic composition of soil microorganisms is mainly represented by heterotrophic bacteria and *micromycetes*.

Microflora of the soil around the landfill, where the waste was accumulated, was detected in a large number of *Bacillus* genus species of heterotrophic bacteria, as well as *Brevibacterium* sp., *Pseudomonas* sp., *Micrococcus* sp., *Rodococcus* sp., representatives of *Enterobacteriaceae* genus *Salmonella, Klebsiella, Streptococcus,* representatives of *Staphylococcus* relatives, microscopic fungi *Mucorales, Aspergillus, Fusarium* and *Candida* were found. Particularly the number of nitrogen-fixing bacteria was found in A1 and A2 from the predominant pathogen species *Fusarium* sp. and *Aspergillus* sp. and the first phase nitrifying bacteria *Nitrosomonas*, the number of nitrogen-fixing bacteria was found in samples A3 and A4, and a small number in sample A1 and A2 sample in soil ecosystems

contaminated by municipal and livestock wastes, representatives of genus *Fusarium*, from bacteria *Escherichiacoli* (*E. coli*), *Streptococcus* sp., *Staphylococcus* sp. were dominant. A lot of them were found at point A2, the titre of which was 10^7 CFU/mL. CFU High titre of heterotrophic bacteria, endobacteria and *micromycetes* was found in samples A1 and A2, A3 from 10^8 to 10^9 kL/mL. In samples A1–A4, the titre ranged from 10^6 to 10^9 cL/mL, and in control samples -10^9 cL/mL.A decrease in the amount of microorganisms depending on depth is associated with an increase in limiting factors impact: deterioration of gas-air regime, decrease in temperature and humidity level. Lack of some bacteria indicates the thickness of soil, absence of microbiological processes of natural conditions, which is confirmed by weakly alkaline indicators of pH 6.5–6.8. The prevailing microorganisms around the waste containers disposed by the citizens were representatives of *Aspergillus* genus, followed by *Fusarium* sp. and *Penicillium* sp. representatives constituted from 17 to 48%, and representatives of other relatives −23% (**Figure 2**).

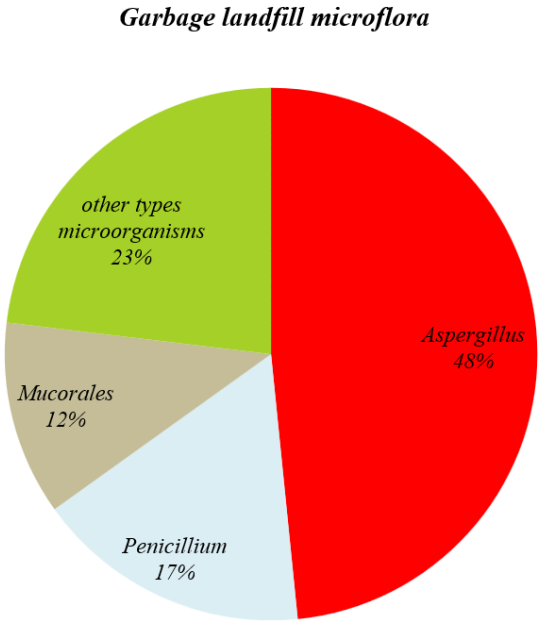

Figure 2. Microorganism indicator.

The microflora of soil samples around the polygons consisted of the following microorganisms' groups: *micromycetes*, actinomycetes, heterotrophs, phase I–II nitrifiers, nitrogen-fixing bacteria.

3.2. Taxonomic and micromorphological characteristics of isolated microorganisms

Fusarium: Colonies are flaky (cotton-like), white to pink and red at first. Hyphae are septate, colourless. Conidiophores yielding microconidia are simple or branched.

Aspergillus: Colonies are pure white to slightly yellowish, woolly to velvety. The rate of growth is moderate. Hyphae are septate, colourless. Conidial heads are spherical in the beginning, with radial arrangement of conidia chains.

Penicillium: Colonies are grey-birch, to pistachio-coloured, 34–35 mm in diameter, with radial grooves, velvety, greyish turquoise centre and white periphery. Hyphae are septate, colourless.

Candida sp.: agar develops white, large, creamy, rounded colonies on the surface. Cells present oval-shaped buds, with pseudohyphae and hyphae divided into septa. Aerobes. The budding occurs at 25–27 ℃. Yeast and pseudohyphal cells are formed at 25–27 ℃. Surface of colonies is elevated, shining, transparent, with different colours.

Trichoderma sp.: cultivated on agar exhibited a high growth rate. The colonies initially exhibited a white colouration, which subsequently transformed into an olive-green hue by the fourth day. A loose, felt-like, colourless mycelium formed at the colony's centre, accompanied by an increase in the development of aerial mycelium towards the periphery. Pigmentation was observed. The hyphae were septate, transparent, and smooth-walled, measuring 3.5 μm in diameter. The conidiophores displayed a relatively simple branching system.

Bacillus sp.: the colonies are small and round with convex edges and a smooth surface. They are 2–3 mm in diameter and have a milky colouration. The structure is transparent and homogeneous, with a soft consistency. The cells are bacilliform, measuring 1 mm in diameter, and display motile characteristics. They are Gram-positive.

Pseudomonas sp.: colonies with a rounded shape, 1–2 mm in size, grow in GRM nutrient medium. Stems of dark pale yellow colour, elevated, elastic in consistency, 3–5 mm in size, straight or curved shaped cells, 0.5–1.0 × 1.5–5.0 μmin size. Negative by Gram. It does not move. Aerobe. Optimal seed temperature is 4-35 ℃.

Micrococcus sp.: crops form small round convex colonies with a smooth and even edge on GRM, 1–4 mm in diameter, cream-coloured, non-transparent, with a homogeneous structure. Cells are coccoid, solitary. Cells immobile, gram positive, non-spore-forming. Aerobe, it grows in the range of 10–45 ℃. Optimum temperature is 28–32 ℃.

Escherichia coli: colonies are yellow-cream-coloured, edges are even, texture is soft. Gram-negative, cells bacilliform, large, facultative anaerobes.

Enterobacter sp.: colonies grow on Endo medium with yellow, pink-yellow colour formation, colonies are round, edges of colonies are smooth, size 2–2.7 μm, colonies are slimy, with or without metallic lustre. Cells are straight, 0.6–1.0 μm in diameter, with peritrichial arrangement of flagella, some strains contain a capsule; non-spore-forming. Aerobe or facultative anaerobe. Optimal growth temperature is 37–38 °C.

Rodococcussp.: colonies grow on a hard agar surface in a rounded shape, with smooth (sometimes rough) edges and soft texture. Colonies are bright cherry red, light yellow, yellowish-brown, brown, red-yellow, sometimes colourless. Gram positive, immobile, acid resistant, and does not form spores. Cellular forms have sticks, cocci (coccabacilli), branched vegetative myceliums. Pleomorphic, morphological forms are changeable. It can grow to +30 ℃, +10 ℃, +40 ℃ during incubation period. pH medium 6–7. The most important feature is a three-stage

morphogenetic developmental cycle (cocci-bacilliform, filamentous or branched cells-cocci). Incubation seeding time is 48 h.

Klebsiela, Clostridium, Pseudomonas, Xanthomonas, Alcaligenes, Caulobacter, Achromobacter, Stenotrophomonas, Azotobacter were identified as bacteria involved in the processes of decomposition in organic substances.

Typical soil inhabitants of "Shymkent-Kokys polygons" are species of heterotrophic bacteria genera *Bacillus*sp.,*Brevibacterium*sp.,*Pseudomonas*sp., *Micrococcus* sp., *Rodococcus sp., Enterobacteriaceae,* representatives of the genus *Mucorales, Aspergillus, Fusarium, Candida, Penicillium, Trichoderma* from microscopic fungi (**Figure 3**), especially at points A4 and A3 dominant species of pathogenic microorganisms *Fusarium* sp. and conditionally pathogenic *Aspergillus* sp., as well as from nitrifying bacteria of the first phase of *Nitrosomonas* sp., the number of nitrogen-fixing bacteria was found in samples A1 and A2, small amounts A3.

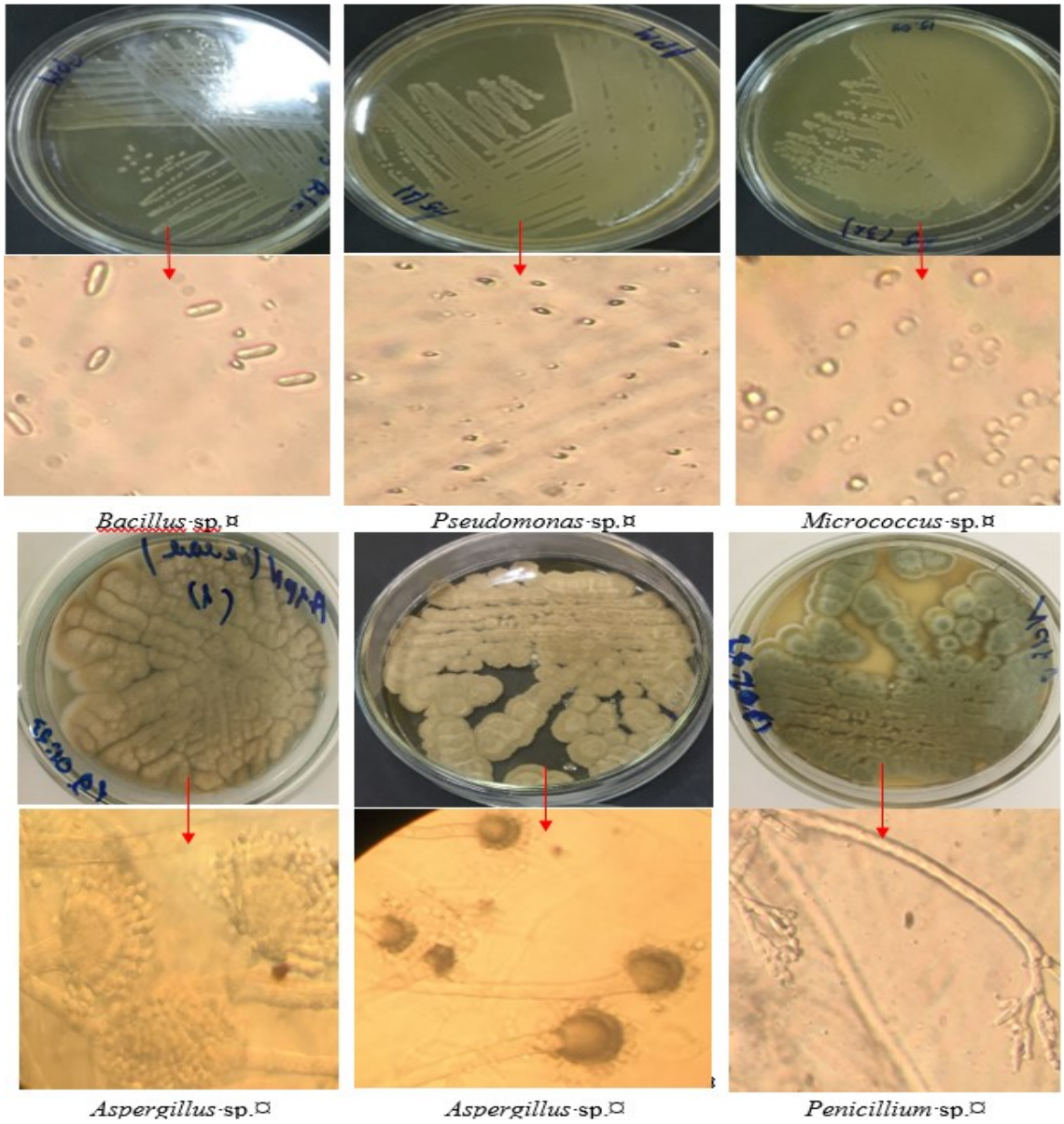

Figure 3. Colonies of microorganisms in isolated soils of the Shymkent-Kokys landfill.

As a result of microbiological studies, a total of 30 isolates were separated, of which 16 (53.3%) isolates were classified as bacteria, which corresponds to 50% of the total number of separated microorganisms, 3 (10%) isolates were classified as actinomycetes and 11 (36.6%) isolates belong to *micromycetes*.

3.3. Genetic identification of *micromycetes*

Amplification with ITS primers resulted in a PCR product of approximately 550 bp (**Figure 4**).

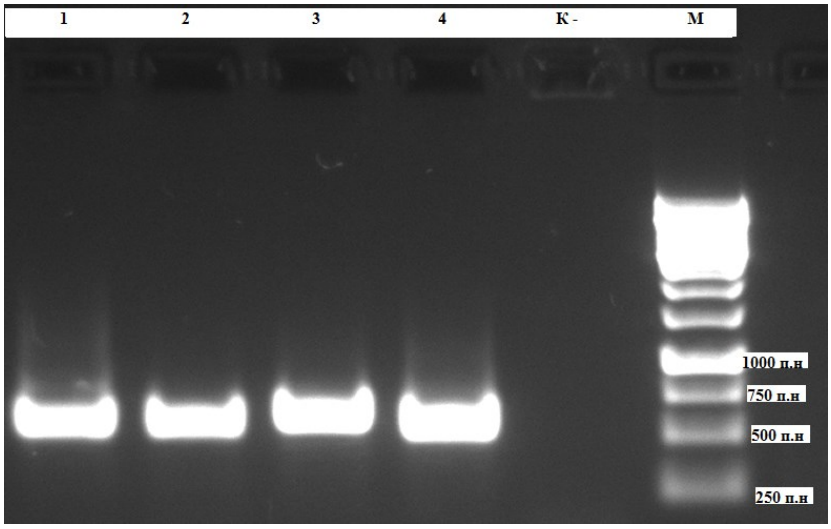

Figure 4.PCR product obtained with ITS primers.

Note: 1–4—fungi samples; M—DNA marker 1 Kb.

As a result of the analysis, the nucleotide sequence of the ITS-region of the studied fungal samples was obtained. The obtained data were compared with the data from the NCBI International database. A phylogenetic tree was constructed with the closest related strains allowing taxonomic identification of the studied strains.

KPMA2 *Trichoderma polysporum.*

Nucleotide sequence obtained by sequencing the ITS region:

GCGTCGCAGCCCCGGACCAAGGCGCCCGCCGGAGGACCAACCAAAA
CTCTTTTGTATGTCCCCTCGCGGACTTTTATAATTCTGAACCATCTCGGCG
CCCCTTGCGGGCGTTTCGAAAATGAATCAAAACTTTCAACAACGGATCTC
TTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCG
CCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAAC
CCCTCCGGGGGGTACGGCGTTGGGGATCGGCCCTTTACGGGGCCGGCCCC
GAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCAC
ACTCGCATCGGGAGCGCGGCGCGTCCACGTCCGTAAAACACCCAACTTCT
GAAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATA

Phylogenetic tree constructed by comparing the ITS region of the study sample with the sequences of reference strains deposited in the International Blast database. The degree of homology with the closest strain M T072083.1:76-569 *Trichoderma polysporum* QH09-16 was 100%, which allows us to assign the studied strain to this species.

KPMA3—*Trichoderma harzianum.*

Nucleotide sequence obtained by sequencing the ITS region:

GCCCCGGACCAAGGCGCCCGCCGGAGGACCAACCAAAACTCTTTTT
GTATACCCCCTCGCGGGTTTTTTATAATCTGAGCCTTCTCGGCGCCTCTCG
TAGGCGTTTCGAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTC
TGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTAT
TCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCG
GGGGGTCGGCGTTGGGGATCGGCCCTCCCTTAGCGGGTGGCCGTCTCCGA
AATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAC
TCGCATCGGGAGCGCGGCGCGTCCACAGCCGTTAAACACCCAACTTCTG
AAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATC

a) Phylogenetic tree constructed by comparing the ITS region of the tested sample with the sequences of reference strains in the International Blast database.
b) The degree of homology with the closest strain MK738146.1:111-605 *Trichoderma harzianum* isolate Mc2151 was 100.00%.
c) As a result of the research, a strain of microorganisms with high activity to hydrocarbons was isolated and identified from the landfill soil. Based on the above data it should be concluded that microorganisms promising for polyethylene waste utilisation have been isolated.

3.4. Screening and selection of microorganisms

In the course of research work, the following 10 strains were selected from 30 pure isolates of microorganisms for use in biocomposting SDW: KPA1, KPA2, KPA3, KPA4, KPA5, KPMA1, KPMA2, KPMA3, KPMA4, KPA2M1.

Table2. Changes in the number of bacteria and *micromycetes* at different doses of natamycin.

Growth time	**Natamycin concentration mg/L**			
	Control	**100**	**300**	**800**
Exposure time	Number of heterotrophic bacteria CFU/mL			
1–5 watch	3.4 ± 0.2	*	*	*
3 days	5.7 ± 0.6	4.5 ± 0.6	5.8 ± 0.6	6.6 ± 0.6
7 days	4.1 ± 0.2	4.9 ± 0.2	5.1 ± 0.2	4.1 ± 0.2
15 days	5.5 ± 0.3	5.6 ± 0.3	6.5 ± 0.3	7.5 ± 0.4
30 days	5.2 ± 0.3	5.8 ± 0.3	7.2 ± 0.3	7.2 ± 0.4
	Number of *micromycetes* CFU/mL			
1–5 watch	0.94 ±0.3	*	*	*
3 days	0.8 ±0.4	0.7 ± 0.3	0.8 ± 0.4	-
7 days	2.5 ±0.2	0.7 ± 0.3	0.3 ± 0.2	-
15 days	0.4 ±0.3	0.5 ± 0.3	0.2 ± 0.1	-
30 days	0.9 ±0.4	0.8 ± 0.4	0.1 ± 0.1	-

* no determination was made; - no growth.

As the basic composition of solid domestic waste an organic waste consists of cellulose, only selected bacterial strains with a high capacity to degrade cellulose are supposed to be used for composting. It has been suggested that the use of micro-mycetes in organic waste composting can cause many problems, cause phytopathogenic diseases in plants, and have toxic effects on the product they produce, as evidenced by numerous worldwide studies (Bren et al., 2023; Liu et al., 2023; Novikovaet al., 2023). To inhibit the growth of *micromycetes*, the bacteria were inoculated in Getchinson's nutrient medium including different concentrations of natamycin (**Table 2**).

As a result, micromycete growth at a concentration of 800 mg natamycin, during 7–15 days, was completely stopped. Accordingly, a high growth titer of heterotrophic bacteria was observed. Further, the activity of cellulose cleavage ability of heterotrophic bacteria was investigated. During 3–15 days, the number of cellulose-active microorganisms was calculated using the limiting cultivation method. The highest cellulose bacterial activity was observed during 15 days of cultivation and their total titer was 10^7–10^8. This was due to creation of certain selective conditions. Representatives of the genus *Bacillus* with high cellulose activity using selected bacterial strains were selected. In this regard, the next work stage involved screening for cellulose activity among selected strains of related *Bacillus* and *Pseudomonas* species. The bacteria were cultured in Getchinson's medium for 15 days. Cellulose activity was determined in the culture fluid at 3, 7 and 15 days. The highest cellulose activity was 95%–97% in strains KPA1, KPA5, with medium cellulose activity of KPA3, KPA4, KPA2—71–82%, KPA1, KPA5 amounted to 0.85 mg of glucose in 1 mL, which was 3.5% ± 0.4% times higher than that of all strains tested.

By species identification the strains KPA1, KPA2 belong to *Pseudomonas* sp. strains KPA3 and KPA4, KPA5—*Bacillus* sp. Besides heterotrophic bacteria, a group of *micromycetes* also possess cellulose properties, but to decompose the organic matter of compost mixtures and to increase the humus content, it was assumed to get a preparation consisting only of bacteria, without the use of *micromycetes*. It is well known that micro-mycetes have cellulose properties, but the study results show that *Pseudomonas* sp. KPA1, KPA2 strains and *Bacillus* sp. KPA3, KPA4, KPA5 strains have high fungicidal properties when their antagonistic abilities were studied. The micromycete group KPMA1, belongs to the genus *Penicillium* sp., KPMA2—*Trichoderma polysporum*andKPMA3—*Trichoderma harzianum* strain, KPMA4 *Aspergillus* sp. and KP2M1 strain is classified as *Fusarium* sp. Microorganisms extracted from the soil of Shymkent city landfills will be studied in the future to explore the possibility of obtaining biological preparations.

4. Conclusion

The microflora composition of the household waste landfill in Shymkent consists of *micromycetes*, heterotrophic bacteria, nitrifiers of I–II phase, denitrifiers, and actinomycetes. It was found that the indicator of microorganisms' distribution is high at depths of 10–20 cm and 20–30 cm in the horizontal plane of soil.

A total of 30 pure isolates were obtained from the soil microflora of Shymkent landfill. Of these, 16 were bacteria, representing 53.3% of the total, including nitrogen-fixing, nitrifying, and dinitrifying bacteria. Additionally, three actinomycetes (10%) and 11 *micromycetes* (36.6%) were also isolated. Following screening and selection procedures, strains with high biotechnological potential for biocomposting were identified.

As a result of the research, it was found that strains of microorganisms KPA1, KPA2 *Pseudomonas* sp. strains KPA3, KPA4, KPA5 *Bacillus* sp. extracted from soils on the territory of "Shymkent-Kokys" are able to utilize domestic wastes with high cellulose content. When cultivating these strains of microorganisms for 3, 7 and 15 days, it was found that strains KPA1, KPA5 utilize cellulose-containing organic waste up to ±95.2%–±97.8%.

Author contributions: Conceptualization, AA, SK and AZ; methodology, AT; software, AA, SK and SA; validation, KT, AA and AI; formal analysis, AA, SK and BK; investigation, AT; resources, KT, AA and AI; data curation, AA, SK and AI; writing—original draft preparation, AA, AT and SK; writing—review and editing, SK and AT; visualization, AA, SK, AI and BK; supervision, AZ, AS and AA; project administration, AT; funding acquisition, AA, SK and AT. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

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