

# Efficiency of Specific Biopreparations in Organic Waste Management

Lilia E. Matrosova<sup>1</sup>, Mikhail Ya. Tremasov<sup>2</sup>, Yuliya V. Cherednichenko<sup>2</sup>, Elena L. Matveeva<sup>1</sup>, Alexander A. Ivanov<sup>2</sup>, Malik N. Mukminov<sup>3</sup>, Arkadiy V. Ivanov<sup>2</sup> and Eduard A. Shuralev<sup>3\*</sup>

<sup>1</sup>Institute of Economics, Management and Law, 42 Moskovskaya St, Kazan, Tatarstan, 420111, Russian Federation; mehrdad.khosroo@gmail.com, Elena.L.Matveeva@yahoo.com

<sup>2</sup>Federal Center for Toxicological, Radiation and Biological Safety, Nauchny Gorodok-2, Kazan, Tatarstan, 420075, Russian Federation; Mikhail.ya.12@gmail.com, Yuliya.V.Cherednichenko@gmail.com, AlexanderA.Ivanov@yahoo.com, ArkadiyV.Ivanov@yahoo.com

<sup>3</sup>Institute of Environmental Sciences, Kazan Federal University, 18 Kremlyovskaya St, Kazan, Tatarstan, 420008, Russian Federation; MalikN.Mukminov@gmail.com, eduard.shuralev@mail.ru

## Abstract

**Background:** Biological approach is becoming more popular in the protection of the environment by organic waste of agricultural holdings due to economic efficiency and absence of additional damage to the ecosystem. Microorganisms of biopreparations possess significant fermentative properties and high antagonistic activity against of many pathogenic and opportunistic bacteria and toxigenic filamentous fungi. In our study we have isolated millions of coliform bacteria and Enterococci, thousands of Salmonella, Proteus and Staphylococci in 1 g of initial substrate. After the usage of biopreparations the quantity of coliform bacteria and Enterococci was less than 10 cells/g, and no Salmonella, Staphylococcus or Proteus were found. Application of the biopreparations has prevented the loss of nutrients. Therefore, nitrogen content was higher by 50-60% than in the control. The quantity of nitrifying, ammonifying and cellulose-fermenting microorganisms increased by 15.9%, 6.6% and 15.4%, respectively. Productivity of grain and vegetable crops increased by 10-20%. An important advantage of the biopreparations usage, that also we found, is the elimination of specific odor within a couple days due to the ability of their microorganisms to assimilate nitrogen from urea and neutralize the substrate against the bacteria causing putrefaction, anaerobic processes and the emission of ammonia and hydrogen sulphide. **Conclusion:** The application of developed new biopreparations will allow producing high-quality environmentally friendly agricultural products.

**Keywords:** Environment, Fertilizer, Harvest, Manure, Microorganisms, Soil

## 1. Introduction

The rapid growth of the world population, undoubtedly, requires the implementation of scientific and technological advances in all areas of production and human activities as well as intensive development of industry and agriculture, which ultimately deteriorates the environmental quality<sup>1,2</sup>. Anthropogenic pollution of the environment has become a serious problem and has acquired a global scale. Every year, soil degradation and

destruction of soil structure tend to increase, which is accompanied by a decrease in abundance and imbalance of representatives of micro- and meso-fauna. Frequent uncontrolled use of mineral fertilizers aimed at increasing the productivity of agricultural crops disturbs the stability of agroecosystems. Since mineral fertilizers contain substantial amounts of toxic elements, their systematic use increases the content of heavy metals in soils.

The soil fertility is reduced simultaneously with the accumulation of large amounts of manure around

\*Author for correspondence

livestock enterprises, which constitutes epizootological danger and spreads bad smell over large areas<sup>3-5</sup>. After the use of organic waste, soil is substantially contaminated with microflora, which creates a certain environmental and sanitary hazard. It was noted that the use of unprocessed manure is impractical because the losses of nitrogen in it during the storage for 2–3 months reach 50–60% and more.

Many of the methods proposed for organic waste management are not environmentally friendly and lead to the formation of secondary products that are often even more dangerous. The most appropriate, efficient, and environmentally friendly technologies are the biological methods, in particular, those that use the potential of microorganisms<sup>6,7</sup>. A number of original articles describe the possibility of using microorganisms for organic waste management<sup>8-10</sup>. Microorganisms synthesize enzymes and other biological compounds that decompose organic matter. It was confirmed that the technologies based on microbial communities are less expensive than the conventional methods. The production and use of preparations based on them do not lead to environmental pollution. The development of microbial technologies for managing organic waste with its subsequent use as fertilizers will allow efficient use of valuable biological resources.

The key role in the destruction of organic matter is played by bacteria, yeast, and filamentous fungi. Microorganisms are involved in the decomposition of organic matter and soil self-purification, formation of soil fertility, transformation of humus, and balancing of minerals and cellulose. It is also known that microorganisms are involved in the destruction of many organic compounds that are environmental pollutants<sup>11-13</sup>.

The Federal Center for Toxicological, Radiological and Biological Safety (Kazan, Russia) performs studies on the development of technological methods for the processing of organic waste and its use as a fertilizer. Microorganisms of different taxonomic groups were purposefully isolated from natural biotopes (dung, manure, soil, waste water, etc.), and the optimal compositions for utilization of organic matter were selected. During the screening, special attention was given to the preservation of the viability of destructive microorganisms after their inoculation in different substrates. On the basis of microbial consortia, biopreparations and utilization technology with the use of different types of organic materials for processing were developed.

The technological process of utilization consists in a multilayered addition of the biopreparation into the entire volume of deposited substrate (litter manure and semiliquid slurry manure with a moisture content of 86–92%). In the case of liquid manure with a moisture content of 92–97% and manure waters with a moisture content of over 97%, the biopreparation is added immediately into the collector, cesspool, or treatment facility tank. This paper describes the results of using the biopreparations developed at the Center for organic animal waste management.

## 2. Materials and Methods

Litter and liquid poultry manure was treated with the biopreparations UF-1 and Ecos, differing in the microorganism consortium composition. For testing, manure piles (500 tons) were formed. A layer of stockpiled manure (0.5m high) was uniformly moistened with biopreparations in a proportion of 10 ml per ton substrate. The preparations were preliminarily diluted with water in a ratio of 1:1,000. Then, the treated surface was coated with substrate, and the procedure was repeated. To evaluate the efficiency of biopreparations, samples of the surface and deep layers of the piles were taken every 10 days and subjected to physicochemical and microbiological studies. The mass fractions of moisture and dry residue were determined by the conventional procedure; the pH value was measured potentiometrically. The total nitrogen content in the substrates was determined according to Kjeldahl. This method is based on the mineralization of the fertilizer by heating with concentrated sulfuric acid in the presence of hydrogen peroxide, followed by distilling off the ammonia into a boric acid solution and subsequent titration with sulfuric acid. The total phosphorous and potassium content was determined photometrically after the mineralization of the substrate by heating with concentrated sulfuric acid in the presence of hydrogen peroxide. The organic matter and ash content was determined thermogravimetrically. The principle of this method consists in measuring the weight loss of an organic fertilizer sample after calcination at 8000°C. Toxic elements were determined by atomic absorption spectrometry with a Perkin Elmer Analyst spectrometer, mercury was detected by the cold-vapor atomic absorption spectroscopy, arsenic was measured colorimetrically, and pesticides were assayed by gas liquid chromatography. The sanitary and microbiological assessment of manure was performed on the

basis of the results of determination of the total microbial contamination and the presence of coliform bacteria, *Enterococcus*, *Salmonella*, and *Staphylococcus*. The number of microorganisms of various groups was determined by sowing a homogenized substrate by serial dilutions on elective culture media with subsequent counting the grown colonies. Microorganisms were identified using the Maldi Biotyper system. The effect of the obtained fertilizer on the agrochemical properties of soil and the grain yield was studied in a field experiment performed on gray forest moderately loamy soil (humus 2.7%,  $P_2O_5$  182.0mg/kg,  $K_2O$  131.0mg/kg, hydrolytic acidity 2.9 mg eq/100 g, total absorbed bases 20.8 mg eq/100 g, and soil base saturation 95.1%). The mineral fertilizer  $N_{120}P_{90}K_{75}$  and the cattle manure (with an equivalent nutrient content) were used for comparison. The pH value and the hydrolytic acidity of soil samples were determined potentiometrically, and the amount of absorbed bases was measured by titration. Mobile phosphorus and potassium compounds were determined according to Kirsanov by extracting them from soil with an acetic acid solution.

### 3. Results and Discussion

The study of the sanitary-bacteriological state of the samples of liquid manure (moisture content  $71.15 \pm 2.96\%$ ), which were taken in different sites, showed a high degree of their microbial contamination. The manure contained coliform bacteria, *Enterococcus*, *Salmonella*, *Staphylococcus*, and *Proteus*. The treatment of manure with the biopreparations significantly reduced its bacterial contamination. A significant decrease in the number of coliform bacteria after the treatment with UF-1 (major component – *Streptomycetes*) was observed on day 10 of the experiment. At the end of the experiment, on day 30, the amount of coliform bacteria was less than 10 cells/g.

A similar tendency was observed for *Enterococcus*, *Salmonella*, *Staphylococcus*, and *Proteus*, the quantity of which also decreased. For example, the amount of *Enterococcus*, *Salmonella*, *Staphylococcus*, and *Proteus* in the treated manure on day 10 of the experiment was lower than in the untreated substrate by 2.5, 3.5 and 1.0 log CFU. On day 20 of the experiment, no *Salmonella*, *Staphylococcus*, and *Proteus* in the treated manure were found. The number of *Enterococcus* on day 10 was less than 100 CFU per 1g of substrate and decreased to a level of 10 cells/g on day 30 of the experiment.

When the poultry manure was treated with the biopreparation Ecos (major component - bacteria of the genus *Bacillus*), the number of coliform bacteria, *Enterococcus*, *Salmonella*, *Staphylococcus*, and *Proteus* on day 10 decreased by 2.0, 1.0, 4.0, and 1.0 log CFU, respectively. On day 20 of the experiment, no *Salmonella*, *Staphylococcus*, and *Proteus* in the treated manure were found. The amount of coliform bacteria and *Enterococcus* on day 20 was lower than in the control by 4.0 log CFU and became less than 10 cells/g on day 30 of the experiment. Throughout the study period, no significant changes in the number of enterobacteria and staphylococci in the control (untreated) samples were detected. On day 30 of the study, 1g of the substrate contained more than 100,000 CFU of coliform bacteria, *Enterococcus*, and *Salmonella* and more than 100 CFU of staphylococci and *Proteus*. The substrates treated with the biopreparations represented a loose dark brown mass, without characteristic specific odor and a slight amount of impurities. The control samples represented a conglutinated brown mass with a characteristic peculiar smell, containing high quantities of undecomposed substrate and impurities. The physicochemical analysis of the treated liquid manure showed that it contained high quantities of nutrients as compared to the untreated manure. For example, the content of nitrogen in the substrate treated with UF-1 and Ecos was 5.47% and 4.80%, which was greater than in the control sample by 50.1% and 41.7%, respectively ( $p < 0.001$ ).

In the next experiment, we performed utilization of the litter poultry manure (moisture content  $49.80 \pm 1.46\%$ ). Sawdust was used as a bedding material for housing the poultry. The baseline level of the coliform bacteria, *Enterococcus*, and *Salmonella* was more than 3.8, 2.7, and 2.0 log CFU/g, respectively. The bacteriological analysis of the substrate treated with UF-1 on day 10 showed a reduction in the number of coliform bacteria and *Enterococcus* by 1.5 log CFU, relative to the control. On day 15, these values decreased by 2.4 and 1.9 log CFU, respectively. The number of coliform bacteria on day 20 of the experiment was less than 10 cells/g. Enterococci and *Salmonella* were not detected in the treated substrate. When the substrate was treated with the biopreparation Ecos, the number of coliform bacteria and *Enterococcus* decreased to a level of 10 cells/g on day 25 of the experiment. Changes in the number of enterobacteria in the control variant were not observed throughout the study period. The use of biopreparations prevented the loss of nutrients in the treated

substrate. The content of nitrogen after the treatment with UF-1 and Ecos was higher than in the control by 60.0% and 55.0%, respectively ( $p < 0.001$ ) (Table 1). Significant changes in the content of phosphorus and potassium were not detected.

Atomic absorption and colorimetric analysis showed that the treated manure was not a source of contamination of crop products with hazardous toxic compounds. The content of toxic elements was at the level of maximum permissible concentrations. The residual quantities of pesticides bayleton and arcerid were not detected. The fertilizer obtained on the basis of liquid manure was tested in field trials. After the addition of the fertilizer in the soil, the content of mobile phosphorus ( $P_2O_5$ ) and exchangeable potassium ( $K_2O$ ) increased, the hydrolytic and exchangeable acidity decreased, and the base saturation of the soil increased. By the influence on the nutrient regime (content of mobile phosphorus  $P_2O_5$  and exchangeable potassium  $K_2O$ ) and the physicochemical properties of soil (hydrolytic and exchangeable acidity and soil base saturation), the obtained fertilizer at a dose of 6 t/ha was identical to 20 t/ha of the untreated manure (Table 2).

It is known that the biological state of soil is characterized by the abundance of the major groups of filamentous

fungi and bacteria, which have a positive effect on its fertility. It was of interest to estimate the effect of the obtained fertilizer on the population level of cellulolytic organisms and ammonifying and nitrifying bacteria. It was found that the addition of the obtained fertilizer markedly stimulated the growth of microorganisms involved in the conversion of carbon- and nitrogen-containing compounds. The use of fertilizers obtained as a result of treatment of poultry manure with the biopreparations UF-1 and Ecos accelerated organic matter mineralization, as evidenced by the increase in the population level of the ammonifying bacteria assimilating mineral sources of nitrogen and its organic forms. The number of nitrifying and ammonifying bacteria and cellulolytic microorganisms after the addition of the fertilizer obtained as a result of the treatment of manure with UF-1 at a dose of 6 t/ha, was greater by 15.9% ( $p < 0.05$ ), 6.6%, and 15.4%, respectively ( $p < 0.05$ ), than that after the addition of manure (20 t/ha) and greater by 34.2% ( $p < 0.001$ ), 11.8%, and 32.6% ( $p < 0.001$ ) than after the addition of the mineral fertilizer. After the addition of the fertilizer obtained by treating with Ecos, the number of nitrifying and ammonifying bacteria and cellulolytic microorganisms was greater by 13.4%, 5.1%, and 12.2% than after the addition of

**Table 1.** Physicochemical composition of the litter poultry manure treated with the biopreparations

Parameter	Variant		
	UF-1	Ecos	Control
Mass fractions of moisture, %	43.10 ± 5.04	44.50 ± 8.16	45.70 ± 6.95
pH	5.48 ± 0.12	5.50 ± 0.08	5.51 ± 0.11
Total nitrogen content, %	5.25 ± 0.13 *	4.67 ± 0.25 *	2.10 ± 0.12
Total phosphorus content, %	2.60 ± 0.31	2.50 ± 0.22	2.50 ± 0.36
Total potassium content, %	2.00 ± 0.25	1.90 ± 0.28	1.90 ± 0.19
Mass fraction of organic matter calculated for carbon, %	41.40 ± 0.36	40.96 ± 0.24	40.00 ± 0.35

\*  $p < 0.001$

**Table 2.** Effect of obtained fertilizers on the agrochemical properties of soil

Parameter	Variant			
	$N_{120}P_{90}K_{75}$	Manure, 20 t/ha	Fertilizer, 6 t/ha (UF-1)	Fertilizer, 6 t/ha (Ecos)
$P_2O_5$ , mg/kg	209.00 ± 6.11	212.00 ± 7.03	214.00 ± 7.29	215.00 ± 7.36
$K_2O$ , mg/kg	154.00 ± 5.43	157.00 ± 5.69	158.00 ± 5.78	158.00 ± 5.76
Hydrolytic acidity, mg eq/100 g	2.50 ± 0.08	2.30 ± 0.06	2.20 ± 0.02	2.10 ± 0.02
Exchangeable acidity (pH)	5.10 ± 0.11	5.10 ± 0.13	5.06 ± 0.10	5.06 ± 0.10
Soil base saturation, %	96.40 ± 3.82	96.80 ± 3.97	97.00 ± 4.05	97.10 ± 4.20



manure (20 t/ha) or by 27.7% ( $p < 0.001$ ), 6.7%, and 24.9% ( $p < 0.001$ ) than after the addition of the mineral fertilizer. The total microbial count after the addition of the fertilizer based on UF-1 and Ecos was greater by 12.9% and 8.3%, respectively, than after the addition of manure and greater by 35.3% and 29.8%, respectively ( $p < 0.001$ ), than after the addition of the mineral fertilizer.

Due to the effect and after effect of the organic fertilizer used in doses of 3, 6, and 9 t/ha, the total yield of spring wheat, annual grasses, barley and oats as a result of crop rotation for 4 years increased by 10.6, 18.4, and 25.9 centner/ha of grain units, respectively (UF-1-based fertilizer) and by 9.9, 17.9, and 24.7 centner/ha of grain units, respectively (Ecos-based fertilizer). The cattle manure added in this period in a dose of 20 t/ha increased the crop yield by 17.5 center /ha of grain units, which corresponds to the yield increase resulting from the addition of the organic fertilizer in a dose of 6 t/ha. The addition of the mineral fertilizer in a dose corresponding to the content of elements in 6 tons of the organic fertilizer increased the yield by 10.4 centner/ha. The fertilizer had a positive influence on the quality of grain crops. After the addition of the obtained fertilizer in a dose of 6 t/ha, the grain protein content of the spring wheat was 14.1% (UF-1-based fertilizer) and 14.0% (Ecos-based fertilizer) versus 13.8% after the addition of 20 t/ha manure. The potato yield after the use of the fertilizers based on UF-1 and Ecos was higher than after the use of the mineral fertilizer by 20.0% and 19.2%, respectively ( $p < 0.01$ ).

## 4. Conclusion

The results of this study show the effectiveness of the obtained biopreparations accelerating the organic waste degradation in the poultry manure management. The microorganisms contained in the biopreparations exhibit well-expressed enzymatic properties and a high antagonistic activity against many pathogenic and opportunistic bacteria (including the bacteria of the genera *Escherichia*, *Salmonella*, *Enterococcus*, *Proteus*, and *Klebsiella*) and toxigenic filamentous fungi. They stimulate the growth of saprophytic microflora exhibiting biological activity, which is involved in the soil-forming processes and the organic matter degradation. The used microorganisms have a number of functional and ecological advantages, such as the ability to grow in a wide range of pH and temperatures and resistance to chemical contaminants. An important advantage is also the elimination of the

unpleasant peculiar smell of substrate within several days. Thus, it can be assumed that the use of the obtained biopreparations is associated with the following:

- The inoculated microorganisms exhibit a high competitive ability and inhibit the pathogenic microflora, ensuring the safety of the substrate in the hygienic and sanitary aspect.
- The inoculation of the microorganisms in the substrate stimulates the growth of the original microflora, which exhibits useful properties and is involved in humification.
- The substrate is enriched in the biologically active substances produced by the microorganisms.
- The microorganisms contained in the biopreparations are able to assimilate nitrogen from urea and exhibit antagonistic activity against the pathogens that produce metabolites that cause bad smell; as a result, the substrate is deodorized.

The use of the developed biopreparations makes it possible to significantly improve the environmental situation around the livestock and poultry enterprises, reduce the economic costs for storage and disposal of manure masses, improve soil fertility and crop yield, and obtain organic food and animal feed. The agricultural enterprises become able to run a closed natural cycle (field–farm–processing–field), making additional profits from the complete and deep processing and minimizing the environmental burden of industrial activity.

## 5. References

1. Peres C, Agathos S. Biodegradation of nitroaromatic pollutants: From pathways to remediation. *Biotechnol Ann Rev*. 2000; 6:197–220.
2. Benali M, Kudra T. Thermal dewatering of diluted organic suspensions: Process mechanism and drying kinetics. *Dry Technol Int J*. 2002; 20:935–51.
3. Kim J, Luo F, Jiang X. Factors impacting the regrowth of *Escherichia coli* O157:H7 in dairy manure compost. *J Food Protect*. 2009; 72:1576–84.
4. Lay J, Fulton RM, Hester PY, Karcher DM, Kjaer JB. Hen welfare in different housing systems. *Poult Sci*. 2011; 90:278–94.
5. Wilkinson KG, Tee E, Tomkins RB, Hepworth G, Premier R. Effect of heating and aging of poultry litter on the persistence of enteric bacteria. *Poult Sci*. 2011; 90:10–8.
6. Galitskaya P, Mamaeva E, Biktasheva L, Selivanovskaya S. Distribution of *Molnia*, pesticide along soil profile and its

- influence on soil organisms. *Biosciences Biotechnology Research Asia*. 2014; 11:151–7.
7. Selivanovskaya S, Galitskaya P. Ecotoxicological assessment of soil using the *Bacillus pumilus* contact test. *European J Soil Biology*. 2011; 47:165–8.
  8. Anilkumar P, Chithra K. Land use generator based solid waste estimation for sustainable residential built environment in small/medium scale urban areas. *Indian Journal of Science and Technology*. 2016; 9:6–12.
  9. Heringa SD, Kim J, Jiang X, Doyle MP, Erickson MC. Use of a mixture of bacteriophages for biological control of *Salmonella enterica* strains in compost. *Appl Environ Microbiol*. 2010; 76:5327–32.
  10. Zhang J, Wu P, Hao B, Yu Z. Heterotrophic nitrification and aerobic denitrification by the bacterium *Pseudomonas stutzeri* YZN-001. *Bioresour Technol*. 2011; 102:9866–9.
  11. Wackett L. Environmental biotechnology. *Trends Biotechnol*. 2000; 18:19–21.
  12. Palanichamy C. A sustainable energy option to the expanding Chennai metropolitan area. *Indian Journal of Science and Technology*. 2015; 8:22–32.
  13. Khan M, Zaidi A, Wani P, Oves M. Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environ Chem Lett*. 2009; 7:1–19.