Environmentally proved methods of cascade conversion of organic waste using a microbial complex with filamentous fungi

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Abstract. The article deals with the problem of microbial bioconversion as an effective way to utilize organic waste in relation to the industrial poultry industry. At modern poultry farms, with the implementation of an intensive technology for growing poultry, the main type of waste that could limit the scale of production is significant scale of waste litter. The cellulose and lignin components that make up the waste material and litter mass are hardly decomposable substrates. The article contains information on the results of examining the parameters of microbial processes that ensure the degradation of organic matter, including, in addition to the actual poultry litter, a significant proportion of natural components. The advantages and dynamics of the qualitative and quantitative composition of the aboriginal microflora of waste have been defined. The methods of the developed recycling technology, which is a cascade process of destruction of the waste material components, are described. Destructive processes are provided by the work of a kind of biological conveyor where various systematic and ecological-trophic groups of microorganisms have been applied. Consecutively realized fermentation provides the most efficient conversion of the substance by analogy with natural reduction and soil-forming processes. The efficiency of the use of ammonifying and nitrifying bacteria, as well as the cellulolytic and lignolytic potential of actinomycetes and filamentous fungi variates at the stage of decomposition that is optimal for each destructor has been examined. Bioconversion allows to ensure a waste mass loss of up to 30-33% of the original, to reduce emissions into the atmosphere of significant amounts of ammonia and amines, which have a characteristic pungent odor, thereby reducing social tension in the areas located closely to the storage sites. The final product of the cascade biodegradation is biocompost, which corresponds to the physicochemical parameters of organomineral fertilizer.

1. Introduction

One of the important problems in poultry industry of the Russian Federation is the protection of the environment from pollution by poultry waste.

Litter masses are sources of nutrients, which, when released into the soil and when washed off, could significantly change the physicochemical parameters of groundwater and surface waters, contributing to the eutrophication of water systems [1]. The ingress of nutrients into the soil leads to changes in the composition of the components of biocenoses and the characteristics of their biotopes [2]. Atmospheric air could also be affected by manure waste, or, in particular, by gas-phase vapors. For the economic component of the technological process of industrial poultry breeding, long-term deposition of waste is the limiting factor for expanding production. One of the effective practice to
overcome this problem could be composting or bioconversion, usually originated with a complex of microorganisms [3-5]. Thus, it is important to find and adapt a complex of microorganisms to the available raw materials for realizing the discussed the technology. An important environmental problem in the agricultural sector is the decline in soil fertility. The proposed solutions to this problem today are diverse, many of them are based on the inclusion of non-traditional crops in the crop rotation or the use of conversion organic materials and waste [6-8].

The purpose of this work is to solve the problem of poultry waste utilization, as well as to obtain an innovative product through their microbial bioconversion - organo-mineral fertilizers. It would improve the environment and increase soil fertility. The first task of the research was the selection of microorganisms of various systematic and ecological-trophic groups capable in enzymatic destruction of organic compounds. The second task was to create variants of microbial compositions capable in realizing stepwise decomposition of organic waste: at the first stages with the aim to carry out ammonification and nitrification, and at the next stages it aimed the destruction of cellulose and lignin components to humic derivatives and microbial transformation of insoluble forms of phosphorus into mobile forms.

The proposed technology is based on the use of unique microbial compositions characterized by stability and providing a deep level of waste destruction. Such compositions and stages of technology implementation are new and have no close analogues. The technology of microbial conversion of waste minimizes the loss of nutrients (nitrogen, phosphorus, sulfur, and others) in the process of destruction, which, on the one hand, reduces the scale of their emissions into the atmosphere, and on the other hand, determines the transition to mobile forms available for plants as part of the fertilizer.

2. Materials and research methods
The current research was carried out on the basis of the Department of Biology, Biological Technologies and Veterinary and Sanitary Examination, as well as the interfaculty biochemical laboratory of Penza State Agrarian University. The litter and litter mass obtained from fattening turkeys was studied as a waste of poultry farming. Isolation of pure microbial cultures from different objects was carried out according to the method of Pasteur or Drygalsky. Identification of isolated microorganisms was organized by conventional methods of microbiological analysis.

The activity of a complex of bacteria of the genus Bacillus was examined as a functional group of ammonifiers; as nitrifiers - the genus Nitrosomonas; for the destruction of cellulose and lignin components, the filamentous fungi of the genera Thelavia, Cellulomonas, and Myceliophthora and actinomycetes of the genus Nocardia were used. To determine the degree of destruction of poultry litter waste by microbial compositions of different compositions, the authors used the gravimetric method. Statistical analysis of the results obtained was carried out using the Microsoft Excel program.

3. Research results and their discussion
At the initial stage of the research, the composition of the aboriginal microflora of poultry litter materials was examined. Turkey litter waste and litter mixture delivered to the storage site were observed as the initial substrate. Later, to define the dynamics of microbial processes, samples of litter material, deposited up to one year in time, were taken as samples for the current research.

The results of the research made it possible to identify certain patterns in the dynamics of the total number of microorganisms and their species composition (Figure 1).

Meat-peptone agar was used to isolate bacterial microflora, and Sabouraud's nutrient medium was used for fungal microflora. So, in the freshly used litter, depending on its basis (sawdust or cereal straw), a rather high titer of microorganisms is noted: from 4.8×108 to 5.2×109 CFU/g of dry substrate. The main diversity of species composition falls on the residents of the digestive tract of poultry (bacteria of the genus E. coli, anaerobic clostridia, bacteroids, yeast cells in small numbers), as well as a certain proportion of bacteria, which are typical for zymogenic microflora of such substrates. The predominant form of the latter group is the genus Bacillus.
The detected presence of representatives of this genus deserves special attention, since they influence on the ammonification processes as the early stage in the destruction of the nitrogen-containing mass of waste. Indeed, the stepwise degradation of the litter mixture starts in connection with the process of ammonification where the presence and preservation of bacteria in the deposited mass, in particular \textit{Bacillus subtilis}, is fundamentally and very important. A single colony of filamentous fungi was isolated in the material delivered to the landfill directly from the feeding farms, and no representatives of actinomycetes were found. This fact could be explained by the prevalence of readily available nutrient factors (undigested food components and feed residues) in the fresh litter and litter mixture, which are mainly utilized by bacteria. Another reason for the low content of fungi, perhaps, the high pH of the substrate (at the level of 8.5-9.5), due to the ammonification processes, accompanied by the release of free ammonia. The shift of the pH range to the alkaline region is usually considered as one of the unfavorable factors preventing the spread of fungi in this ecological niche.

The study of the substrate, which underwent primary fermentation, made it possible to reveal a certain tendency in the change in both the total number and the species composition of microorganisms. After every two months from the beginning of the deposition of poultry waste and litter material, samples were taken from it for observation and subsequent assessment of the species composition and properties of microorganisms. In the course of these research, it was found that the total number of microorganisms, within six months, decreases from 10 to 50 times compared with the initial state and ranges from $1 \times 10^7$ to $1 \times 10^8$ CFU/g of dry substrate. That fact could be explained by the thermal phase that took place in the chronological sequence of the composting process. Also, an important change in the parameters of the substrate should be connected with a decrease in pH due to a decrease in the intensity of ammonia release and, as a consequence, an increase in the proportion of fungal microflora and actinomycetes in the microbial cenosis. The activation of the development of the latter could be considered as an indicator of the depletion of available components of the substrate and a change in the direction of microbiological processes towards the processing of the components that were more difficult to decompose (cellulose, hemicelluloses, and lignin).

On the basis of pre-selected functional species of microorganisms, the physiological and biochemical potential of which is capable of providing high efficiency of composting of poultry litter mass, functional microbial compositions of microorganisms were compiled. The composting process...
is essentially the same as the rotting process. It is characterized by a cascade of stages that are implemented in a specific chronological order. Taking into account changes in the physicochemical parameters of the compostable mass (temperature, oxygen level, pH of the environment, etc.), artificial introduction of the corresponding microbial species is carried out, the vital activity of which provides qualitative and quantitative changes in organic material, as a result it turns into compost. At the first stages of destruction, available components decompose: easily degraded polysaccharides, low molecular weight carbohydrates, proteins, nitrogenous substances, which, as a rule, is accompanied by heating of the substrate, as well as a shift in the general biochemical balance towards ammonification.

At later stages, the substrates become more difficult to utilize components, localized mainly in the litter material (wood shavings, sawdust, wood filings, cereal straw). By their chemical nature, the main substances of these materials are polymers such as cellulose, hemicelluloses and lignin. Thus, the application of species providing ammonification at the initial stages, and cellulose and lignolytic complexes of microorganisms at the later stages, seems to be an expedient approach. accompanied by heating of the substrate, as well as a shift in the general biochemical balance towards ammonification.

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Within the established timing of application, the corresponding complexes of microorganisms in the frame of the process, an important influence is devoted to pH, which could undergo significant dynamic changes. At the initial stages of active fermentation, pH is in the alkaline region, and with further biodegradation, it shifts to the acidic side. This situation is explained by the fact that in the process of ammonification, the released ammonia, interacting with water, forms a solution of ammonium hydroxide with alkaline properties. At later stages, the sequential oxidation of hardly decomposable compounds occurs, resulting in products with acidic properties (carboxylic acids and phenolic compounds). Thus, the change in pH is mostly determined by the activity of the microorganisms and the gradual change in their formations. In addition to changes in the species composition of microorganisms, shifts in pH could also contribute to the transformation of the forms of substances, the mobility of elements, and, as a consequence, the degree of assimilability of such important biogenic elements as phosphorus and nitrogen. However, to accelerate these changes, it seems possible to correct pH of the substrate with acidic solutions, which could provide better adaptation of certain microorganisms and the efficient application of the physiological and biochemical potential. Thereby, since the final product - compost - is an integral result of the activity of all microorganisms at all stages of biodegradation of the degradable material, then the regulation of the process through the sequential introduction of the appropriate microbial complexes with simultaneous correction of pH would provide a high-quality product in the shortest possible time. To test this assumption, a series of experiments were carried out. Samples of the poultry waste and litter mass were brought to 60% moisture and placed in plastic containers in an amount of 5 kg. The moistened waste substrates were exposed under standard conditions (20 °C, 760 mm Hg) for two weeks, during that period of time, with periodic stirring, the substrates were fermented due to the enzymatic activity of the aboriginal microflora. Then, as a control, a variant was used without introducing any additional microbial factors, the destruction of the substrate in them was carried out only at the expense of the aboriginal microflora. In the first variant of the experiment, in addition to the native microflora, a mixture of bacteria of the genera Bacillus and Nitrosomonas was added at the rate of 1.0-1.5×10^9 CFU/g of dry substrate. The second option involved the introduction of a composition consisting of the studied species of fungi of the genera Thelavida, Cellulomonas, and Myceliophthora, which is a liquid substance with cells of microorganisms and the remains of a nutrient medium, to the substrate in the second week of destruction, and in the third week, species of actinomycetes of the genus Nocardia were added. The third variant of the experiment was organized in
the same way as the previous one, but to accelerate the adaptation of these species, the substrate was also corrected with a 10% solution of sulfuric acid until pH at the final stages of destruction was at the level of 6.3-6.8. The use of this approach made it possible to ensure a decrease in the release of ammonia, due to its binding in the form of ammonium sulfate and, as a consequence, to achieve a partial preservation of such an important biogen as nitrogen in a form accessible for biological assimilation. The results of the experiment were taken after three months of destruction. In the control and experimental variants, the main assessed parameters were organoleptic (color, smell, consistency), and the dynamics of pH was also determined (Figure 2 a, b).

Figure 2. Dynamics of loss of substrate (a) and pH of the medium (b) in the process of microbial destruction: control (destruction due to aboriginal microflora); experimental variant 1 (aboriginal microflora + a complex of bacteria of the genus Bacillus and Nitrosomonas); experimental variant 2 (aboriginal microflora + fungi of the genera Thelavía, Cellulomonas and Myceliophthora in the second week and actinomycetes of the genus Nocardia in the third); experimental variant 3 (aboriginal microflora + fungi of the genera Thelavia, Cellulomonas and Myceliophthora in the second week and actinomycetes of the genus Nocardia in the third + decrease in pH), a - the moment of acid addition
The dynamics of the substrate mass loss was taken as the main parameter in determining the integral enzymatic activity of the process. The results of the experiment made it possible to prove the efficiency of the proposed microbial composition and the described method.

Thus, the decrease in the mass of waste in the control was 22.0%, and in the experimental variants, respectively, 27.0; 34.5 and 42.0%. The smell of the fermented mass in the second and third variants of the experiment acquired an earthy character, and in the third variant even a weak ammonia smell was not detected. The consistency of the substrate in the third variant became medium - and finely dispersed, the fermented mass acquired a dark brown color. At the same time, pH of the substrate in the control, first and second variants of the experiment was at the level of 7.5-7.7, while in the third variant it decreased to 6.3-6.8.

It was found that the maximum decrease in the substrate is observed in the third variant of the experiment and is associated with the addition of a 10% solution of sulfuric acid in order to fix pH at the level of 6.3-6.8 (Figure 2a).

In the control, first and second variants, pH was in the alkaline zone and limited the development of fungal microflora, while the nutrient resources necessary and available for bacteria were already used up to a greater extent. Due to the aimed regulation of pH and the application of species of fungi and actinomycetes, destructive processes are activated, this also contributes to the deep destruction of ligno and cellulose components. This is accompanied by a noticeable change in the color and consistency of the substrate, it becomes soft, loose, plant residues significantly lose their strength, become easy to break, acquiring a characteristic brown color, as having lost their cellulosic component.

The existence of actinomycetes stimulates soil-forming processes, which is illustrated by a typical (earthy or soil) odor.

It is also necessary to draw specific attention to the dynamics of the substrate decrease and pH during the period of microbial destruction (Figure 2a, b).

4. Conclusion
The main resident among the microorganisms of the natural microflora of organic waste, i.e. turkey litter and waste material, is the spore-forming bacterium Bacillus subtilis. It is advisable to combine individual microbial species into complexes for the effective destruction of organic waste at the stage of application into the substrate in the form of individual seed suspensions. In this case, it would be efficient to combine bacterial species with each other into a separate complex, fungi and actinomycetes also into a separate complex.

The result of microbial complexes application into the compostable substrate to achieve the maximum rate and degree of its destruction should be consistent with the stages of degradation. So, bacterial complexes should be introduced at the first stages of composting, but the complexes of fungi and actinomycete species are efficient at later stages. The composting process is quite effectively initiated by the aboriginal microflora, which gives an a satisfying intermediate result in the form of ammonification, the substrate overcoming the thermal phase, and the achievement of the necessary sanitary and biological indicators. In some cases, this makes it possible to avoid the application of bacterial preparations at the initial stage.

In the current experiments, a decrease in the substrate was achieved when a complex of fungi and actinomycetes was added after natural bacterial fermentation, which was 32% higher than in the control group. Due to the intensive processes of destruction of the lignocellulosic litter components, the characteristic unpleasant odor is replaced by the natural odor of the soil. When introducing species of fungi and actinomycetes, in order to accelerate the time to reach the optimal pH index, it could be corrected with a 10% solution of sulfuric acid to 6.3-6.8. Under the conditions of such pH indexes, it would be useful to add phosphorite flour, where the phosphates would change into a soluble form and additionally stimulate the processes of biodegradation in the litter material. However, further research is required to confirm this hypothesis.
References

[1] Smith V H 2003) Eutrophication of freshwater and coastal marine ecosystems: a global problem
Environ Sci Pollut Res Int. **10**(2), 126–139

[2] Chekaev N P and Vlasova T A 2020 Application of anhydrous ammonia as nitrogen fertilizer, its influence on soil properties and yield of agricultural crops Scientific papers-series A-agronomy **63**(1) 56–61

[3] Koryagin Y V, Kulikova E G and Kuznetsov A Y 2020 A groecological evaluation of application the microbiological fertilizers in lentil cultivation technology Scientific papers-series A-agronomy **63**(1) 361–365

[4] Nielsen C, Rahman A, Rehman A U, Walsh M K and Miller C D 2017 Food wasteconversion to microbial polyhydroxyalkanoates Microb Biotechnol **10**(6) 1338–1352

[5] Kee S H, Chiongson J, Saludes J P, Vigneswari S, Ramakrishna S and Bhubalan K 2021 Bioconversion of agro-industry sourced biowaste into biomaterials via microbial factories - A viable domain of circular economy Environmental Pollution **271** 116311

[6] Kshnikatkina A N, Kshnikatkin S A, Alenin P G, Shehanin A A, Prakhova T Y, Prakhov V A, Medvedev A P and Voronova I A 2021 Biological diversity of non-traditional oil crops. IOP Conference Series: Earth and Environmental Science, **659**(1) 012091

[7] Efremova S, Kulikova Y, Konovalov V and Politaeva N 2019 Utilization of wastes from grain processing industries IOP Conference Series: Earth and Environmental Science **337**(1) 012033

[8] Anastopoulos I, Omirou M, Stephanou C, Oulas A, Vasiliades M A, Efstathiou A M and Ioannides I M 2019 Valorization of agricultural wastes could improve soil fertility and mitigate soil direct N(2)O emissions Journal of Environmental Management **250** 109389