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MICROBIAL SUCCESSIONS AT CHICKEN MANURE COMPOSTING WITH CELLULOLYTIC MICROORGANISMS

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Chicken manure composting with selected cellulosolytic microorganisms provides different effects on the development of microorganisms of certain ecological trophic groups. Introduction to the organic substrate bacteria of the Bacillus genus promoted the increase of the cellulolytic bacteriaquantity, while addition of the bacteria of Trichoderma genus stabilized the number of micromycetes in the compost. Microorganisms that have the greatest positive effect on the development of compost microbiota were selected.

Key words: *introduction of microorganisms, compost, chicken manure, microbial successions, cellulolytic microorganisms, Bacillus sp., Trichoderma sp.*

Composting is an aerobic process that results in partial mineralization and profound transformation of organic matter caused by the compost organisms' metabolism. Although composting is a microbiological process, existing technologies of organic matter conversion practically do not take into the account its microbiological features. Determination of the diversity and structure of microbial groups of compost is of considerable interest to researchers, primarily aimed to address rising environmental issues [1–2]. Besides, the study of the microorganisms'succession characteristics in composted substrates is essential for the efficient management of the composting process, due to the key role of bacteria and microscopic fungi in this process, while appearance of specific microorganisms indicates the quality of matured compost [3–4].

In our previous studies we have investigated the changes in the number of individual ecological trophic groups of microorganisms in chicken manure compost at optimized C : N ratio (introduction of straw and peat in estimated quantities) [5]. However, the presence of straw in a composted substrate (heavily fermented component) prolongs composting process. In order to accelerate the fermentation of organic matter, it is advisable to introduce cellulolytic microorganisms. Nevertheless, the development characteristics of different groups of microorganisms, including cellulolytic, under such condition remain uncertain. In this regard, the objective of given study was to clarify the succession changes in the groups of microorganisms under the introduction of selected strains of cellulolytic microorganisms into the chicken manure composting.

Materials and methods. The study of the microorganisms succession during the chicken manure composting with selected cellulolytic microorganisms was carried out under the model experiments conditions. Composting was carried out in plastic containers with 5 kg of chicken manure (70 % moisture level). 0.7 kg of shredded straw and 1.9 kg of peat were added to manure substrate in order to optimize C : N ratio at the level of 20 : 1, s. The repetition of the experiment is fourfold.

The substrate moisture level was maintained during compostingon the 70–75 % level (stirring once per two weeks).

Bacteria were cultivated under the periodic culture conditions on the culture medium of the following composition: molasses (50 %) — 30 g; $(NH_4)_2SO_4$ — 0.1 g; KH_2PO_4 — 0.25 g; K_2HPO_4 — 0.25 g; $MgSO_4$ — 0.25 g; $CaCO_3$ — 0.3 g; water — 1,000 dm^3; pH 7.0.

The suspension of fungi spore-micelle was grown on a bevelled wort-agar in test tubes and flushed with sterile water.

The introduction of microorganisms into the composted substrate was carried out in 2 months after their initial fermentation at the rate of 1×10^9 cells of bacteria / g of compost, micromycetes — 1×10^5 CFU / g of compost.

The proposed experimental design was followed during the study of the influence of selected cellulolytic microorganisms on chicken manure composting with peat and straw:

1. Control (a mixture of chicken manure with peat and straw)

Introduction of the bacteria to the mixture of chicken manure with peat and straw:

2. *Bacillus sp.* SB 1
3. *Bacillus sp.* S 13
4. *Trichoderma sp.* PD 3
5. *Trichoderma sp.* L 1
6. *Trichoderma sp.* 129.

The number of microorganisms in compost was determined using MPA for ammonifying bacteria; starch-ammonia agar — for microorganisms that utilize mineral forms of nitrogen; and wort agar for micromycetes [6]. The number of cellulolytic bacteria was determinedusing liquid medium by Imshenetsky and Solntseva [7]. To assess the emissions of carbon dioxide, the method of enclosed cameras was used [8]. The emission of CO_2 in the compost gas samples was determined on a gas chromatograph with a heat conductivity detector.

Statistical analysis of the results was performed using a dispersion analysis. The results of model experiments were calculated using the two-factor dispersion correlation analysis (Statistica 6.0), and Microsoft Office Excel 2003–2007 software.

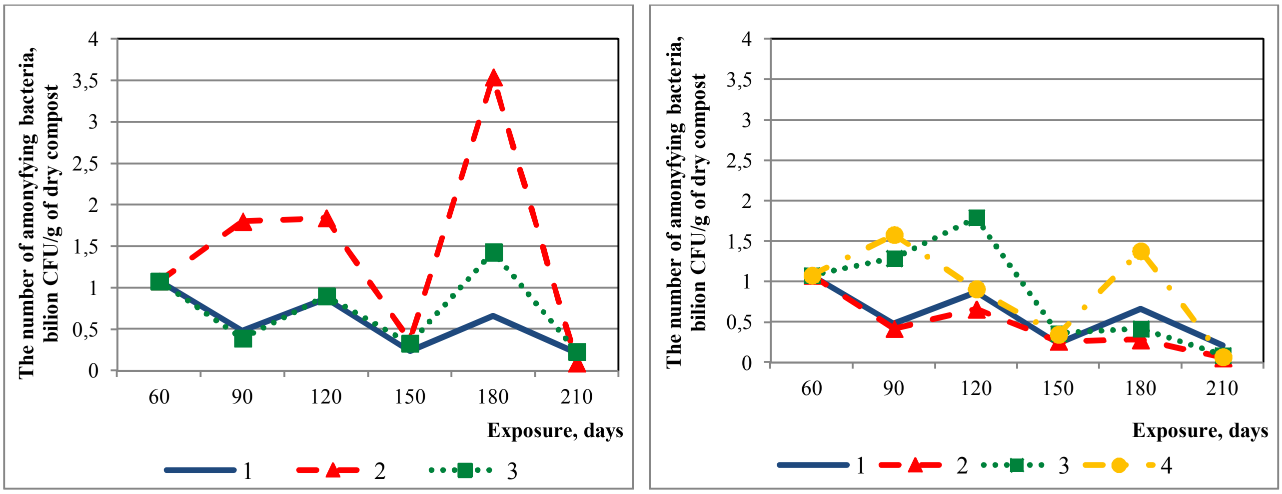
Results and discussion. In our previous studies, we have isolated and selected active strains of cellulolytic microorganisms from different substrates (chicken manure, compost based on chicken manure, biohumus (vermicompost product), hay, half-deciduous leaves, soil). As it was determined, the bacteria of the *Bacillus* and *Trichoderma* genus were the most active among the studied microorganisms. The ability of active strains of microorganisms to grow on the chicken manure was pre-tested using the genetic labelling method [9]. Based on the obtained data, the strains of cellulolytic microorganisms with the highest survival rates, namely, *Bacillus sp.* S 13, *Trichoderma sp.* PD 3 and *Trichoderma sp.* 129 were included to the experiment design together with the *Bacillus sp.* SB 1 and *Trichoderma sp.* L1, that preserve the stable quantity in the substrate during a long period of composting and accelerated rates of mineralization of organic matter, but did not a high survival rates.

The obtained results have shown that the introduction of microorganisms into the mixture of chicken manure, peat and straw had a different effect on nitrogen transformation processes resulted from the heterogenic development of microorganisms in the compost. Thus, the assessment of the number of ammonifying bacteria and microorganisms that uptake predominantly mineral forms of nitrogen indicates the stimulation of their development after the introduction of selected microorganisms. An increase in the number of ammonifiers was observed during 3–4 months (90–120 days) and 6^{th} month (180 days) of composting (Fig. 1). Thus, their number had increasedfrom 1.10 billion CFU/g of compost on the second month of composting to 1.30–1.80 billion CFU/g of compost on the third-forth month of composting.

After 6 months of composting, the another increase in the number of ammonifying bacteria was observed (up to 3.54 billion CFU/g of compost) indicating that there are two peaks in the development of ammonifiers, which can be explained by the nonuniformity of the organic substrate and the prolonged mineralization of its individual components.

The dynamics of the number of micromycetes in the compost after the introduction of bacilli is characterized by a gradual insignificant increase, which can be explained by the active development of the introduced bacteria, and partial inhibition of the development of micromycetes. However, this will require further investigation and clarification.

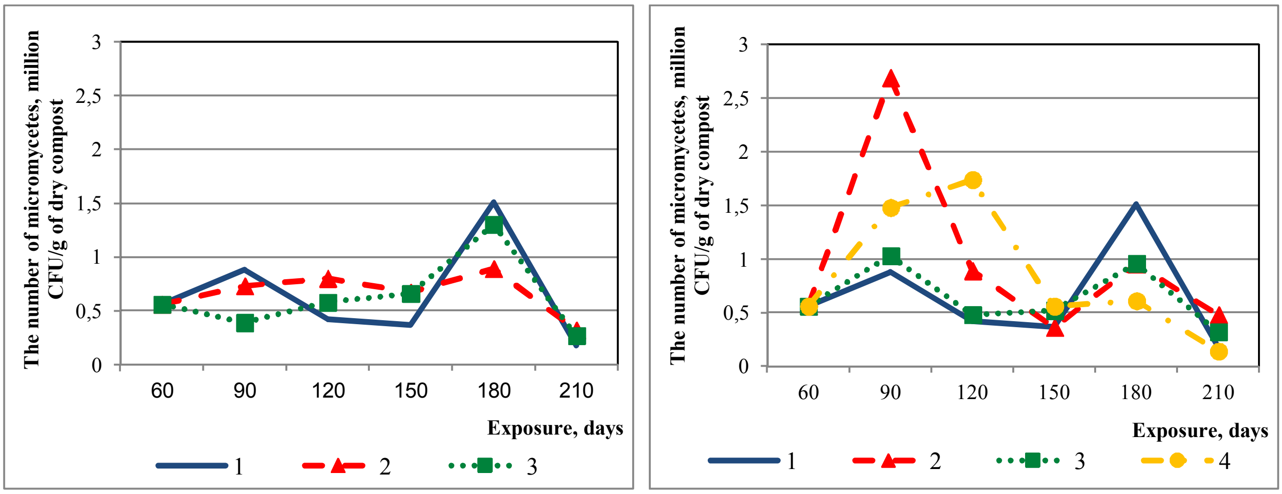
After the introduction of *Trichoderma* species to the compost, the initial increase in the number of micromycetes from 0.50 million CFU/g (2^{nd} month of composting) to 1.03–2.70 million CFU/g of dry compost was observed (varied by the isolate) which was later stabilized on a certain level (Fig. 2). The highest indicator of the number of micromycetes was determined in the variant with *Trichoderma sp.* PD 3, that may indirectly indicate the active



1 — Control (a mixture of manure with peat and straw).
Composting under the following influence:
2 — *Bacillus sp.* SB 1;
3 — *Bacillus sp.* S 13.

1 — Control (a mixture of manure with peat and straw).
Composting under the following influence:
2 — *Trichoderma sp.* PD 3;
3 — *Trichoderma sp.* L 1;
4 — *Trichoderma sp.* 129.

Fig. 1. Dynamics of the number of ammonifying microorganisms in the chicken manure compost depending on introduced microorganisms.



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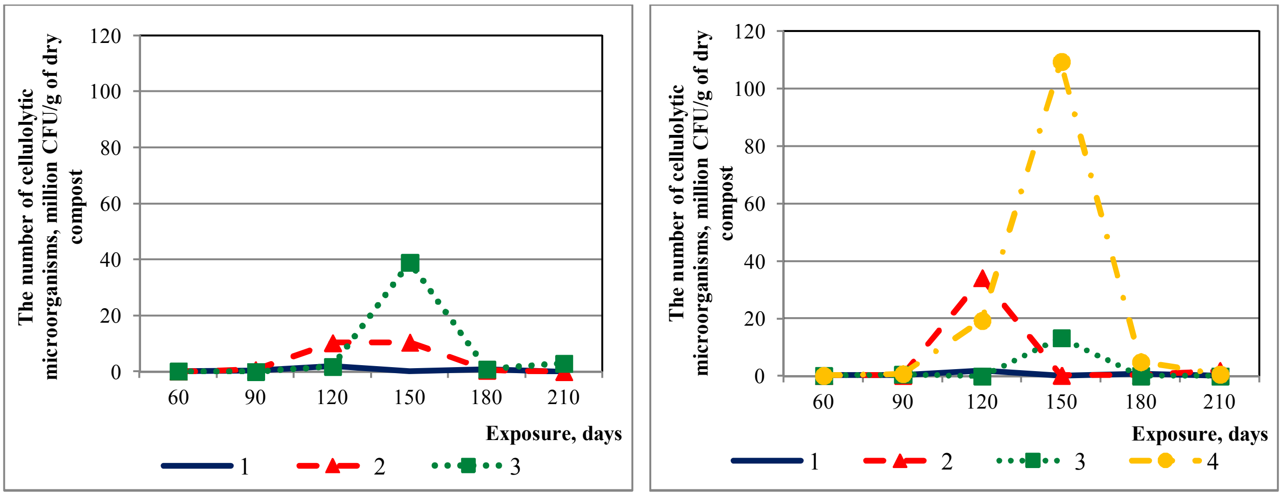
1 — Control (a mixture of manure with peat and straw).
Composting under the following influence:
2 — *Trichoderma sp.* PD 3;
3 — *Trichoderma sp.* L 1;
4 — *Trichoderma sp.* 129.

Fig. 2. Dynamics of the number of micromycetes in the chicken manure compost depending on introduced microorganisms.

development of the introduced fungi in the substrate.

The increase in the number of cellulolytic aerobic bacteriain the manure was observed after 3 months of composting. It should be noted that the introduction of *Bacillus sp.* S13 had ensured the increase of the cellulolytic bacteria number — up to 39 million CFU / g of dry compost (Fig. 3). At composting of the mixture of manure with micromycetes of the *Trichoderma* genus, the increase in the number of cellulolytic bacteria along with a gradual decrease in the number of fungiwas observed.

It is worth noting that the introduction of



1 — Control (a mixture of manure with peat and straw).
Composting under the following influence:
2 — *Bacillus sp.* SB 1;
3 — *Bacillus sp.* S 13.

1 — Control (a mixture of manure with peat and straw).
Composting under the following influence:
2 — *Trichoderma sp.* PD 3;
3 — *Trichoderma sp.* L 1;
4 — *Trichoderma sp.* 129.

Fig. 3. Dynamics of the number of cellulolytic microorganisms in the chicken manure compost depending on introduced microorganisms.

Trichoderma sp. 129 fungus ensured the highest development rate of cellulolytic bacteria — 109.3 million CFU / g of dry compost.

Approaching the final stage of composting (7 months) the decrease in the number of representatives of all the studied groups of microorganisms (to the lowest rates for the entire period of composting) was observed. Obviously due to the complete mineralizationof the organic matter, which has limited the sources of nutrition for the development of microorganisms. According to the microbiological data, the composting period can be considered complete.

CO_2 emissions can be used as an integral indicator of the development of microorganisms. The production curves of carbon dioxide generally characterize the peculiarities of the composting process. Thus, the highest CO_2 emissions were observed in the compost after the introduction of *Bacillus sp.* S 13 and *Trichoderma sp.*PD 3 (Table). At the end of the composting period, CO_2 emissions were

Table. ***Carbon dioxide emission dynamics,*** *nmol CO_2 / g of compost per hour*

Variants	Exposure					
	2 months of composting (before introduction of microorganisms)	3 months	4 months	5 months	6 months	7 months
Control (a mixture of manure with peat and straw)	174.9 ± 11.6	143.7 ± 4.4	114.8 ± 10.2	100.8 ± 4.6	27.3 ± 2.9	8.72 ± 0.38
Introduction of microorganisms to the compost						
Bacillus sp. SB 1		149.5 ± 5.1	112.0 ± 1.3	195.1 ± 2.3	70.5 ± 2.4	6.29 ± 0.7
Bacillus sp. S 13		388.1 ± 8.7	171.2 ± 1.8	150.5 ± 2.3	68.9 ± 5.7	2.61 ± 0.3
Trichoderma sp. PD3		36.1 ± 0.8	67.7 ± 0.3	263.5 ± 3.9	72.1 ± 1.5	4.23 ± 0.2
Trichoderma sp. L 1		41.2 ± 2.2	69.7 ± 0.9	130.9 ± 2.9	65.6 ± 1.9	2.17 ± 0.2
Trichoderma sp. 129		22.7 ± 2.9	61.9 ± 1.2	154.7 ± 3.3	16.6 ± 0.9	1.40 ± 0.2

reduced, which may also indicate completion of the process of fermentation of organic matter.

Based on the data obtained on the changes in the number of representatives of certain ecological trophic groups of microorganisms and CO_2 emissions during the composting process, the selection of *Bacillus sp.* S 13 and *Trichoderma sp.* PD 3, is recommended for the further study of the effectiveness of chicken manure composting with peat and straw.

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МІКРОБНІ СУКЦЕСІЇ ПРИ КОМПОСТУВАННІ КУРЯЧОГО ПОСЛІДУ ЗА ІНТРОДУКЦІЇ ЦЕЛЮЛОЗОЛІТИЧНИХ МІКРООРГАНІЗМІВ

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Компостування субстрату на основі курячого посліду з селекціонованими целюлозолітичними мікроорганізмами забезпечує різний вплив на розвиток мікроорганізмів окремих еколого-трофічних груп. Інтродукція до органічного субстрату представників роду Bacillus позитивно впливає на збільшення чисельності целюлозоруйнівних бактерій, а за використання представників роду Trichoderma відбувається стабілізація чисельності

МИКРОБНЫЕ СУКЦЕСИИ ПРИ КОМПОСТИРОВАНИИ КУРИНОГО ПОМЁТА ПРИ ИНТРОДУКЦИИ ЦЕЛЮЛОЗОЛИТИЧЕСКИХ МИКРООРГАНИЗМОВ

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Компостирование субстрата на основе куриного помета с селекционированными целюлозолитическими микроорганизмами обеспечивает разное влияние на развитие микроорганизмов отдельных еколого-трофичеких групп. Интродукция в органический субстрат представителей рода Bacillus положительно влияет на увеличение численности целюлозолитических бактерий, а при использовании представителей рода Trichoderma *мікроміцетів у компості. Відібрано мікроорганізми, які мають найбільший позитивний вплив на розвиток мікробіоти компосту.*

Ключові слова: *інтродукція мікроорганізмів, компост, курячий послід, мікробні сукцесії, целюлозоруйнівні мікроорганізми, Bacillus sp., Trichoderma sp.*

происходит стабилизация численности микромицетов в компосте. Отобраны микроорганизмы, проявившие наибольшее позитивное влияние на развитие микробиоты компоста.

Ключевые слова: *интродукция микроорганизмов, компост, куриный помет, микробные сукцессии, целюлозоразрушающие микроорганизмы, Bacillus sp., Trichoderma sp.*

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