Agronomy Research **20**(X), xxx–ccc, 2022
https://doi.org/10.15159/AR.22.059

Optimum ratio of complex biological product and fertilize (NPK) and the contribution of fungi and bacteria to the general decomposition and mulching of coniferous wood waste

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Received: August 7th, 2021; Accepted: December 15th, 2021; Published: September 12th, 2022

Abstract. The use of a complex biological product (CBP) based on native microbiological consortiums of coniferous forest litter accelerated the composting process of coniferous wood waste. The contribution of micromycetes and bacteria to the activation of coniferous wood waste composting processes using the different fertilizers rates was studied. A fractal analysis has confirmed the formation of a micromycetes-bacterial system in the treatment with optimal rates of NPK and CBP. In this case the better decomposition of wood waste was observed. It was noted that micromycetes of the genus *Penicillium* dominated in the composts obtained with CBP addition. This compost was not phytotoxic. Thus, for coniferous sawdust decomposition and its further humification, it is necessary to use both micromycetes and bacteria. The use of organic material resulting from wood waste decomposition with CBP and optimal rates of NPK is an effective way to increase the content of organic substances in soils and their potential fertility.

Key words: humification, sawdust, microorganisms, fractal portrait.

Abbreviations: CBP – complex biological product; GMC – graph of maximum correlations; FGM – fractal group of microorganisms.

INTRODUCTION

The bark and wood of coniferous trees decompose to form humus substances in the forest floor as a result of natural processes of microbiological destruction (Kononova et Bel'chikova, 1961). Coniferous wood contains about 50% of hard-to-decompose lignin, up to 40–55% of cellulose and 0.2% of nitrogen, which microorganisms can transform into humus forms of substances (Orlov, 1990; Nenajdenko & Mazirov, 2002; Zavarzin, 2004).

For a long time, a self-organizing biological system of micromycetes and bacteria with effective transformation of organic substrates has been formed in litter of coniferous forests, which most effectively transforms complex organic substrates (Young & Crawford, 2004; Crawford et al., 2012). The use of these biological systems in the manufacture of biological products accelerate the processing of waste on woodworking enterprises into organic fertilizers (Lozanovskaya et al., 1987; Six et al., 2006; Baldock, 2007; Fageria, 2012; Singh & Nain, 2014; Hatfield & Walthall, 2015). That improves the environmental situation around these enterprises, as well as gets some economic effect from the introduction of fertilizers in agricultural production. Otherwise, deposits of wood waste will release toxic phenolic compounds into the environment, worsening the environmental situation around them.

One way to enhance the decomposition of crop residues can be obtained by processing of microbiological inoculants containing high efficiency of microorganisms-destructors (Rusakova, 2016; Wu et al., 2017). Meanwhile, for the decomposition of organic substrates and further humification, it is necessary to use a micromycetes and bacteria (Kaszab et al., 2011; Sviridova et al., 2016; Fan et al., 2017; Leow et al., 2018). In the content of organic fertilizers obtained from wood waste humus substances are present (Vallini et al., 1997). Enzymatic systems of micromycetes of the genera *Trichoderma, Aspergillus, Penicillium* and cellulolytic bacteria can participate in the biosystem transformation of wood wastes into humus substances (Imsheneczkij, 1953; Nannipieri et al., 2003; Emcev & Mishustin, 2006).

Destruction of lignin during decomposition of wood wastes is carried out in the presence of oxygen by multi-stage biochemical transformation of organic wood molecules into low-molecular compounds by enzyme systems of fungi and bacteria. In this case, the contribution of micromycetes to decomposition of wood wastes is of particular importance, since their mycelium is able to penetrate into the cell structures of wood and provide direct contact of bacteria and their enzymes with organic molecules of plant cells from the inside (Skryabin et al., 1986; Vedenyapina et al., 2010). Thus, only in the self-organizing biological system (Young & Crawford, 2004; Crawford, 2012) when bacteria and micromycetes act together, the most rapid and organized transformation of wood wastes into humus substances will occur (Kumar & Shweta, 2011; Vorobyov et al., 2011).

In addition, there was an evidence that lignin degradation into phenols and other humic acid precursor substances can promote the formation of humic acid (Wu et al., 2017).

To decompose the bark of coniferous trees from the forest floor of coniferous forests, micromycetes and degrading bacteria were isolated, which served as the basis for a complex biological product (Patent No. 1792974 ‘Method of decomposition of wood’) (Sviridova et al., 1993). To develop the application technology for CBP, a multi - sided study of the mechanisms of functioning of isolated lignin destructors when converting wood waste into humus substances and the principles of organizing micromycetes-bacterial biosystems in which biochemical processes must be supported by effective and coordinated actions of biosystem components was required.

The goal of these studies was to determine the optimal rates of CBP and fertilizers (NPK) and investigate the contribution of micromycetes and bacteria to joint decomposition and humification of coniferous wood waste.

MATERIALS AND METHODS

Content of CBP

The CBP contains lignin- degrading microorganisms isolated from decaying wood located on the soil of forest litter (Sviridova et al., 2001). The total number of microorganisms of CBP is about 10^7 CFU mL^{-1}, of which 10^3 CFU mL^{-1} of micromycetes, 5.0×10^6 CFU mL^{-1} of proteolytic bacteria, 4.0×10^6 CFU mL^{-1} of amylolytic bacteria, 1.0×10^6 CFU mL^{-1} of oligotrophs, and 9.0×10^4 CFU mL^{-1} of cellulolytic bacteria. Dominant cultures of CBP were identified and deposited in ARRIAM collection (RCAM): *Penicillium chrysogenum* (Thom 1910) OH 4 RCAM 00741 and *Pseudomonas fluorescens* 7 RCAM 00537. Sequences was obtained and deposited in GenBank, (GenBank Accession Numbers: MT156331 for *Penicillium chrysogenum* and MT156340 for *Pseudomonas fluorescens*). The dominant micromycetes *Penicillium chrysogenum* (Thom 1910) OH 4 RCAM 00741 and bacteria *Pseudomonas fluorescens* 7 RCAM 00537 possessed of high catalase, oxidase, cellulase, proteolytic activities and were aerobes.

Identification of dominant cultures

Identification of dominant cultures was performed by PCR-amplification and sequencing of 16s rRNA fragment (for bacteria) and internal transcribed spacer (ITS) region (for micromycetes). The 16S rDNA gene was amplified using primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGWTCCARCC-3’). The ITS region of the nuclear ribosomal RNA gene was amplified with primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'–TCCTCCGCTTATTGATATGC-3’) (White et al., 1990). PCR-fragments were extracted from 1% agarose gel (Onishchuk et al., 2015) and sequenced using the ABI PRISM 3500xl (Applied Biosystems, Waltham, MA, USA) according to the manufacturer’s instructions. Homology searches were carried out using the BLASTn program (Altschul et al., 1990) in NCBI GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The dominant microorganisms were identified also with using a morphological and biochemical characteristics by standard methods (Raper et al., 1949; Holt, 1980; Bilaj et al., 1982; Tepper et al., 1993).

Experimental design

The model composting experiments with coniferous sawdust of and different NPK rates were carried out during 20 days in aerobic conditions with daily mixing. The sawdust of coniferous trees from sawmill (Leningrad region, Russia) was used. 15 g of air-dry mass sawdust was placed into plastic container and was distributed in triplicate The humidity of compostable sawdust was maintained to 60%, average temperature of the composting was +20 °C. The NPK rate (per 100 g of air-dry mass of sawdust) was corresponded to: NH_4NO_3 - 5.7 g; KH_2PO_4 - 2.6 g; $CaCO_3$ - 0.15 g and CBP rate is 10 mL per 100 g of air-dry mass of sawdust. Sawdust was treated with NPK and CBP.

The scheme for composting included the following experimental treatments: 1) NPK; 2) 1/2 NPK; 3) NPK + CBP; 4) NPK + 1 / 2CBP; 5) 1/2 NPK + CBP; 6) 1/2 NPK + 1 / 2 CBP.

Analytical methods

Microbiological and chemical analyzes of the composted mass of sawdust were carried out at the end of the experiment, on 20 days of composting. A decrease in C_{org} may be explained by the decomposition of organic matter. Therefore, the degree of decomposition of composted sawdust was evaluated by the loss of organic C. The degree of humification was evaluated by the amount of humic substances formed (fulvic acids and humic acids), as well as by the change in the physical characteristics of composted sawdust (by darkening, compost friability and degree of sawdust destruction).

Assay the composts toxicity

We used a biological test method to assay the toxicity of the composts. The radishes were grown for 3 days in Petri dishes with an extract from the finished compost. 1 g of a compost sample was taken and placed in a flask with 50 mL of water, mixed thoroughly, and then radish seeds were soaked in the resulting filtered solution for one day. After that, radish seeds at the rate of 25 pieces were placed on Petri dishes (4 replicates in each treatment) with filter paper humidified with 10 mL of water.

Microbiological tests

Accounting for various groups of microorganisms in samples of compost treatments was carried out according to the formation of colonies on standard media (Netrusov, 2005). The numbers of colonies of microorganisms were counted on: micromycetes - on Chapek medium with lactic acid, proteolytic bacteria - on meat - peptone agar; amylolytic bacteria - on starch-ammonia agar; oligocarbofiles - on starvation agar and cellulolytic bacteria - on a liquid Hetchinson's medium with filter paper (Tepper et al., 1993). 10 g of each compost samples were placed into flasks with 100 mL distilled water and shaken on for 30 minutes. Serial dilutions from 10^{-2} till 10^{-6} for each sample were prepared 1 mL dilutions were plated to the Petri dishes. Deep passages were made. Plates were incubated at +25 °C during six day and for cellulolytic bacteria - during twenty one days, and colony forming units (CFUs) were counted.

Assay of chemical parameters of composts

The chemical parameters of composts were determined by the following methods: Total nitrogen content (Ntotal) in compost was determined by digesting the samples in sulfuric acid (H_2SO_4) followed by Kjeldahl analysis on Kjeltek apparatus (Tekator, Sweden) (Bremner & Mulvaney, 1982). Nitrates content ($N\text{-}NO_3$) was assayed by photometric method with the phenoldisulfonic acid on KFK-2 electro photocolorimeter (Peterburgskij, 1975). CO_2 content was assayed on a gas chromatograph ('Tsvet-110', Russia), katharometer was a detector, helium was a carrier gas.

Humus fractionation

Humus fractionation was performed and the content of humus compounds was determined by the modifications method (Canellas & Façanha, 2004). Samples were treated with 2 mol L^{-1} o-phosphoric acid to separate the free fulvic acids fraction. Humic fraction was extracted by adding 100 mL of 0.1 M NaOH plus 0.1 M $Na_4P_2O_7$ to 10 g sample and shaken for 16 hours. The dark-colored supernatant solution was separated by centrifugation at 3,000 g for 30 min. The residue was resuspended in 50 mL of 0.1 M NaOH plus 0.1 M $Na_4P_2O_7$ then shaken for 4 hours. The solution was centrifuged

again and the supernatant was added to the supernatant collected previously. The extracted alkaline solution containing dissolved humic and fulvic acids was acidified to pH 1.0–1.5 with H_2SO_4. After 12 hours at 8 °C the extract was separated into soluble and insoluble parts by centrifugation. After fractionation the content of organic carbon in the soluble humified organic matter was determined.

Content of organic carbon (C_{org}) - by oxidation with potassium dichromate by Tyurin method (Orlov & al., 1995). Compost samples were dichromate digested with 10 mL 0.4 N oxidizing solution ($K_2Cr_2O_4$:H2SO4=1:1) by applying in external heat of 150 °C for 20 min in presence of catalyzer Ag_2S0_4; the using oxidizing agent was determined by titration with $Fe(NH_4)_2(SO_4)\cdot 6H_2O$ using phenylanthranilic acid as an indicator.

Data analysis

The experimental data were analyzed using variance, correlation and fractal statistical analysis (Rajzin, 1980; Lakin, 1990; Kronover, 2006). Cluster analysis was used to determine the main factor in grouping experiment treatments into clusters. Euclidean distance was used for data points. The distance between the points and the clusters was estimated using the distant neighbor algorithmic method (Sneath & Sokal, 1973). Correlation analysis was used to calculate the values of the matrix of pair correlations between the measured chemical and microbiological characteristics of composts, using vectors of variation of the values of these characteristics in six experimental treatments. The maximum correlation graph was constructed based on the lower limit of the module of the values of the correlation coefficient is equal to 0.5 (Vorobyov et al., 2006). Fractal analysis modified was used to assess the participation of various physiological groups of microorganisms in the joint transformation of organic substrates.

When three physiological groups of microorganisms arrange on a straight line they are selected as fractal object and named as fractal group of microorganisms (FGM). The frequencies of microorganisms in FGM are the numerical power series (for example, 0.1; 0.01; 0.001). We assume that FGM is an indicator of the biological system organization of microorganisms. When the FGM arrive, three points into fractal portrait arrange on a straight line. Microorganisms of different physiological groups are represented by points on the fractal portraits. The coordinates of these points depend on the logarithms of their frequencies (Vorobyov et al., 2019). This method allows determining of groups microorganisms involved in biological system processes.

The index of microbial bioconsolidation was calculated on the basis of a topological analysis of the location of groups of microorganisms in fractal portraits (Fig. 3). The total number of groups of microorganisms (N0 = 4) represented in the portraits was taken into account, excluding the point located at the origin (X = 0, Y = 0). The number of groups of microorganisms (NF) represented in the portraits and on the dotted lines was taken into account, excluding the point located at the origin (X = 0, Y = 0). The Index of Microbial Bioconsolidation (IMB) was calculated using the following formula:

$$IMB = NF / N0$$

The IMB values for the treatments of the experiment are presented in Table 1. In the same table, the correlation coefficients of IMB with the measured characteristics of the compost are presented.

Table 1. Data of microbiological and chemical analyzes of composts of sawdust conifers with using NPK and CBP*, and ANOVA data, and correlation coefficients with the Index of Microbial Bioconsolidation

No.	Indicators	Experimental treatments						±SE	One-way ANOVA data	Correlation coefficient with IMB
		NPK	1/2 NPK	NPK + CBP	NPK + 1/2 CBP	1/2 NPK + CBP	1/2 NPK +			
		1	2	3	4	5	6			
1	Micromycetes, 10^3 CFU g^{-1}	130c**	77ab	27a	26a	28a	37a	25	$p < 0.002$	-0.61
2	Proteolytic bacteria, 10^3 CFU g^{-1}	2,260c	270a	600ab	500a	830b	4,470d	150	$p < 0.0001$	0.28
3	Amylolytic bacteria, 10^3 CFU CFU g^{-1}	110b	27a	20a	170c	50a	87b	20	$p < 0.0001$	-0.58
4	Oligocarbofiles, 10^3 CFU g^{-1}	3.7a	3.3a	4.0a	6.0a	12.7b	21.0c	2.0	$p < 0.0001$	0.62
5	Cellulolytic bacteria, 10^3 CFU g^{-1}	450c	45a	95ab	45a	45a	8a	30	$p < 0.0001$	-0.49
6	Nitrogen total, %	1.8b	1.0a	1.9b	1.6b	1.1a	0.9a	0.2	$p < 0.0001$	-0.29
7	N-NO_3, g per 100 g compost	1.7a	4.4c	4.3c	4.0bc	3.0b	3.5b	0.4	$p < 0.0001$	0.20
8	Corg of compost, %	42.0a	43.5a	42.1a	42.2a	42.3a	42.1a	1.0	$p < 0.48$	-0.26
9	Compost readiness, points (maximal 10)	5	3	10	7	7	8	-	-	0.68
10	C-CO_2, % (per 1 g of compost and per	0.29a	0.35ab	0.45c	0.33a	0.30a	0.30a	0.03	$p < 0.0003$	0.28
	Index of Microbial Bioconsolidation	0.6	0.6	1.0	0.5	1.0	1.0			

*Notes: 20 days of composting.

**Letters next to numbers indicate differences at $p < 0.05$ and according to the Dunkan test.

Microbiological and chemical data of the experiments

The microbiological and chemical data of the experiments on the humification of wood waste are shown in Table 1. The content of microorganisms in composts content was about 10^6 CFU g^{-1}. The participation of different groups of micromycetes and bacteria in the transformation of coniferous sawdust was revealed. In treatments No. 3–6 (with addition of CBP) the most effective composting of sawdust was noted. In these treatments, the dominant microorganisms were micromycetes *Penicillium chrysogenum* (Thom, 1910), which reached up to 80–90%. The number of *Trichoderma viride* (Pers, 1794) amounted 10%. Among bacteria *Pseudomonas fluorescens* was dominated. The increased abundance of *Penicillium chrysogenum* (Thom, 1910) and *Pseudomonas fluorescens* in the microbial community of composted material indicated their joint action in sawdust decomposition. These bacteria also were dominant in microbial community of CBP.

It was noted that the application of NPK increased the total number of microorganisms due to increasing of micromycetes, proteolytic bacteria, and cellulolytic bacteria. In treatments No. 1 and No. 2 (without CBP) the number of phytopathogenic micromycetes *Fusarium oxysporum* (Schltdl 1824) and *Fusarium culmorum* (Wm.G. Sm., Sacc. 1892) reached to 50%. Whereas as the number of micromycetes of other genera consisted: for *Penicillium* - 20%, for *Trichoderma* (*T. viride* Pers., 1794) - 10–20%, for *Aspergillus* (*A. niger* Tiegh., 1867) - 10%, for *Mucor* - 1–5%. In that case a dominant bacteria were *Bacillus.* Thus, in treatments without CBP the micromycetes of the genus *Fusarium* prevailed.

The decrease of C_{org} content in composts average by 6% (from 48 to 42%) in comparison with the sawdust was revealed, that in all composts there was a decrease in C_{org} from 48% (initial sawdust) to 42% (experience). It confirms the active decomposition of sawdust when using of NPK. The experimental treatment with addition of NPK (in maximal rate) and CBP had the best indicators for substrate decomposition, the destruction of sawdust was better and compost has high degree of destruction.

A small content of fulvic acids was detected in all treatments. In the treatment No. 1 (NPK) the content of fulvic acids was 0.03%. In treatment No. 2 (1/2 NPK) the content of fulvic acids was 0.01%, but humic acids were not found. However, in the treatments with addition of CBP the presence of humic acids was detected. In treatment No. 3 (CBP + NPK) the content of fulvic acid was 0.12% and the content of humic acid was 0.02%. In treatments No. 4–6 the content of fulvic acids was 0.07% and humic acids was 0.01%.

The composts obtained in experimental treatments with CBP stimulated biomass of radish seedlings up to 10% and radish seeds germination was 100%. In the same time in treatments No. 1 and 2 (without CBP) radish seeds germination was only 95–97%.

It was noted that the intensity of CO_2 emission during composting was lower in the treatment without the use of CBP and with a full dose of nitrogen (No. 1) - at the level of 0.20–0.29%. This may be due to denitrification processes, which can occur at low respiration rates. This is also indicated by a decrease in the content of $N\text{-}NO_3$ in the compost of this treatment relative to others. In the treatment with a half dose of nitrogen (No. 2), the respiration rate gradually increased to 0.35%, which may be due to longer processes of microbiological decomposition of the sawdust.

With a decrease of the application rate of CBP (1/2 CBP), the respiratory rate also decreased. The increased activity of CO_2 emission in the early stages of composting in

treatments with CBP indicate an intensification of the decomposition of sawdust by the CBP community of micromycetes and bacteria initiated by CBP.

It was noted that in compost of treatment No. 1 (NPK) was a sufficiently high contents of micromycetes, proteolytic bacteria and cellulolytic microorganisms, but the degree of humification of sawdust was lower than in treatments with CBP. In treatment No. 6 (1/2 NPK + 1/2 CBP) with a good degree of humification of the substrate, a higher content of micromycetes was noted than in other treatments with addition of CBP, but the number of cellulolytic microorganisms was lower than in treatment No. 3 (CBP and NPK), i.e. degrading processes slowed down.

Meanwhile, in the optimal treatment No. 3 (NPK + CBP), the number of proteolytic and amylolytic microorganisms was average, but the content of cellulolytic microorganisms was twice higher compared to treatments No. 2, No. 4, No. 5 and No. 6 with a fairly high number of micromycetes.

Probably, a biosystem of microorganisms formed in this ratio could be optimal for the processes of humification. This may indicate the influence of the micromycete-bacterial complex of CBP (contains a biosystem community of lignin destructors) on the activation of the processes of transformation of lignocellulosic substrates into humic substances.

RESULTS OF DATA ANALYSIS

As a result, of the cluster analysis of the characteristics of the experimental treatments, a dendrogram was constructed (Fig. 1) and the factors uniting the experimental treatments into clusters were determined. It noted that the treatments with NPK (No. 1and No. 2) and without CBP did not form a cluster, two clusters formed when CBP was add. Treatments No. 3 and No. 4 (with full dose of NPK) united into the first cluster. Treatments No. 5 and No. 6 (with 1/2 NPK) conform the second cluster. Thus, the main factor in the clustering was NPK rates, which unite these treatments into clusters.

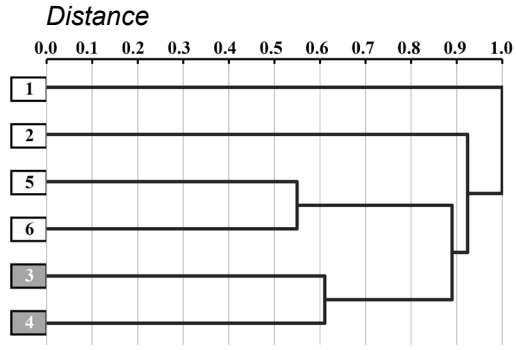

Figure 1. A dendrogram of the proximity of experimental options, built on their microbiological and chemical characteristics: 1) NPK; 2) 1/2NPK; 3) NPK+CBP; 4) NPK+1/2BP; 5) 1/2NPK +CBP; 6) 1/2NPK +1/2CBP (distance in the Euclidean space according to the principle of 'distant neighbor').

The analysis of pair correlations of the compost's characteristics and microbial community data (Table 1) made it possible to single out the highest absolute coefficients of correlations and construct a nondirected graph of maximum correlations (GMC, Fig. 2).

It was taken into account the absolute value of the correlation coefficients should exceed 0.5. GMC demonstrates the relationship ($r = 0.89$) of micromycetes (1) with cellulolytic bacteria (5), which indicates their joint participation in the decomposition of lignocellulose from sawdust. The absence of a connection between amylolytic bacteria (3) and other components of the biosystem means that this group of microorganisms

does not take an active part in biosystem processes of composting. Their activity requires readily available carbohydrates that have not formed in the right amount. GMC demonstrates that proteolytic bacteria (2) and oligocarbofilic bacteria (4) act in a close correlation ligament (r = 0.76), actively participating in the processes of transformation of the organic substrate.

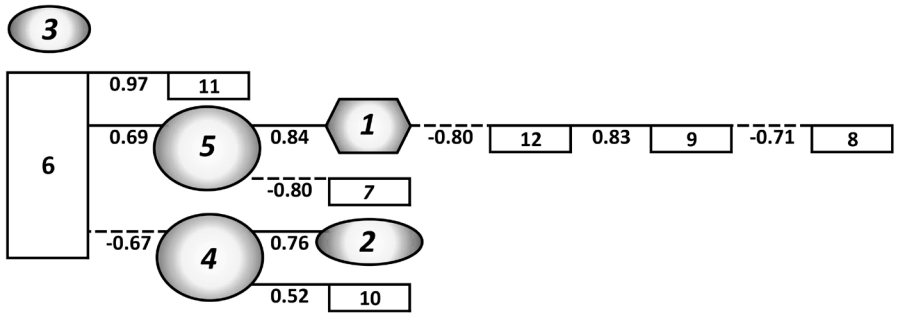

Figure 2. A graph of the maximum correlation coefficients between the chemical and microbiological characteristics of compost during the humification of wood waste: 1 – micromycetes; 2 – proteolytic bacteria; 3 – amylolytic bacteria; 4 – oligotrophs; 5 – cellulolytic bacteria; 6 – N_{total}; 7 – $N\text{-}NO_3$; 8 – Corg. of compost; 9 – the degree of compost readiness; 10 – $C\text{-}CO_2$; 11 – dose of N; 12 – the rate of application of the biological product.

Oligocarbofilic bacteria (4) with active oxidoreductases participate in the decomposition of lignin together with proteolytic bacteria (2) (r = 0.76) and decrease the N_{total} (6) (r = -0.67). Micromycetes (1) and cellulolytic bacteria (5) also act in conjunction (r = 0.84) destroying polymer organic molecules in compost. In this case $N\text{-}NO_3$ (7) is consumed and the connection with N_{total} (6) (r = 0.69) is strengthened with an increase in its dose. Thus, two groups of microorganisms (2)–(4) and (1)–(5) perform the main function of the destructive biosystem. The use of CBP (12) accelerates composting processes, which is reflected in an increase in the level of humification of compost (9) (r = 0.83). A decrease in the total number of micromycetes (1) when using CBP (12) indicates that micromycetes function in the composition of biosystems with a smaller number (r = -0.81), but with a greater efficiency of further biochemical transformations of organic substrates and with reducing of consumption of energy and nutrient resources.

To assess the extent of participation of the functional groups of microorganisms in biosystem processes, we used a fractal analysis of microorganism's amount. For this, the fractal portraits of microbial communities were constructed (Fig. 3). In fractal portrait points represent the groups of microorganisms with coordinates depending on the frequency of occurrence of the corresponding microorganisms. The topological analysis of the arrangement of points in a fractal portrait showed that some groups of three points (triplets) are located on straight lines. We assume that this arrangement of points is a nonrandom event, and it indicates the formation of a biosystem by the corresponding groups of microorganisms. The absence of (1)–(5) or (2)–(4) microorganism’s groups in triplets in treatments No. 1 and No. 2 (without CBP, Fig. 3a and 3b) demonstrated the absence of CBP influence on biosystem processes. The location of micromycetes (1) in the upper part of the fractal portrait demonstrate low efficiency of waste humification

the in treatments No. 1 and No. 2 (Fig. 3, a and 3, b). In treatments No. 3 (CBP + NPK, Fig. 3, c) and No. 6 (1/2CBP + 1/2NPK) (Fig. 3, f), all points depicting trophic groups of microorganisms are covered by straight dashed lines, i.e. these microorganisms form common biosystems. We believe that in these treatments, the biosystems are formed and micromycetes (1) take part in biosystem's processes in the first positions, at the very beginning of composting, since they are located in the lower part of the fractal portraits.

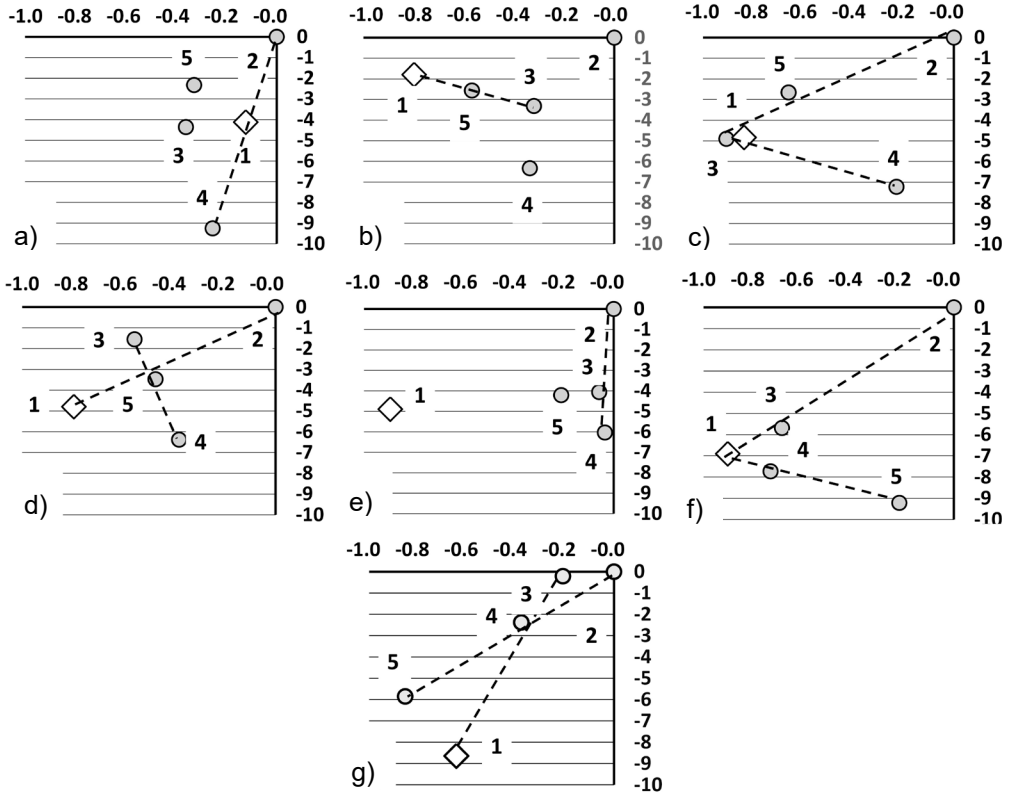

Figure 3. Fractal portraits of trophic groups of microorganisms participating in the processes of humification of wood waste according to experimental options.
Treatments of experience: a – NPK; b – 1/2 NPK; c – NPK +CBP; d – NPK + 1/2 CBP; e – 1/2 NPK +CBP; f – 1/2 NPK +1/2 CBP; g – Complex Biological Product (CBP).
Groups of microorganisms: 1) micromycetes, 2) proteolytic bacteria, 3) amylolytic bacteria, 4) oligocarbofiles, 5) cellulolytic bacteria.
Notes: The numbering of the points in the portrait corresponds to the numbering of microorganisms in Table 1. On the X-axis graphs, the fractional part of the $log_2(p_i / p_{max})$ value is plotted, and along the Y-axis, the full $log_2(p_i / p_{max})$ value is plotted, where pi and $p_{max} = p_2$ are the frequency of occurrence of microorganisms in compost with the number (i = 1, 2, 3, 4, 5) and the frequency of occurrence of dominant microorganisms, which are proteolytic bacteria - (i = 2).

DISCUSSION

The microbiological transformation of wood wastes from the woodworking industry into substances makes it possible to solve ecological problem and agricultural

fertilizing. To increase the efficiency of microbiological transformation of wood waste into humic substances, it is necessary that the microbial community forms an active destructive biosystem. Microorganisms in the composition of sawdust destructive biosystems transform organic substrates in an organized and orderly manner, evenly distributing the enzymatic load among the participants of a conversion processes. Since microorganisms in the composition of biosystems transform organic substrates in an organized and orderly manner, evenly distributing the enzymatic load among the participants in the conversion process. For example, in consortium of cellulolytic and oligocarbofilic bacteria in soils, bacteria are consistently responsible for the hydrolytic degradation of complex high-molecular nitrogen-free organic substances (cellulolitic), as well as for the redox processes of transformation of residual products into humus links (oligocarbofiles). Such active destructive biosystem was formed in sawdust composts obtained with addition of CBP (Kozlov et al., 2017). For fast humification of composts the optimal ratio between the numbers of different groups of microorganisms should be set. The active destructive CBP biosystem (which containse a micromycetes, proteolytic, cellulolitic and oligocarbofilic bacteria) activated the processes of transformation of lignocellulosic substrates into humic substances. The increasing of activity of CO_2 emission in the early stages of experiment in treatments with addition of CBP indicated the increasing of decomposition of sawdust.

Before composting experiments, a long cultivation of the microbial community was carried out, and the micromycetes-bacterial biosystem was selected for CBP. The fractal portrait (Fig. 3, g) confirmed the systemic consolidation of microorganisms in the CBP.

It is noted, that the compost from other treatments (without addition of CBP) decreased the total mass of radish seedlings below the control on 5%. Because in these composts dominated the phytopathogenic micromycetes of the genus *Fusarium*. Whereas the toxicity of composts obtained with addition of CBP was eliminated. Because in biosystems of these composts *Penicillium chrysogenum* (Thom, 1910) and *Pseudomonas fluorescens* were dominated. The possibility of suppressing soil plant pathogens using compost has been widely studied (Noble & Coventry, 2005: Pugliese et al., 2010). Compost quality is also related to the concentration of humic substances in the final product; thus, determining humification progress is essential for evaluating composting process (Kulikowska & Sindrewicz, 2018). Composts obtained with addition of CBP had a high degree decomposition and small content humic acids, which depend on the composting time. Humic substances increase a soil fertility (Vergnoux et al., 2011).

The dendrogram (Fig. 1) demonstrates the dependence of sawdust composting and transformation into humic substances on rates of NPK and 1/2 NPK, because two different cluster are formed with these rates. When using CBP, the NPK and 1/2NPK rate determines the biochemical characteristics of the destructive processes and the number of microorganisms involved in these processes. Apparently, the low dose of NPK is necessary to start of destruction and to grow of microorganisms in the required quantitative ratio.

The graph of maximum correlations (Fig. 2) shows the formation of two binary microbial complexes in a destructive biosystem: (1)–(5) - micromycetes and cellulolytic bacteria, (2)–(4) - proteolytic bacteria and oligocarbofiles. The greatest emission of CO_2 and decomposition of the composted mass were recorded in experimental treatment No. 3. Based on this data it can be argued that in experimental treatment No. 3 the

(2)–(4) microbial complex acts maximal effectively, which determines the highest intensity of the processes of transformation of the organic substrate in humic substances. At the same time, the (1)–(5) microbial complex increases the nitrogen content in humic organic mass that increases its fertilizing properties.

Fractal portraits of microbial communities of compost masses showed that in the experimental treatments No. 3, 4, and 6 (Fig. 3, c; 3, d; 3, f) biosystems similar to CBP biosystem (Fig. 3, g), were formed. But, in the experimental treatments No. 1, 2 (Fig. 3, a; 3, b) this biosystems were not formed. This indicates that the addition of CBP stimulated the formation of microbial biosystems in composts.

This confirmed also by emitting of a greater amount of CO_2 (45, 33, 30% per g of compost and per day) in these experimental treatments.

The correlation of IMB with the compost decomposition scores (feature 9, $r = 0.68$) indicates an increased importance of the biosystem organization of the microbial community for accelerated composting of wood waste.

CONCLUSION

The micromycetes closely interacted with cellulolytic, oligocarbofiles and proteolytic bacteria during composting of sawdust of coniferous trees. The micromycetes closely interacted with cellulolytic, oligocarbofiles and proteolytic bacteria during the process of coniferous sawdust decomposition. As a result, fulvic acids and humic acids were formed.

The addition of CBP during coniferous sawdust composting resulted in production of a high-quality non-toxic compost and in micromycetes-bacterial biosystem (similar to the biosystem in CBP) formation. The optimal rates of CBP and fertilizers (NPK) for composting were determined. The fractal portrait demonstrated the formation of a micromycetes-bacterial system when NPK and CBP were applied.

ACKNOWLEDGEMENTS. This work partially was supported by a subsidy of the Ministry of Education and Science of the Russian Federation No. 0664-2019-0025 and FGUS-2022-0003.

The research was performed using equipment belonging to the Core Centrum ‘Genomic Technologies, Proteomics and Cell Biology’ of All-Russia Research Institute for Agricultural Microbiology.

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