CHOOSING MOST PROSPECTIVE PROCESS OF MAKING NEW BIOFERTILIZERS

Galina Yu. Rabinovich1, Daria V. Tikhomirova2

1,2Federal State Budgetary Scientific Institution All-Russian Research Institute of Reclaimed Lands, Emmauss, Russia

12016vniimz-noo@list.ru, 22016vniimz-noo@list.ru

Corresponding Author: Galina Yu. Rabinovich

https://doi.org/10.26782/jmcms.spl.10/2020.06.00033

Abstract

The Department of Biotechnologies at the VNIIMZ (Tver oblast’, Russia) has developed the method of making new organic biofertilizer BiGuEM based on chicken (poultry) manure and turf. The peculiarity of the new method is that it involves alcalizing the turf-manure mix, followed by adding various kinds of biostimulants. The basic method of making BiGuEm has been patented, and its modified versions are currently being patented one by one.

This work was aimed at evaluating the results of screening assays for choosing the best way of producing BiGuEm that had gained an edge on the other processes upon the addition of the new biostimulant to the initial fermented mass. In the end, that biostimulant demonstrated the highest efficiency. The choice of the most efficient BiGuEm production process was made by a set of methods of biochemical, microbiological, and agrochemical analyses conducted, considering their behavior.

Three variants of producing BiGuEm were studied, and it was recognized that the best one was a modified process called S3 and run using a complex-component stimulant, including the combination of citric acid and acetic magnesium. That process corresponded to the maximum reductive-oxidative coefficient (ROC) that indicated the active catabolic orientation of transformative conversions, reached 0.91 at the end of bioprocessing, and signaled, through mobilized microbial flora, about the accumulation of available nutrients in the biofertilizer. In addition, a significant increase in the level of invertase activity was observed by the end of fermenting at the synchronous receding activity of cellulase, which pointed at the advancing replacement of substrates for its activity with low-molecule compounds. It was found out that the highest fractions (% per abs.dr.subs.) in the biofertilizer produced by S3 belonged to such fertilizer elements as phosphorus (P2O5) (2.52) and potassium (K2O) (1.44). The high carbon content of up to 31.85 indicated that the resulting biofertilizer possessed a considerable energy potential. According to the interpretation of the set of the results, the production of BiGuEm using citric acid and acetic magnesium was...
related to one of the most prospective processes for further elaboration and testing on different agricultural crops.

**Keywords:** BiGu Embio fertilizer; bioprocessing; oxidoreductase and hydrolase; reductive-oxidative coefficient’ microbial flora; fertilizer elements.

I. Introduction

The procedures and strategies required for adoption in agricultural production will not only allow controlling the quality and amount of applied fertilizers but also localize and handle their amount (XXXVII,XVII,XIX). A cheap, efficient, and sustainable kind of waste processing is composting defined as the natural decomposition of organic substances with microorganisms in a controlled environment.

The processing of organic raw materials into highly efficient fertilizers with an enhanced level of nutritional content and ecofriendliness through the use of microorganism potential acquires a growing socioeconomic and environmental significance for the whole global community. Many organizations dedicating their activity to solving the problem in question are involved in developing efficient methods of bioprocessing organic raw materials. There are a lot of internationally known kinds of composting from diverse organic materials(IX,VI,XXIX,XVII)VIII XI; XXVI;XXXVIII; II;XXVIII,XXI,III). The application of organic fertilizers produced by this method increases the content of organic substances and nutrients in soil, which improves its physical, chemical, and biological characteristics, increases the agricultural output, and improves its quality (X,XXIII,XXVI,XX).

The technologies of making organic fertilizers from agro-industrial waste are constantly being enhanced, which is proven by invention of new methods of bioconversion (fermentation) as the basis of composting (XXXI). Russia has the world’s highest potential amounts of raw materials for producing organic biofertilizers. Despite a considerable reduction in the applied amounts of organic fertilizers, which stems from a decreasing livestock population in most Russian regions and an impairing material and engineering foundation for producing and applying organic fertilizers, their share in the total input of nutrients for practical farming remains relatively consistent because of the reduction in using mineral fertilizers in recent years (XLI).

Poultry manure is one of the kinds of valuable organic raw materials with a high nutrient content and better in many respects than cattle and pig manure (XXV, XXVII). The composting process may inactivate pathogenic microorganisms contained in chicken manure, and then these manure become safe and useful for application in soil (V, XXIX).

It is known from (XXXVI, XXIV, XLII, XXIII, XX, XVI) that organic fertilizers with manure improve the energy value and yield of farming crops, make them more immune to diseases and insects, extend product storage life, and increase the microbiological activity and nutrient content in soil.

The All-Russian Research Institute of Reclaimed Lands is the developer and holder of patents for several organic raw materials processing biotechnologies applied in various forms of bioconversion and further recommended for multipurpose application in agriculture. One of such technologies is the six-day method of making Bi-
GuEm (XXXIII) formed from chicken manure and turf as the basic carbon-containing raw material of composting. BiGuEm is a homogeneous dark brown bulk mass with a slight specific smell of alkali, favorable acidity, fairly highly content of agronomically useful microbial flora, fertilizer elements in the form accessible to plants and microbial flora, and also phytohormonally active substances (XXXIII, XXXIV, XXXV). The BiGuEm production technology is updated by variations in using various biostimulants as mixes of organic and inorganic compounds in various concentrations. Currently, the staff of the Department of Biotechnologies is actively pursuing this line of work by modifying the process and producing more efficient analogous biofertilizers. The study presented below arose from the need for evaluating the results of screening BiGuEm production processes by means of different stimulants, the choice of the best process, and the decision on its further practical application.

II. Materials and Methods

The planned tests were conducted in 2016-17 at the Department of Biotechnologies of VNIMZ. All the BiGuEm production processes were run in the fermenter by bioconversion divided in two phases. The bioconversion is preceded by preparing the initial mix of organic raw materials, including poultry manure and turf taken at a ratio of 50:50. The initial mix is thoroughly stirred and chaffed to particles with a grain size of no more than 10 mm. The turf-manure mix is processed (alkalinized) for 24 hours at 20 to 22°C using 0.5 % KOH to destroy high-molecular organic compounds. The alkalinizing activates the enzymic activity of oxidoreductases and hydrolases.

The first phase of bioconversion continues for 96 hours at 36 to 39°C; this is why, this phase is attended by an active growth of mesophilous microbial flora, including the one turned in spores in the preceding alkaline hydrolysis of the turf-manure mix as well as sanitary-indicative microbial flora that feels very comfortable in the specified range of temperatures. In the second phase of bioconversion going on for 24 hours at 55 to 60°C the biofertilizer achieves a required level of ecofriendliness. The samples of the fermentable mass taken one day after the biofertilizer’s unloading from the fermenter contain no enterobacteria (representatives of sanitary-indicative microbial flora) and much lower amounts of fungi indicative of the presence of pathogenic agents. Such an effect is unachievable in the mesophilous phase because the temperature of 36 to 39°C favors the growth of sanitary-indicative microbial flora and spoilage microorganisms. The product formed in the approved basic bioconversion process is ecofriendly and shows characteristic quality figures.

In the course of making the biofertilizer various stimulants are usually added after alkalinizing. To make them more convenient for perception, the compared BiGuEm production processes were designated in short as basic, BS1, and S3. The only stimulant of microbiological activity used in the basic process was flour milling waste. The complex-component stimulant additionally applied to the fermentable substrate in BS1 was a vinoacetous mix of potassium and sodium and citric acid with a concentration of 0.1 % per initial raw mass. In S3 it was decided to not change the chosen concentration of both components; however, the vinoacetous K-Na salt was replaced with magnesium acetate. Thus three processes of producing the new bioferti-
lizer were selected, and their testing allowed choosing the optimal process with the best complex-component stimulant.

The bioconversion processes with various complex-component stimulants were tested by collecting initial samples, fermentable mix, and final product (actually, the biofertilizer proper) by biochemical, microbiological, and agrochemical analyses.

The three kinds of BiGuEm for screening processes were produced from an identical raw material extracted from one source. This is incredibly important because processes related to bioactive organic materials are heavily dependent on this circumstance as a result of their rapidly changing proper characteristics unlike in minerals and other inert substrates. In this respect, the alkalinized initial raw material was made variable after alkalinizing precisely by applying various substrates.

Despite their differences, the purpose of all the three processes when implemented ensures the formation of biofertilizers of similar quality and efficiency. Should an inappropriate test procedure be chosen, this circumstance may make it more difficult to choose the best process. Therefore, special significance was attached to the procedure of evaluating the bioconversion processes and the quality of produced biofertilizers.

The tests were annually put up with a threefold repeatability. The sampling throughout each process was conducted at the start of fermenting (initial mix), after 48 hours, after 96 hours, and at the end of the process (after 144 hours). The samples were analyzed against several mobile indicators, which involved measuring microbial flora, activity of oxidoreductase (catalase and dehydrogenase) and hydrolase (invertase, cellulase, protease, and urease) enzymes, and agrochemical indicators of final fertilizers. The microbial flora was measured by limiting dilution; the enzymic activity of the samples was evaluated by the gasometric and photocolorimetric analysis (XLIII, XXXII) adapted to organic substrates. The nitrogen, phosphorus, potassium, and carbon content were measured according to GOST procedures 32044.1-2012, 26657-97, 30504-97, 26213-91 used at the mass analysis lab of the Department of Biotechnologies.

The statistic data processing was conducted in Microsoft Excel 2003 and Statgraphics 6.0.

III. Results

The peculiarity of the first few days of the production of BiGuEm is the alkalinizing of the initial turf-manure mix for profoundly destroying high-molecular compounds that activate microbial flora and oxidoreductase and hydrolase enzymes in the following fermenting phases.

It should be noted that nitrogen-transforming microorganisms play the dominant role in converting the organic raw materials in use (Table 1). All the three BiGuEm production processes were attended by a gradual reduction in the ammonifying and amilolytic microbial flora content by the end of the fermenting process. To reflect the rate at which the substrate is converted by nitrogen-transforming microbial flora, it is expedient to use mineralization coefficient KmN formed as the relation between the amounts of amilolytic and ammonifyingmicroflora. The variation in KmN in the tested BiGuEm production processes is shown in Fig. 1. In addition, Table 1 shows
the behavior of the two groups of microbial flora (microscopic fungi and enterobacteria) that are indicative of the formation of ecofriendly biological fertilizers in the course of the compared processes.

Table 1: Behavior of microbiological indicators in the BiGuEm production process

| Process | Variants | Ammonif. mcorgs., mln/g | Amilolyt. mcorgs., mln/g | Fungi. 1 000/g | Enterobacteria. mln/g |
|---------|----------|--------------------------|--------------------------|----------------|-----------------------|
| Basic   | Init. mix | 1 109.02 ± 49.44 | 580.90 ± 19.59 | 9.21 ±0.25 | 477.23 ± 11.87 |
|         | 48 h      | 751.58 ± 24.97 | 527.21 ± 21.57 | 2.16 ± 0.09 | 196.11 ± 7.08 |
|         | 96 h      | 534.52 ± 19.51 | 297.28 ± 6.60 | 0.38 ± 0.02 | 104.94 ± 4.52 |
|         | 144 h     | 407.96 ± 17.71 | 133.99 ± 4.04 | 0.12 ± 0.01 | 0 |
| BS 1    | Init. mix | 1157.24 ± 29.77 | 598.13 ± 11.68 | 7.28 ± 0.16 | 491.39 ± 14.34 |
|         | 48 h      | 877.48 ± 43.89 | 502.04 ± 6.51 | 3.70 ± 0.07 | 234.96 ± 10.67 |
|         | 96 h      | 549.58 ± 24.73 | 252.49 ± 10.26 | 0.45 ± 0.03 | 130.52 ± 5.06 |
|         | 144 h     | 220.59 ± 9.85 | 118.49 ± 3.22 | 0.08 ± 0.01 | 0 |
| S 3     | Init. mix | 1122.51 ± 48.84 | 415.44 ± 7.24 | 8.64 ± 0.24 | 544.87 ± 20.34 |
|         | 48 h      | 688.14 ± 29.89 | 225.72 ± 9.11 | 2.72 ± 0.11 | 246.37 ± 9.29 |
|         | 96 h      | 393.15 ± 16.51 | 163.50 ± 3.70 | 0.40 ± 0.02 | 96.60 ± 2.72 |
|         | 144 h     | 233.48 ± 8.78 | 115.27 ± 3.30 | 0.08 ± 0.01 | 0 |
Fig. 1: Variations in mineralization coefficient $K_{mNd}$ during BiGuEm production

Table 2 shows the results of measuring the activity of oxidoreductase enzymes indicating that the fermentable mass goes through intensive decomposition and synthesis. The most characteristic activity estimate of oxidoreductases was derived using the oxidative-reductive coefficient (ROC). This coefficient is the relation of activity between oxidase and reductase enzymes (converted to provisional units) and reflects the general course and intensity of fermenting (Fig. 22).

According to the ROC calculations, the basic BiGuEm production process and BS1 developed evenly: in both processes that coefficient increased in the first half of bioconversion and linearly decreased by its end. S3 had a high ROC in the first half, which indicated the top level of transformations and pointed at the initially high efficiency of the biostimulant in question and its maximally oriented catabolic essence.

Table 2: Behavior of oxidoreductase in BiGuEm production processes

| Process | Variants | Catalase activity. mIO$_2$/g/min | Dehydrogenase activity. mg TPHF/g/24 h |
|---------|----------|-----------------------------------|----------------------------------------|
| basic   | Init. mix| 28.97 ± 1.04                      | 3.14 ± 0.15                            |
|         | 48 h     | 51.74 ± 2.55                      | 3.74 ± 0.07                            |
|         | 96 h     | 47.68 ± 1.51                      | 3.66 ± 0.07                            |
|         | 144 h    | 22.92 ± 1.43                      | 2.98 ± 0.11                            |
| BS1     | Init. mix| 29.04 ± 1.55                      | 3.06 ± 0.09                            |
|         | 48 h     | 41.78 ± 1.27**                    | 3.54 ± 0.08                            |
|         | 96 h     | 46.06 ± 1.58                      | 5.23 ± 0.10***                         |
|         | 144 h    | 22.90 ± 0.96                      | 3.41 ± 0.16                            |
| S3      | Init. mix| 33.52 ± 1.69*                     | 2.73 ± 0.08*                           |
|         | 48 h     | 54.96 ± 2.30                      | 3.28 ± 0.07**                          |
The activity of hydrolases is represented by enzymes from carbon- (cellulase-invertase) and nitrogen-transforming (protease-urease) arrays (Table 3).

The activity of cellulase in the BiGuEm production processes was undulating and receded mostly in S3. In all the considered variants of producing BiGuEm relatively high values of the invertase activity were registered in the first half of the process; therefore, it was in that period when the maximum disintegration of sugars was observed. Like with the activity of cellulase, a decline in the activity of that enzyme was observed; by the end of the process the decline converted to an increase, especially in the bioconversion using magnesium acetate (S3).

**Table 3: Behavior of hydrolytic enzymes in BiGuEm production processes**

| Process | Variants | Enzyme activity | invertase. mg of glucose/g | cellulase. μg of glucose/g/day | protease. μg of protein/g/h | urease. mg NH₃/g/day |
|---------|----------|-----------------|---------------------------|-------------------------------|-----------------------------|---------------------|
| basic   | Init. mix| 259.25 ± 10.38  | 3.71 ± 0.16                | 2.76 ± 0.14                   | 9.23 ± 0.39                 |
|         | 48 h     | 396.53 ± 7.19   | 1.71 ± 0.06                | 2.70 ± 0.11                   | 12.03 ± 0.27                |
|         | 96 h     | 291.00 ± 9.62   | 2.32 ± 0.05                | 2.41 ± 0.04                   | 3.96 ± 0.15                 |
|         | 144 h    | 303.15 ± 8.03   | 3.24 ± 0.04                | 2.39 ± 0.05                   | 10.50 ± 0.26                |
| BS1     | Init. mix| 219.9 ± 11.35*  | 2.43 ± 0.05***             | 2.55 ± 0.10                   | 9.31 ± 0.36                 |
|         | 48 h     | 511.15 ± 14.56***| 1.95 ± 0.01                | 2.47 ± 0.06                   | 16.00 ± 0.66**              |
|         | 96 h     | 431.00 ± 9.56****| 2.78 ± 0.06***             | 2.40 ± 0.10                   | 10.56±0.23****              |
|         | 144 h    | 448.7 ± 8.22****| 2.28 ± 0.06****            | 2.33 ± 0.08                   | 10.92 ± 0.64                |
| S3      | Init. mix| 197.85 ± 6.29** | 4.77 ± 0.15**              | 2.72 ± 0.09                   | 9.67 ± 0.19                 |

Footnote: the difference between the basic variant and the variants for comparison is valid at * p ≤ 0.1; ** p ≤ 0.05; *** p ≤ 0.01; **** p ≤ 0.001
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In all the BiGuEm production processes the activity of protease gradually declined during the fermenting phase (Table 3), whereas the action of another nitrogen-transforming enzyme called urease took an opposite course, which is highly important to bioconversion.

It is important that the intensity of fermenting processes depends on the moisture content in the organic mix. The recommended moisture level for all solid-phase processes, BiGuEm production included, is 60 to 75 % (XXXIV - XXXV). In all the tested BiGuEm production processes the moisture level at the end of bioconversion would usually decrease to equal 60 % on average. The accumulation of fertilizer elements exposed in Table 4 below was observed in all those production processes and conditioned by a favorable moisture level positively affecting the microbial flora involved in the bioprocessing of the turf-manure mix.

**Table 4: Behavior of basic agrochemical indicators in BiGuEm production processes**

| Process | Variant | Total nitrogen ads, % | P\(_2\)O\(_5\) | K\(_2\)O | Carbon content, % |
|---------|---------|-----------------------|----------------|---------|-----------------|
| Init. mix | 48 h | 1.85 ± 0.04 | 1.90 ± 0.06 | 1.11 ± 0.05 |
|          | 96 h | 2.12 ± 0.04 | 1.88 ± 0.07 | 1.18 ± 0.04 |
|          | 144 h | 1.76 ± 0.04 | 2.19 ± 0.10 | 1.17 ± 0.04 |
| Init. mix | 48 h | 1.87 ± 0.07 | 1.89 ± 0.06 | 1.02 ± 0.05 |
|          | 96 h | 1.81 ± 0.07 | 1.66 ± 0.07 | 1.05 ± 0.03 |
|          | 144 h | 1.86 ± 0.07 | 1.95 ± 0.08 | 1.42 ± 0.07 |
| Init. mix | 48 h | 2.01 ± 0.10 | 2.26 ± 0.05 | 1.32 ± 0.05 |
|          | 96 h | 1.82 ± 0.09 | 2.09 ± 0.08 | 1.12 ± 0.06 |
|          | 144 h | 1.91 ± 0.08 | 2.52 ± 0.09 | 1.44 ± 0.04 |

**IV. Discussion**

In terms of microbiology an important role in formation of biofertilizers is played by agriculously valuable groups of microorganisms. The registered data about the microbial flora size in the studied bioconversion processes revealed the dominant
role of nitrogen-transforming microorganisms (Table 1) involved in bioconversion transformations (XXXIV - XXXV). It follows from the Table that by the end of all the bioconversion processes the size of those groups would gradually dwindle, which was indicative of the gradual sampling of their correspondent substrates. It should be noted that in the basic process of producing the new biofertilizer the ammonifying microbial flora size remained very high even at the end of the process, which indicated that it was insufficiently efficient as compared with the processes involving complex-component stimulants. A similar trend was observed when variations in the size of amilolytic microbial flora able to use mineral forms of nitrogen were investigated.

In the basic process and in BS1, where vinoacetous K-Na was used, the KmN mineralization coefficient (Fig. 1) would rise after 48 hours from the start of the processes and go down by their end. In BS1KmN was higher than in the initial raw material. In S3 using magnesium acetate that indicator behaved somewhat differently but its value in the final product was also higher than in the initial mix. However, in all the three BiGuEm fertilizers and fermentable substrates KmN was below 1, which was indicative of a minor loss of nitrogen in the fermenting processes; that was why, all the kinds of BiGuEm had to accumulate nitrogen, which would eventually endow them with fertilizing value. In addition, despite the low values of mineralization coefficients, the final BiGuEm products appeared to have enough microorganisms, which indicated that the future fertilizers had a high biological activity potential (XXXI, XXXII).

It should also be noted that in all the three processes the microscopic fungi content decreased by the end of fermenting and the final BiGuEm products appeared to have no enterobacteria at all after the fermentable mix passed the temperature peak, which was a favorable sanitary indicator for the formed biofertilizer. It is interesting that in the biofertilizers from BS1 and S3 using the combinations of citric acid with vinoacetous K-Na salt and magnesium acetate, respectively, the fungi content was somewhat lower than in the basic BiGuEm production variant. This has most likely to do with the fact that the stimulated processes trigger virtually all the catabolic reactions engaging the greater part of microbial flora from the bioreactor into metabolism. This being the case, microbial community representatives replace each other in sequence and thus ensure an efficient decomposition of high-molecular substrates that cannot be food for fungi any longer; this is why, by the end of biofertilizer formation in these processes the fungi population can approach zero and is replaced with bacteria and ray fungi able to endow biofertilizers with a fairly good smell (XXXI, XXXIV - XXXV).

In this context, it is important to expose the guiding principles of using the herein suggested biostimulants in the production of biofertilizers.

For example, citric acid (C₆H₈O₇) has good solubility and low toxicity; it is well compatible with a lot of substances and also environmentally safe being a major component of cellular respiration because it has antioxidant and bactericide properties and plays the key role in the Krebs cycle and other metabolic processes (XV). The soft acidulating properties of citric acid play a certain role as well.

The main advantage of using vinoacetous K-Na (KNaC₄H₄O₆*4H₂O) as the biofertilizer production stimulant is that it contains two major physiological elements.
Potassium activates the behavior of vitamins and enzymes, participates in a set of reactions intensifying the biosynthesis of proteins from amino acids and in other cell life sustenance processes. In all these reactions potassium acts as the carrier of electrons. It intensifies the hydrophilic property (water content) of protoplasm colloids, which helps the cells remain young and active (XXX). This is exactly the way potassium can affect the microbial flora involved in bioconversion as part of the biofertilizer formation process. Sodium included in vinocetic K-Na strongly affects the transport of substances through membranes and sustains the continuous operation of the so called Na-K pump (Na+/K+)(IV).

Mg (CH$_3$COO)$_2$ is colorless crystals, well soluble in water, and forms crystalline hydrates. Magnesium as an element plays an important role in the metabolism in cells, where it activates enzymes (kynases) that release and carry phosphorous acid (XXII). Magnesium acetate is an antiseptic and disinfecting agent, and, which is especially important in this study, catalyzes organic synthesis to ensure the formation of full-rate biofertilizers.

It is known from (XXXI) that enzymic is typical of all fermentation processes. This is why, fermentation tests allow identifying the orientation of organic mass transformations conditioned, in particular, by the activity of oxidoreductases and hydrolases.

One of the most efficient procedures was the assessment method, involving the determination of activity of oxidoreductases and the measurement of reductive-oxidative coefficients (ROCs) that explicitly showed the intensity and general orientation of the BiGuEm production process.

In all the variants (Table 2) an intensified activity of catalase and dehydrogenase was observed after 48 hours, which was indicative of intensive decomposition and synthesis in the fermentable mass. By the end of the bioconversion processes (after 144 hours or six days) the activity of both enzymes decreased; however, in BS1 and S3 the activity of dehydrogenase appeared to be even higher than initial, which was indicative of efficiently running biosynthesis processes and continued structuring of BiGuEm in the formative phase.

According to Fig. 2, the curve of variations in the reductive-oxidative coefficient in the course of producing the new biofertilizer was flatter than the other two processes. The process using the biostimulant (citric acid + magnesium acetate) formed the highest peak of this indicator in 48 hours from the start of fermentation and, therefore, was considerably different from the other processes. According to the ROC, this kind of process reflected the highest level of transformative changes and thus indicated the high efficiency of that biostimulant, which was confirmed by the final product’s ROC of 0.91 being close to 1. Therefore, the biofertilizer stabilized with the formation of the decomposition/synthesis plateau, which generally led to an active attenuation of catabolic processes. The relation between the ROC and the activity of catalase was confirmed by the regression equation recorded as $y = 0.288999 + 0.0216471x$, and correlation coefficient $R = 0.85$, which pointed at a moderately strong connection between the variables.

An important role in the production of organic fertilizers is played by identifying hydrolase enzymes that effect hydrolysis in diverse complex organic com-
pounds and thus enrich the fermentable turf-manure mass during bioconversion with mobile and accessible nutrients (Table 3).

Note that turf as the carbon-containing component of the BiGuEm formation process provides the microorganisms of the initial mixes with necessary energy, which is why the fermenting substrate is efficiently decomposed to monomers during bioconversion through the activity of cellulase and invertase as specific enzymes. Cellulases can destroy high-molecular carbon-containing compounds, whereas invertase can destroy their metabolic byproducts. Actually, these enzymes ensure the processing of hard-to-decompose vegetation waste and supply of necessary carbon-containing compounds to microorganisms involved in biosynthesis reactions during bioconversion (XXXII).

It should be noted that in the process’s last day characterized by elevated temperatures of 55 to 60°C the activity of cellulase remained high because this enzymic complex has a high thermal resistance (XXXI). This being the case, the final product of S3 appeared to show the highest activity of invertase compared with the declining activity of cellulase, which meant a high level of carbon transformations in that variant of the process and possible accumulation of monosugars accessible to plants. The tested processes exhibit an antilate relation between the activities of cellulase and invertase; this relation is described using the regression equation recorded as $y = 4.16258 - 0.00437478x$, with correlation coefficient $R = -0.63$, which points at a moderately strong connection between the variables.

Protease responsible for decomposing protein compounds and humic acids to amino acids is a direct participant in ammonification. The activity of this enzyme is closely related to the content of nitrogen-consuming microorganisms from the ammonifying group. All the processes aimed at producing the new biofertilizers showed a minor decline in the activity of protease and reduction in the number of ammonifying agents throughout bioconversion (Table 3).

Urease is the enzyme completing the protein compounds decomposition cycle and actually engaged in close cooperation with protease. Urease catalyzes the decomposition of urea in ammonia and carbonic acid gas and can thus provide the future fertilizer with a plant-friendly form of nitrogen, which positively affects the formation of BiGuEm balanced in terms of nutrition elements. In all the compared biofertilizer production processes the ureolytic activity (Table 3) was on a slight decline in the first half of bioconversion. In 96 hours after the start of the process a sharp decline in the activity level of this enzyme was observed, whereas by the end of the process the enzyme became more active. The maximum activity of urease in BiGuEm was registered during S3 conducted using the complex-component stimulant consisting of citric acid and magnesium acetate.

One of the major indicators for evaluating the behavior of fermenting processes is the quality analysis of final products by the content of fertilizer elements. These indicators are exactly the ones determining the quality of the future biofertilizer. It should be noted that all the tested processes were characterized by a fairly high content of all the basic fertilizer elements, including nitrogen, phosphorus, and potassium (Table 4).
It may be noted that by the content of such fertilizer elements as phosphorus and potassium S3 (Table 4) can be considered one of the best versions of the process, involving the addition of a biostimulant to the initial mix. In addition, the biofertilizer produced in this process appeared to have the highest carbon content, which was indicative of the creation of a fairly rapidly stabilized biofertilizer (the ROC is mostly close to the parity value of 1) with good energy potential.

Thus the addition to the initial mix of complex-component stimulants, especially the one used in S3, changed the intensity (compared to the basic process) of biochemical and microbiological transformations affecting the formation of the new biofertilizer.

The size of the dominant nitrogen-transforming microbial flora would gradually go down by the end of all the bioconversion processes, which was indicative of the gradual sampling of the respective substrates and especially affected the BiGuEm production process specified as S3.

In the biofertilizers produced by BS1 and S3 the fungi population was somewhat smaller than in the basic production process, which might have to do with the higher efficiency with which the microbial community decomposed high-molecular substrates able to be food for fungi. That efficiency was brought in by express stimulation.

The reductive-oxidative coefficient (ROC) reflected the high level of transformative changes in the compared processes and indicated that the decomposition of complex compounds had an edge on their synthesis. Within 48 hours the ROC was especially high in S3 running with the addition of the complex-component stimulant, i.e., citric acid plus magnesium acetate.

In the final product of S3 the highest activity of invertase was registered against the receding activity of cellulase, which was indicative of the efficient mobilizing of carbon-containing compounds, release of a lot of energy spent on biosynthesis, and also of intensive transformation of carbohydrates in this variant, and, therefore, monosugar accumulation.

The addition to the initial mix of the complex-component stimulant, including citric acid and magnesium acetate (S3), facilitated the accumulation in the resulting fertilizer of the highest possible amount of fertilizer elements, including phosphorus and potassium, and also carbon acting as an energy equivalent. The final products of all the compared processes had comparable amounts of nitrogen.

IV. Conclusion

The ultimate solution of the problem of sustainable utilization of large tonnage agricultural waste and other biologically active sources of organic raw materials allows saving natural resources and significantly decreases environmental contamination. All the kinds of developments aimed at making new fertilizers by processing organic raw materials confine to searching for economically sound and environmentally friendly technologies of their production. One of the modern methods of making organic fertilizers that has fully paid for itself through history is bioconversion (fermenting). It runs in controlled mode and relies on involving microorganisms in metabolic transformations related to decomposition and synthesis of high-molecular com-
pounds, which helps form high-quality ecofriendly fertilizers. Various stimulants added to the initial mix after the fermenting process help produce an advanced fertilizer with the most favorable properties typical of highly efficient next-generation biofertilizers.

The developers of particular bioconversion technologies should take stock of their suggested methods of bioprocessing organic raw materials, considering that, when there are too many methods, it is difficult to choose the best one. This being the case, it is also important to quit while one is ahead because it is not always expedient to follow the idea of perfection having no limits because the practicing farmers expect the developers to provide them with the quite specific best technology that will allow optimizing the environmental setting near stock raising farms. This is why, it is necessary to make a pause in a certain phase of research or at the launch of the preferred method of bioconversion and choose the most efficient method and specify it as quite a certain technology. That was exactly the purpose of our screening assay. As shown by the assay, the bioconversion process encrypted as S3 and run using citric acid and magnesium acetate added to the initial turf-manure mix at concentrations of 0.1 % wt. has the best qualities among the compared organic raw materials bioprocessing methods developed and patented by the Department of Biotechnologies at the VNIIMZ.

S3 helped damp down catabolic processes and perform the primary transition of transformative changes to anabolism, which was evidenced by the maximum ROC in the first half of the fermenting process and in the final product (0.91); the early release of carbohydrates in the form of monosugars was observed as a result of the high activity of invertase against the receding activity of cellulase by the time when the final product was formed; in addition, it was registered that the final product contained the highest fractions of mobile fertilizer elements in % per asv (2.52 for phosphorus and 1.44 for potassium) as well as carbon (31.85 %).

On the whole, the foundational inferences from the results is that the BiGuEm biofertilizer produced by S3 can be treated as one of the most promising fertilizers for further elaboration and testing on various farming crops and large-scale implementation.

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