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THE INFLUENCE OF MICROBIAL-ORIGIN GROWTH STIMULATORS ON THE DEVELOPMENT OF VERMICULTURE

Introduction. The need to implement advanced technologies for obtaining a sufficient amount of high-quality protein in Ukraine is an aspect of food security. Biotechnology of vermiculture is one of the ways to transform agricultural raw materials of plant and animal origin into good-quality food products.

Problems Statement. The search for ways to increase the productivity of protein biosynthesis in the process of vermiculture is an urgent problem.

Purpose. The purpose is to study the effect of microbial-origin stimulators in the nutritional substrate on the reproductive properties and biomass accumulation of *Eisenia foetida*.

Materials and Methods. Conventional and advanced physicochemical, microbiological, and technological research methods. Stimulators of microbial origin have been obtained by deep cultivation of *Streptomyces recifensis* var. *lyticus* 2435. The effect of stimulators has been studied by determining the biometric indicators of *Eisenia foetida* culture; the composition of the microbiota of the substrates has been investigated by the method of microscopy.

Results. The proposed growth stimulators are a complex of lytic enzymes (muramidase, 5 types of endopeptidase, 2 types of glycosidase) and accompanying enzymes (proteases, amylases). The maximum indicators have been found in the presence of 0.5% raw biomass of *Streptomyces recifensis* var. *lyticus* 2435 in a proportion of 1 : 15 to the dry matter of the nutrient substrate, the microbial composition of which changes against the initial values: the total number decreases by 13.38%, for the bacteria, by 13.92%, for the micromycetes, and by 31.04%, for the anaerobes. The number of representatives of the genus *Streptomyces* increases 2.17 times; there are no bacteria of the genii *Enterobacter* and *Staphylococcus* in the experimental samples. This fact is probably explained by the enzymatic activity of the digestive system of *E. foetida*.

Conclusions. The studied stimulators of microbial origin have a positive effect on the nutrient substrate fermentation processes and on the reproductive and growth functions of vermiculture.

Keywords: vermiculture, *Eisenia foetida* culture, growth stimulators, culture fluid, substrate, biomass.

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One of the important tasks of the biotechnological industry of Ukraine is the development of protein-containing preparations for various purposes. The solution to this problem is facilitated by the expansion of the range of industrial producers of various preparations. In this regard, *Eisenia foetida* culture has shown a significant advantage over known microbial producers. This species has significant advantages over other types of earthworms, as a producer of various drugs with antibacterial, anticarcinogenic, and cytolytic properties [1–3].

The potential of *E. foetida* is quite powerful. Thus, the use of this culture on an industrial scale will make it possible to create new environmentally friendly and waste-free production of protein-containing products, replenish feed resources with cheap protein additives, obtain biostimulators for agriculture and produce raw materials for the pharmaceutical and perfume industry.

Currently, there have been developing and expanding innovation technologies based on *E. foetida*. Based on the biomass and waste products (metabolites) of *E. foetida*, valuable drugs have been obtained for the treatment of cancer, trypanosome, microbial, viral infections [4], as well as diseases of the immune system [5], inflammations of various origins [6, 7]. New products of *E. foetida* biomass are various enzyme preparations, as well as various types of macromolecules of vermiculture origin, which provide the opportunity to create new nanocarriers in the development of promising medicinal products [8, 9].

It has been determined that the protein obtained from worms, both in raw and processed form, provides high efficiency in fattening all types of animals, birds and fish, and also improves the consumer properties of their meat [10]. So, using *E. foetida*, the problem of improving and preserving the quality of food products of animal origin is solved in a certain way, which is relevant for the present time, since modern consumers of such products place high demands on meat and fish products that are quite expensive. The widespread use of *E. foetida* biomass as a protein

product is also facilitated by the fact that the pheromones of this organism do not have a sharp unpleasant smell, as in representatives of other species of earthworms [11].

Presumably, this explains the development of such a modern direction of biotechnology as obtaining biologically active substances from vermiculture biomass. Thus, there is a well-known method of producing protein-vitamin flour from a hybrid of the red California worm and vermicomposted apple pomace [12]. It is also proposed to obtain a sterilized product from earthworms in the form of a dry powder with preserved enzymatic activity and to prepare a biologically active substance based on vermiculture, containing low-molecular components with a stable and diverse biological effect [8, 12].

Using vermiculture, it is possible to reduce the content of mycotoxins and toxic chemicals in contaminated raw materials to the limit level [13] and obtain safe feed protein products from substandard raw materials [14]. In [10, 15, 16] it has been shown that experimental animals, the main source of whose protein is a feed additive made of red California worm (RCW) demonstrate increased indicators of growth, development, and body weight gain. The positive effect of red California worm on animal fattening has been confirmed by the composition of dry biomass: 56–72% of the proteins that contain essential amino acids recommended by the FAO commissions and the World Health Organization (lysine, combinations of methionine with cysteine and phenylalanine with tyrosine), 12% of fats, out of which 33% are unsaturated and 67% are saturated fatty acids.

Thus, the perspective is not only in the cultivation of vermiculture of *E. foetida* in artificial conditions, but it is considered expedient to find ways to stimulate the development of *E. foetida* to increase its biomass and number.

The research has been carried out according to the following flowchart (Fig. 1).

These experiments have been conducted on the basis of research laboratories of the Department

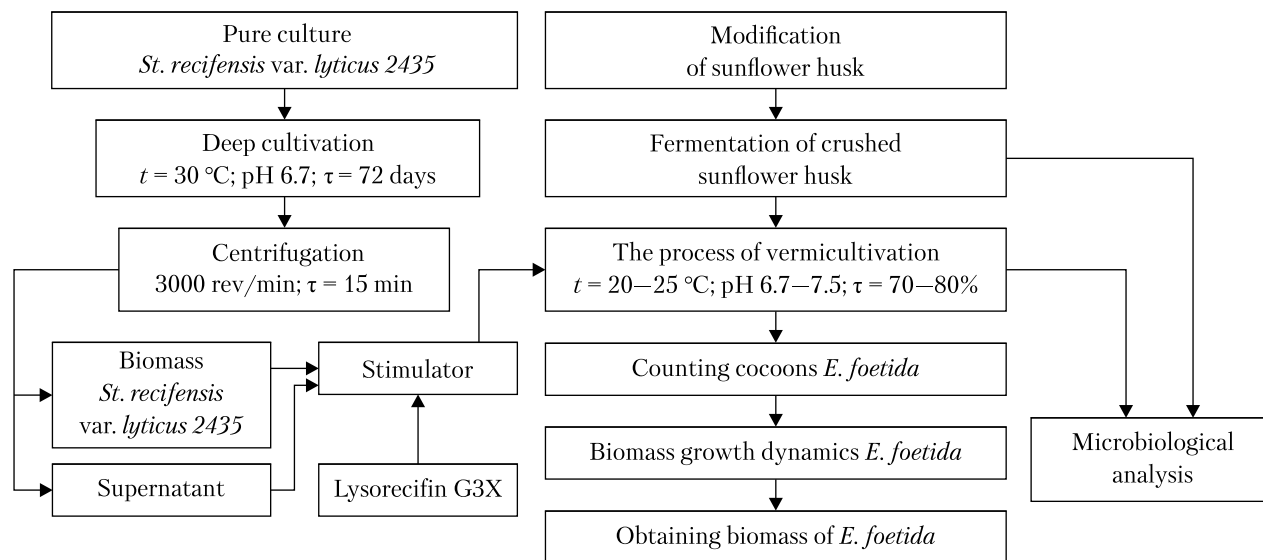


Fig. 1. The research flowchart

Source: prepared by the authors.

of Occupational Safety and Health of the Ukrainian State University of Chemical Technology, as well as the research laboratory of molecular biology of microorganisms and microbial biotechnology of the Research Institute of Biology of Oles Honchar Dnipro National University (DNU). The study used vermiculture of *E. foetida* that is cultivated according to the requirements of TC 3336406.002-95. The *E. foetida* is cultivated on a sunflower husk (SH) substrate (Dream Limited Liability Company), pre-modified to 200–500 μm . The modified raw material is laid for fermentation in burses in the height of 50–60 cm and moistened with water until the moisture content of the substrate reaches 70–80%. During fermentation, in order to improve the aeration and activation of the microbiota of the SH, humidity is maintained throughout the entire volume of the substrate by stirring once a week. The *E. foetida* biomass on a nutrient substrate is accumulated for 160 days at a temperature of 20–25 $^{\circ}\text{C}$; pH 6.5–7.5. An *E. foetida* population of 20 organisms adapted to fermented sunflower husks (the average weight of a worm is 0.1 g), is introduced into each experimental group. The frequency of fresh substrate is

once per 10 days. The stimulators are added twice a week. The development of vermiculture is controlled by weight and computational methods.

Microbiological analysis of the substrate (detection of systematic groups of microorganisms) in certain selective media: meat peptone agar (MPA), salt agar (SA MPA) with 10% NaCl (bacteria), potato ammonia agar (PAA) (actinomycetes), has been carried out. Czapek's medium with pH = 4.5–5.0 before sterilization (micromycetes), Kitt-Tarozzi medium (anaerobic bacteria). The quantitative composition of the microflora of the substrate is presented in CFU/g of substrate [17].

There have been used the stimulators of microbial origin, namely metabolites of *St. recifensis* var. *lyticus* 2435 and the enzyme preparation Lizorecifin of the brand G3X by Enzyme production association (Ladyzhin, the Vinnitsa Oblast). The culture of *St. recifensis* var. *lyticus* 2435 UKM IMV № Ac-5018 is stored in the museum of the Department of Microbiology, Virology and Biotechnology of Oles Honchar Dnipro National University. *St. recifensis* var. *lyticus* 2435 – aerobic gram-positive bacteria that have a mycelial cell structure. Surface culture of *St. recifensis* var.

lyticus 2435 forms dense colonies on agar medium (Gause mineral agar). The shape of the colonies is rounded, convex with smooth or wavy edges. Colonies are formed from two types of mycelium – aerial and substrate. The aerial mycelium has a yellowish-white or grayish-white color, and the substrate mycelium is yellowish-grayish in color. On the aerial mycelium, slightly wavy and short chains of spores are formed in the form of hooks.

This strain is a producer of plant growth stimulators of glycopeptide nature and lytic enzymes (endopeptidases and glycosidases), which are able to destroy the cell walls of some microorganisms. *Lizorecifin* is based on the producer *St. recifensis* var. *lyticus* 2435 that is characterized by a wide spectrum of antimicrobial action, due to the presence of a complex of lytic (five endopeptidases and two glycosidases), concomitant enzymes (proteases, amylase) and thermostable growth stimulating factor of glycopeptide nature [18].

These stimulators are introduced in a concentration of 0.5% and 1% in a proportion of 1 : 15 to the dry matter of the nutrient substrate.

Actinomycetes *St. recifensis* var. *lyticus* 2435 cultivated at a temperature of 30 °C for 72 days in deep conditions on the medium of the follo-

wing composition (%): soybean flour (0.475), glucose (0.063), NH_4NO_3 (0.075), K_2HPO_4 (0.009), CaCl_2 (0.11), CaCO_3 (0.2), and MgCl_2 (0.05). The resulting culture fluid (CF) is centrifuged at 3000 rpm. for 15 minutes, to obtain two stimulating components: raw microbial biomass and supernatant.

The results have been analyzed by the Student's *t*-test. Differences are considered statistically significant if $P < 0.05$ [19].

Several groups of worms have been selected for the study: the reference and the experimental ones. The reference group receives only fermented substrate on the basis of SH, while in the experimental groups, the proposed growth stimulators are added to the main substrate in the indicated amounts. It has been established that the duration of the incubation period of cocoons in the studied population varies from 21 to 56 days. The maximum number of cocoons in the substrates of experimental groups has been detected in the presence of raw biomass of streptomycetic origin in the amount of 0.5% (20 cocoons) throughout the trial period, exceeding the reference values twice. The results of the change in the number of vermiculture (number of adults) in the presence

Influence of Stimulators on the Number of Vermiculture

Experimental group number groups	Option of experiment	Number of vermiculture organisms, pieces							
		20 day	40 day	60 day	80 day	100 day	120 day	140 day	160 day
	Reference main substrate	24 ± 1.2	30 ± 1.5	31 ± 1.6	33 ± 1.7	30 ± 1.5	26 ± 1.3	26 ± 1.3	23 ± 1.2
1	CF Supernatant 0.5%	20 ± 1.1	40 ± 2.0	41 ± 2.1	41 ± 2.1	40 ± 2.0	42 ± 2.1	46 ± 2.3	44 ± 2.2
2	CF Supernatant of 1%	20 ± 1.0	37 ± 1.8	38 ± 1.9	39 ± 1.9	36 ± 1.8	42 ± 2.0	44 ± 2.2	39 ± 1.9
3	Crude biomass of streptomycetes 0.5%	31 ± 1.6	37 ± 1.9	41 ± 2.0	44 ± 2.2	55 ± 2.8	58 ± 3.0	54 ± 2.7	52 ± 2.6
4	Crude biomass of streptomycetes 1%	29 ± 1.4	33 ± 1.2	37 ± 1.6	39 ± 1.5	43 ± 2.1	49 ± 2.0	42 ± 1.8	42 ± 1.8
5	Enzyme preparation G3X 0.5%	28 ± 1.3	33 ± 1.7	36 ± 1.8	36 ± 1.8	32 ± 1.6	40 ± 2.1	41 ± 2.1	37 ± 1.0
6	Enzyme preparation G3X 1%	26 ± 1.2	34 ± 1.6	39 ± 2.0	35 ± 1.7	38 ± 1.9	38 ± 1.9	34 ± 1.7	31 ± 1.6

Note: $P < 0.05$.

of the stimulators under study are presented in Table below.

The analysis of the number of the reference and experimental populations shown in Table indicates the positive effect of the proposed stimulators. At the 20th day, an increase in the number of *E. foetida* individuals has been observed: in the reference group, by 20% versus the initial value of the population, in experimental groups 3 and 4, by 55% and 45%, respectively, in experimental groups 5 and 6 with the enzyme preparation G3X, by 40% and 30% versus their initial value (20 individuals). The maximum number of *E. foetida* has been observed in the presence of raw biomass of streptomycetes origin at a concentration of 0.5%, namely 31 individuals, exceeding the reference values 1.3 times for 20 days. In experimental groups 1 and 2 containing supernatant of culture fluid at concentrations of 0.5% and 1% respectively, changes in the number of individuals for 20 days of the experiment have not been observed. The stimulating effect of this investigated component manifests itself later, namely on the 40th day of the experiment. In group 5, the number of individuals increases twice, and in group 6 the number of worms increases 1.7 times as compared with the beginning of the experiment. The best result on the population size for 120 days (58 individuals) has been reported for experimental group 3, in the reference group the corresponding indicator is only 26 that is less almost 2.1 times than the experimental ones.

Thus, the most effective stimulator among the studied one is the crude microbial biomass, taken in the amount of 0.5% for the formation of cocoons, and for increasing the number of individuals in the population of *E. foetida*. Likely, this stimulator is effective due to the presence of the combination of certain stimulators in the cells of actinomycetes. *Streptomyces* biomass is used by worms as an additional source of nutrition, which contributes to the general development of vermiculture.

The effect of growth stimulators on the accumulation of *E. foetida* biomass has been studied in

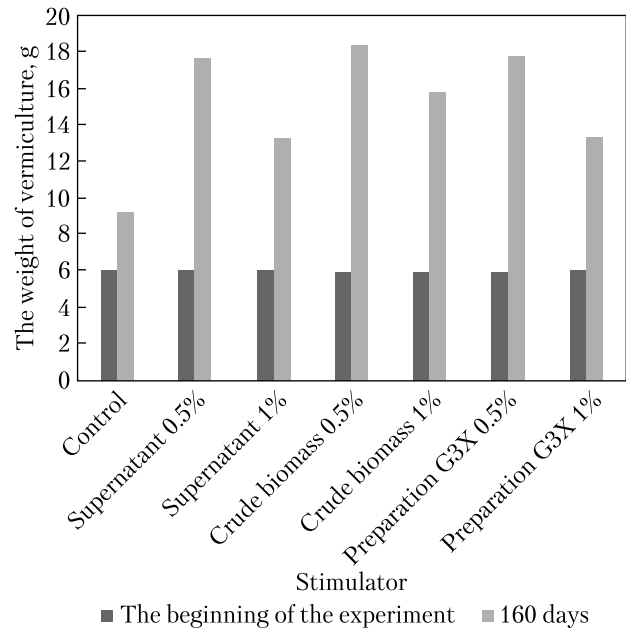


Fig. 2. The effect of stimulators on the growth of *E. foetida* biomass for 160 days

this research. During the cultivation on the substrate from crushed sunflower husks, the biomass of *E. foetida* worms increases from 3.33 g to 12.91 g, depending on the initial weight and physiological state. In the adults, the growth does not exceed 50% monthly. All proposed growth stimulators of microbial origin contribute to the accumulation of *E. foetida* biomass. There has been reported a significant growth in the vermiculture biomass as compared with the initial weight of individuals: 1.8 times, for the reference group, 1.7 and 2.1 times, for experimental groups 3 and 5, respectively, for 20 days. Further, no significant increase in the worm biomass in the reference group has been reported, as it remains at the level of 20 days. The largest weight of biomass of the vermiculture in the final cultivation period has been recorded in experimental group 3. It reaches 18.77 g that, for 140 days, exceeds 3.2 times the initial values and 2 times the reference values. The indicators for the accumulation of vermicompost biomass in all experimental groups and the reference group for 160 days are shown in Fig. 2.

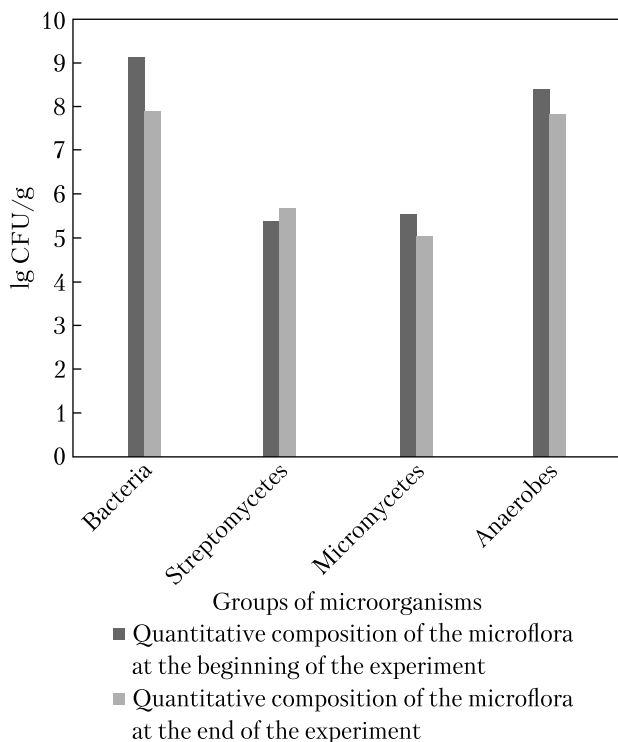


Fig. 3. Changes in the quantitative composition of the microflora in the vermicultivation process

According to the results of the experiment, the best indicators of biohumus biomass growth have been shown by such a stimulating component as biomass of streptomycete origin, taken in an amount of 0.5% (18.3 g); the results are 2 times higher than the reference values.

Microbiological analysis of the substrate has been carried out. Changes in the composition of the substrate microflora have been reported in the presence of the most effective stimulator (crude biomass of streptomycetes) at the beginning and end of the experiment (Fig. 3).

The data have shown that the microbial composition of the substrate after vermiculture changes as compared with the initial values: the total number decreases by 13.38%, for the bacteria, by 13.92%, for the micromycetes, and by 31.04%, for the anaerobes, while that of the streptomycetes increases by 53.86%. The bacteria of the following genus have been identified: *Bacillus*, *Enterobacter*, *Escherichia*, *Streptomyces*, *Fusobacterium*, and *Clostridium* (Fig. 4).

The content of bacteria of the genii *Escherichia*, *Fusobacterium*, *Clostridium* in the substrate decreases 0.7 times, on average, while the number of bacteria of the genus *Bacillus* increases 2.2 times.

The treatment of the substrate by vermiculture with the added stimulator leads to improvement its microflora, so no bacteria of the genii *Enterobacter* and *Staphylococcus* have been detected in the experimental samples, that means these groups of microorganisms have been eliminated. This fact may be explained by the enzymatic activity of the digestive medium of worms. The concentration of bacteria *Streptomyces* sp. increases 2.17 times as compared with that at the beginning of the experiment.

The obtained results largely agree with those of other authors. Thus, in [20, 21], the greatest positive stimulating effect has been shown by the drugs based on the biomass of *St. recifensis*, which is consistent with the data of this research. The positive effect of the studied stimulating components is explained by the mechanism of lytic action of the whole complex of enzymes synthesized by *Streptomyces recifensis* var. *lyticus* 2435. Metabolites of streptomycete lead to a profound destructive effect of microorganisms that live in large numbers on fermented sunflower substrate. The species composition of the microflora of the modified sunflower husk is quite diverse and includes mesophilic bacteria, yeast, fungi, thermophilic bacteria, and actinomycetes [17]. The proposed stimulators contain both bacteriolytic (destroy the cell wall of bacteria) and myco- and yeast lytic (destroy the cell membranes of fungi and yeast) enzymes.

Thus, according to [22], the whole complex of lytic enzymes of streptomycete by the mechanism of action is divided into three types. The first type of enzyme *St. recifensis* var. *lyticus* are glycosidases that break down polysaccharide (glycan) chains in the peptidoglycan of the cell wall of substrate bacteria. These include N-acetylmuramidase (lysozyme), an enzyme that hydrolyzes the

1,4-glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in bacterial cell wall peptidoglycan molecules, as well as N-glucosaminidase, which is a hydrolysis bond between N-acetylglucosamine and N-acetylmuramic acid [23]. The second type of enzyme complex is N-acetylmuramyl-L-alanylamidase (amidase) that breaks down the bond between the muramic acid of the polysaccharide and the peptide part of the peptidoglycan. The third type of lysozymes combines peptidases that hydrolyze the peptidoglycan peptide bonds. Bacteriolytic peptidases have different substrate specificity: some of them break only the glycyl-glycyl bond in cross-linked bridges, while others act on the glycyl-alanine bond, etc.

Thus, the enzymatic degradation of the cell walls of the sunflower husk microorganism is realized by hydrolytic breakdown of certain bonds, which leads to their deep lysis.

During the destruction of the substrate microflora, there is released its intracellular content rich in nutrients, vitamins, micro- and macronutrients, which serve as additional nutrients and growth factors that are easily absorbed by vermiculture and have a positive effect on its development.

In addition, the culture of *St. recifensis* var. *lyticus* is known to synthesize specific substances — avermectins that are used to control acarids [24]. The growth-stimulating effect of these substances on agricultural plants has also been established [15, 22]. The presence of avermectins among the metabolites of streptomycete helps to clean the substrate from parasites that can adversely affect vermiculture.

Thus, it is possible that a combination of lytic and antiseptic factors in the composition of metabolites of *St. recifensis* var. *lyticus* provides their stimulating effect on the development of vermiculture *Eisenia foetida*.

Application of vermiculture technology in the production of *Eisenia foetida* biomass in the agro-

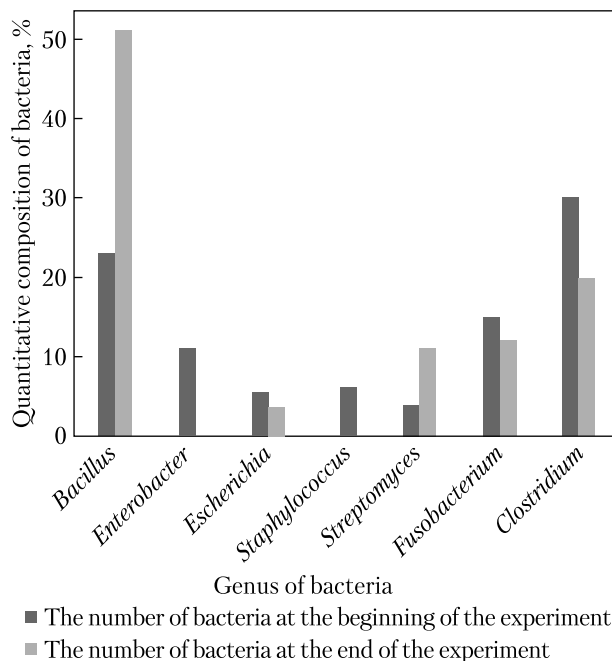


Fig. 4. Changes in the quantitative composition of bacteria in the vermicultivation process

industrial complex in fattening animals in order to increase their productivity to obtain organic food.

It has been shown that the best growth stimulator of *E. foetida* is raw biomass of *St. recifensis* var. *lyticus* 2435 in a dose of 0.5%. This effect manifests itself as a 1.5-time reduction in the time of emergence of cocoons, a 2.9-time increase in the number of adults per 120 days of cultivation, and a 3.1-fold increase in the biomass. Improving the microbial composition of the nutrient substrate by increasing the total number of streptomycetes that synthesize and excrete biologically active substances by 53.86% contributes to the accumulation of *E. foetida* biomass. Thus, the proposed stimulators of microbial origin to a certain extent had a positive effect both on the processes of fermentation of the nutrient substrate and on the reproductive and growth functions of vermiculture.

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ВПЛИВ СТИМУЛЯТОРІВ РОСТУ МІКРОБНОГО ПОХОДЖЕННЯ НА РОЗВИТОК ВЕРМИКУЛЬТУРИ

Вступ. Необхідність впроваджувати в Україні прогресивні технології одержання у достатній кількості якісного протеїну є одним з аспектів продовольчої безпеки. Біотехнологія вермикультивування є одним із способів перетворення сільськогосподарської сировини рослинного та тваринного походження у високоякісні продукти харчування.

Проблематика. Пошук шляхів підвищення продуктивності біосинтезу протеїну в процесі вермикультивування.

Мета. Вивчення впливу дії стимуляторів мікробного походження у складі поживного субстрату на репродуктивні властивості та накопичення біомаси *Eisenia foetida*.

Матеріали та методи. Традиційні та сучасні фізико-хімічні, мікробіологічні й технологічні методи дослідження. Стимулятори мікробного походження одержано глибинним культивуванням *Streptomyces recifensis* var. *lyticus* 2435. Дію стимуляторів вивчали шляхом визначення біометричних показників культури *Eisenia foetida*, склад мікрофлори субстратів — методом мікроскопії.

Результати. Запропоновані стимулятори росту є комплексом літичних ензимів (мурамідази, ендопептидази, глікозидази) і супутніх ферментів (протеази, амілази). Максимальні показники виявлено за присутності 0,5 % сирової біомаси *Streptomyces recifensis* var. *lyticus* 2435 у співвідношенні 1:15 відносно сухої речовини поживного субстрату, мікробний склад якого при цьому змінився відносно початкових значень: загальна кількість бактерій зменшилася на 13,38 %, мікроміцетів — на 13,92 %, анаеробів — на 31,04 %. Чисельність представників роду *Streptomyces* збільшилася у 2,17 рази, бактерії родів *Enterobacter* і *Staphylococcus* у дослідних пробах відсутні, що, ймовірно, пов'язано з ферментативною активністю травної системи *Eisenia foetida*.

Висновки. Досліджені стимулятори мікробного походження позитивно впливали на процеси ферментації поживного субстрату, репродуктивну та ростову функції вермикультури.

Ключові слова: вермикультура, *Eisenia foetida*, стимулятори росту, культуральна рідина, субстрат, біомаса.