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STUDY ON MODIFIED SUNFLOWER HUSK FERMENTATION PROCESS FOR VERMICULTIVATION

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Abstract. *The quantitative and qualitative composition of microorganisms, making up the modified sunflower husk microflora while fermentation, as well as a modified solid-nutrient substrate for accumulation of Eisenia foetida biomass during vermicultivation, was studied. Species' composition of microorganisms changes during the fermentation process: mesophilic organisms are sequentially replaced by mycelial ones, and then by thermophilic spore-forming bacteria and actinomycetes. Complex multispecies preparation - EM Probiotic has been used to intensify fermentation.*

Keywords*: biomass, vermiculture, solid- phase substrate, cultivation, microflora.*

1. Introduction

The main objective of vermicultivation is to solve the problem of biological object's ability to adapt to a specific substrate. There is an adaptation assessment based on morphological criteria: the worm's behavior in the substrate, the size of the worm's body, the number of specimens being at different stages of ontogenetic development, the response to exogenous factors, body color etc. As raw materials, almost all processes for the preparation of Eisenia foetida biomass use manure of livestock, sewage sludge, microbiological and food processing waste [1 - 7].

Biochemical characteristics of vegetable raw sunflower husk (SH) were studied earlier and, taking into account accumulation of large amounts of SH and sufficient level of its nutrients, they create preconditions for effective use of SH in the development of biotechnology of protein feed additives production using vermicultivation [8].

Vermicultivation process consists of two stages: fermentation of SH and development of new vermiculture population on fermented substrate under the influence of abiotic factors. The stage of substrate fermentation is very important because it includes the transfer of vegetable raw nutrients to the state, available for Eisenia foetida use. Such transformation of the substrate components pass through its natural microflora. From the literature it is known that the gram-negative bacteria are more important in the diet of worms than gram-positive ones, and fungi are more available nutrition source than bacteria. Such types of bacteria as Flavobacterium lutescens, Pseudomonas fluorescens, Pseudomonas putida have toxic effect on the worms [9].

Optimization of fermentative transformations is necessary to intensify vermicultivation process as a whole, and to enhance the microbiological processes that take place in the substrate. It is recommended to use modified SH [8]. Modification consists in grinding the starting material, thus increasing its surface area, and further improves the reactivity between glucoside bonds of sunflower husk polysaccharides and worm enzyme system.

The aim of this work is to conduct research in identification of the microorganisms that make up the grinded SH microflora, laid on fermentation, as well as modified solid-nutrient substrate for Eisenia foetida biomass accumulation.

2. Materials and methods

Vermiculture Eisenia foetida has been used as an object of study in the experiments carried out at the Institute of Biotechnology of Ukrainian State University of Chemical Technology (SRI "Biotechnology" SHEI "USUCT", Dnipro, TU 3336406.002-95).

In the first stage of the research, SH substrate, pre-modified to the size of 200-500 microns, was laid on the fermentation in containers of 50-60 cm height. The fermentation was carried out in two ways: the first - without the probiotic addition to the grinded SH, and the second - with the presence of biologically active substance EM Probiotic (produced by the Commercial Production Center “Amrita”, Dnipro). Moisture capacity of the material significantly affects the rate of fermentation, so grinded SH was moistened with water to achieve 70-80 % of substrate moisture capacity at liquor ratio of 1:2. During fermentation, stirring of the substrate in its entire volume was performed once a week to improve aeration, activation of SH microflora and moisture equalization. Eisenia foetida biomass accumulation on nutrient substrate was being carried out for 56 days. Adapted to fermented SH Eisenia foetida population was laid in the amount of 100 specimens (average weight of a worm - 0.1 g) Quantitative and qualitative composition of microflora was assessed by plating SH washing-off on meat peptone agar (MPA) medium; and to determine micromycetes - on wort agar (WA) medium, followed by cultivating at 37 (± 1) °C and 28 (± 1) °C respectively. The total number of bacteria was determined after 48 hours, and micromycetes - after 7 days. Three weeks later, it was defined the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM). Spore forms of bacteria were determined in pasteurized washing-off of SH (fermented and non-fermented), which were plated on complex nutrient MPA+WA medium in the proportions (1:1) [10]. The results obtained were processed by methods of mathematical statistics [11].

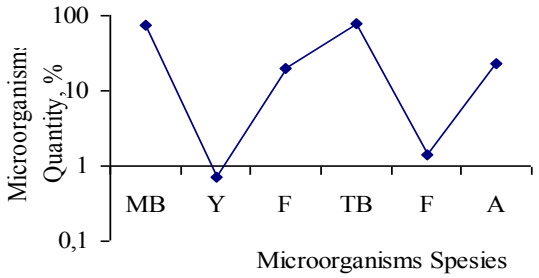
3. Results and discussion

Before laying SH on fermentation, it was determined the number of mesophilic aerobic and facultative anaerobic microorganisms, as well as the presence of actinomycetes and micromycetes [10]. The results indicated that there were mainly mesophilic microorganisms and a small amount of epiphytic microflora, so-called field fungi of p. Cladosporium, p. Thamnidium, p. Alternaria. From the spore-forming microorganisms it was identified specimens of p. Bacillus, aerobic microorganisms p. Cellfalcicula, p. Cellvibrio, p. Cytophaga and a small amount of yeast. The total number of microorganisms was $10^6 \ldots 10^7$ per 1 g of SH. Microbiological monitoring was

performed before laying the nutrient substrate and during the substrate fermentation process.

The fermentation process of the modified SH was conditionally divided into four stages: mesophilic, thermophilic, cooling and afterripening. At mesophilic stage SH was at ambient temperature of 20…25 °C, pH of the substrate was sub acidic 6.2 ... 6.5. On the second day, the temperature in the middle of the container was 35 °C, epiphytic microorganisms dying and quickly replacing by fungi p. Aspergillus, p. Penicillium. The development of filamentous microorganisms contributed to raising the temperature to 40 ° C, which meant gradual transition of the fermentation process to thermophilic stage. In this case, the substrate was acidified to pH 6.0 due to the formation of organic acids. On the third day, the temperature rose to 45 °C. The gradual increase of the substrate temperature was due to the rapid development and accumulation of bacteria. At the same time, the heat release rate was equal to the heat loss rate, so that on the fifth day, the temperature reached a maximum - 50 °C. The composition of microflora predominantly accumulated thermophilic spore-forming microorganisms p. Bacillus and actinomycetes, which participated and continued fermentation processes of substrate conversion. The substrate pH became alkaline due to the emission of ammonia formed by the breakdown of proteins. Though the amount of bacteria was very large in vermisubstrate, 10^8…10^9 cells per g wet wt, they formed less than half of the total microbial biomass due to the small size (1.8 microns). Actinomycetes grew much slower and became visually noticeable in the later stages of the process, when their number considerably increased, forming white plaque on the surface of the SH fermented mass. This feature is considered to be typical for actinomycetes on various substrates. Their number was lower than the number of bacteria and equaled 10^5…10^8 cells per g wet wt.

In the cooling stage, which followed the maximum temperature, the pH was reduced, but remained slightly alkaline at 7.3 ... 7.5. Thermophilic fungi of the colder zones again filled the entire volume of the substrate and, together with actinomycetes, consumed polysaccharides, hemicellulose and cellulose, destroying these polymeric compounds to monosaccharides. At afterripening stage, when microorganisms used readily absorbed compounds, the temperature was decreasing. Heat release rate became very low as the microorganisms were not breeding any more, and the substrate temperature dropped to ambient temperature in a week. Therefore, SH fermentation process was carried out by microflora of diverse species composition. The quantitative ratio of microorganisms groups is represented in the figure 1.



MB - mesophilic bacteria, Y - yeast, F - fungi, TB - thermophilic bacteria, A – actinomycetes

Figure 1– Species Composition of Microflora of Modified SH Fermentation Process

The substrate fermentation was performed in the presence of complex preparation EM Probiotic, which contains a set of bifidobacteria in the amount of 10^5-10^9

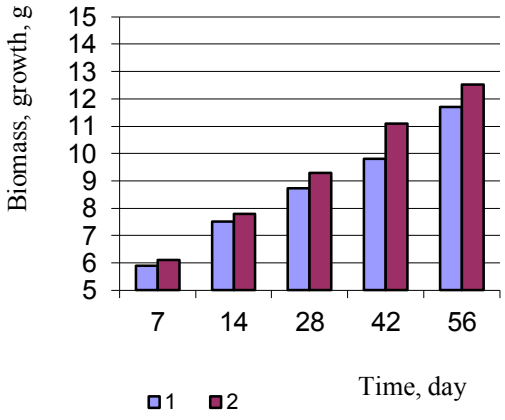
microbial cells / cm^3, anaerobic gram-negative bacilli (Bacteroides) - 10^3-10^5 microbial cells / cm^3, coryneform bacteria - 10^8 microbial cells / cm^3, specimens of Acetobacter - 10^{12} microbial cells / cm^3, yeast - 10^2-10^4 microbial cells / cm^3. EM Probiotic being added into the grinded SH, the time of plant substrate fermentation was reduced due to the multispecies microbial composition of the preparation. Thus, without adding active microorganisms, SH substrate fermentation time equals 10-14 days in the cold season and 10-12 days in the warm period. When adding biologically active substance EM Probiotic, SH substrate fermentation time was reduced to 6-7 days in the cold period, and up to 5-6 days in the warm season.

The research has shown that the optimum amount of EM Probiotic on the stage of substrate fermentation equals 100 g of active substance per 10 liters of water. Increasing of the substance concentration does not affect the fermentation duration and the growth rate of biomass Eisenia foetida. Therefore, introduction of microorganisms complex EM Probiotic provided conditions of symbiotic interaction between microorganisms of SH substrate and, respectively, contributed to the rapid passage of the fermentation process.

On the second stage of vermicultivation, the fermented substrate prepared by various methods was used to develop of a new population of Eisenia foetida under the influence of abiotic factors. Each container was laid with various types of SH fermented substrate in the amount of 250 g; substrate humidity of 70 %, pH - 7.5. The substrate in each container was laid with worms specimens to study the suitability of grinded fermented SH as nutrient substrate for vermiculture breeding.

Visual observations indicate that the worms begin to move gradually into the substrate depth and process it. Moldy microorganisms, representatives of p. Aspergillus, p. Penicillium, were found to appear during vermicultivation on the substrate surface in the first days after the worms' settlement, but disappear in two weeks. The results show that harmful microorganisms are being destroyed during the worms' processing of the investigated substrates that contributes to the substrate decontamination.

Further observations of the worms' activity and survival showed that modified SH can be used as a nutrient substrate for Eisenia foetida and is suitable for the culture habitat. The first worm cocoons were found in a month, the larvae hatched three weeks later. Dynamics of mature Eisenia foetida biomass growth, depending on the substrate fermentation process based on the modified SH is shown in Figure 2.



1 – without the addition of EM Probiotic; 2 - with the addition of EM Probiotic

Figure 2 - Dynamics of Eisenia foetida biomass population growth depending on the kind of fermented substrate

The experiental data have shown that Eisenia foetida biomass on modified SH substrate without addition of probiotic cultures of EM Probiotic has grown by

1.98 times, and with its addition – by 2.1 times comparing to the initial biomass. Thus, positive dynamics of Eisenia foetida biomass growth indicates the suitability of the substrate based on the modified SH.

4. Conclusions

Therefore, SH fermentation is an exothermic biological oxidation process, wherein the mixed population of aerobic microorganisms at elevated temperature - 50 °C and humidity - 80 % subjected the organic substrate to biodegradation. Being biodegraded, the SH substrate had physical and chemical transformations followed by various decomposition products: ammonia, carbon dioxide, low molecular weight peptides. There was also accumulation of dead microorganisms biomass, some amount of living mesophilic microorganisms and products of chemical interaction between these components. The composition of the fermented SH provides vermiculture with a sufficient level of nutrition, and is a suitable habitat for Eisenia foetida.

The composition of microflora substrate also changes during the fermentation. At the beginning of fermentation, mesophilic aerobic bacteria dominated in SH, their number gradually decreasing. However, the number of thermophilic microorganisms, actinomycetes and fungi, was increasing. All this determined the essence of fermentation process and quality of the resulting fermented substrate. The process of substrate fermentation carried out in the presence of complex preparation EM Probiotic occurred more intensively and decreased almost twice compared to untreated SH.

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