*Journal homepage: www.fia.usv.ro/fiajournal*
*Journal of Faculty of Food Engineering,*
*Ştefan cel Mare University of Suceava, Romania*
*Volume XIII, Issue 1 – 2014,* pag. 60 - 67

# DEPENDENCE OF THE BIOSYNTHETIC ABILITY OF THE PRODUCERS OF BIOLOGICALLY ACTIVE SUBSTANCES ON QUALITY OF NUTRIENT MEDIUM AND SUBSTRATUM

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Received March 6th 2014, accepted 28th 2014

***Abstract.*** *The need for formulations of biologically active substances of natural origin currently increasing.The industrial production of such products biotechnological method important, but limited by a number of unsolved problems and shortcomings.So, this paper studied the effect of the qualitative composition of culture media and substrates for biosynthetic activity and carotene producing filamentous proteins and red Californian worm - producing biologically active substances and feed protein.Producers offered for mycelial growth medium containing a carbon source enzymatic hydrolysates flour of various grains, and as a source of nitrogen gluten or soy milk.Found that filamentous organisms - producers of carotenoids and proteins accumulate high amounts of both biomass and target metabolites on nutrient media containing hydrolysates of buckwheat, oat or corn flour in the presence of gluten. Used for the cultivation of vermiculture modified sunflower husk, which Eisenia foetida, as a producer of protein drugs and medical fodder has balanced essential amino acids, vitamins, enzymes and trace element composition of the biomass.These results show that the optimization of culture media and substrates provides increased biomass yield and biologically active substances in the few times producers of various taxonomic groups.*

***Key words:*** *mucoraceous mold, enzymatic hydrolyzates, biomass, activity, carotene, higher edible fungus, vermiculture, nutrient media, substrata.*

## 1. Introduction

Requirement of different sectors of the national economy and medicine in bioactive substances, especially of the natural origin increasingly and stably rises nowadays, what is acknowledged by several interesting works [1-8].
However the problem of industrial productivity of specimens on the basis of biologically active substances remains unsolved, that is why actual.

The difficulty of the given situation can be explained by imperfection or lack of elaborated technologies. Those existent in many cases have a number of faults. The necessary conditions for successful realization of elaborated technologies of receiving biologically active substances are: presence of the select industrial producer of the appropriate target substance and raw material sources which is enough for growing of the given producer. Though, main genetic properties

of the producers determine quality of the bioactive substance which is produced. Whereas, quantity of the received target product depends not only on genetic of the definite producer, but also, in higher degree, on chemical composition of nutrient media and substrata on which this industrial producer is cultivated.

Such dependence refers to the producers of bioactive substances from different taxonomic groups. Thus, lower mucoraceous molds from the class of Zygomycetes Blakeslea trispora and Choanephora conjuncta are used as industrial producers of carotene substances [9, 10]. But the composition of synthesizable carotenoid and primary outgoing of one of them depend on the composition of zymotic nutrient media. For example, with presence of cellobioze in the nutrient medium, outgoing of carotenoids increases sevenfold [9]. Presence of ion-substances in cultivated liquid provides primary synthesis beta-carotenes, but pyridine-substances stimulate accumulation of lycopene [11, 12]. Higher edible fungus from the class Basidiomycetes Pleurotus ostreatus potentially can be used for production of bioactive substances of the protein nature [6, 7, 13]. Outgoing of protein is defined by qualitative and quantitative composition of the nutrient media and substrata on which the producer is grown. Thus, in caprosome of oyster mushroom rising on natural substratum, for example, on wood, the quantity of protein is not more than 20%. On the liquid nutrient media optimized by the sources of nitrogen and carbon, content of protein can reach 30–50% [13]. Producers of biologically active substances of different chemical nature may be not only specimens of microorganisms and fungus, but also of animals. Thus, private active components were isolated from the biomass of red Californian worm Eisenia foetida. They are capable to enforce the immunity of a human being, decrease a risk of cardio-vascular and oncological conditions, increase resistance of organism to germ and viral diseases [8, 18, 19, 20]. The given organisms are producers of biologically active substances which have intracellular localization. However, it is known that the processes of accumulation of biomass of producers and synthesis of targeted bioactive substances in their cellular are stimulated by different nutrient components of media and substrata. Whereas, when intercellular bioactive substances are received in the industrial conditions, it is economically expedient and important to accumulate both biomass and targeted products in them as much as possible. Consequently, the given task is actual and may be solved by the way of optimization of qualitative compositions of nutrient media and substrata for appropriate producers.

The purpose of the given work is identification of the dependence of intensity of synthesis of some biologically active substances from quality of nutrient media and substrata of proper producers of different taxonomical groups.

## 2. Materials and Methods

As an object in experiments one used lower mucoraceous mold IMB F-100019 variants (+), (–) Blakeslea trispora (Institute of microbiology and virology National Academy of Science of Ukraine, Kyiv) – producer of beta-carotene, higher edible fungus Pleurotus ostreatus (Pleurotus ostreatus) strain НК-35 (Department of microbiology, Institute of botany, Kyiv) – as producer of protein substances, as well as vermiculture Eisenia foetida (Research institute "Biotechnology" State University, Dnepropetrovsk Specification 3336406.00 2–95) – producer of biologically active

components, protein biomass. Growing of mucoraceous mold Blakeslea trispora was implemented in several stages. Work culture was received by separate passage of museum swarms on the wart-agar nutrient medium and sufficient growing for 7 days. Mother culture was received by the following also separate passage on the liquid nutrient media in flasks with capacity of 300 ml. The composition of mother nutrient media %): corn extract – 13, green treacle – 7. Time of growing of the mother culture is three days on the termobiological termostatical shakers УВМТ-12-250, working at a speed of 220 – 240 rpm/min. The received (+), (–) mother inoculum was carried for combined growing in to zymotic flasks with capacity of 300 ml. Correlation (+), (–) strains for combined cultivating is 4:1. The composition of the zymotic media is different. Extract-treacle nutrient media contain (%): corn extract – 6, green treacle – 6, potassium dihydrogen phosphate – 0.05, thiamine chloride – 0.0002, vegetable oil – 4. Alternative nutrient media include: enzyme hydrolyzate of different types of flour and shorts, as well as gluten or soya milk in different concentrations. All used nutrient media were under thermal sterilization at 120°C for 45 min [14]. Duration of fermentation was 5 days on the microbiological shaker УВМТ-12-250, working in the mentioned above regime of hashing. The temperature on all stages of cultivation 24–26°C. At the end of fermentation the received cultural liquid (CL) was separated on centrifuge T–23 at 3000 rpm/min for 10 min. CL was analyzed according to quantity of biomass and beta-carotene using known methodology [14, 15]. The experiment was repeated 5 times. The culture of the fungus Pleurotus ostreatus was kept on the wart-agar nutrient media in flasks from which they were replanted into the shaking flasks with capacity of 300 ml, containing 50 ml enzyme nutrient medium. The composition of the experimental media was pointed above, pH of media – 6.8–6.9. To receive enzyme hydrolyzes domestic enzyme preparations were used «Alphalad» and «Glucolad» (Plant of enzyme preparations, Ladyzhsk c., Ukraine) with concentration 1:1900 and 1:2000 respectively. Temperature Regime of hydrolyze 60°C for 10–15 min. Duration of deep growing Pleurotus ostreatus – 5 days on the microbiological thermostatic shaker, the speed of hashing – 220–240 rpm/min., temperature of cultivating 26–28°C. At the end of fermentation in CL the quantity of biomass and protein was determined [14, 16]. Filamentous mass was separated from liquid fraction CL by the way of centrifuging at 3000 rpm/min for 15 min. The experiment was repeated 7 times. The received results were processed with help of methods of mathematical statistics [17]. Cultivation of Eisenia foetida was held on the substrate of modified sunflower husks (SH) grained till fractions 200–500 microns, wet with water in correspondence (hydromodule) 1:2. Extract was laid on the fermentation in special capacities (plastic clamps) as high as 50–60 sm. The process of fermentation lasts 12–14 days under influence of enzymes of microorganisms introduced by microflora of sunflower husks. To increase aeration of fermenting mass, activation of microbiological work of microorganisms, leveling humidity about the whole volume, precaution of rotting in the depth of a clamp, hashing of substrate was held once a day. Bioprocessing by the producer of the fermenting substrate is held 45 days with accommodation density Eisenia foetida 5–10 thousand specimens per 1 square meter and at an altitude of the lay of substrate of 30sm. Humidity of substrate is kept at a level 70–80%, temperature – 20–25°C, pH 6.5–7.5. Additional forage as thick as 5 sm added in 10–15 days after settlement of the

basic substrate with culture Eisenia foetida. With accommodation density 100 hundred per square meter, selection of biomass in a quantity of 75–80% was done. Quality and harmlessness of the biomass of population Eisenia foetida, adapted on the modified oil husks were determined with the help of the instruction of the Ukrainian Security Council (USC–15) scientific council of UN on protein problems and veterinary – sanitary regulations. Biological worth of the biomass Eisenia foetida was determined with the help of method of amino-acid score according to known methodology as well as by the degree of digestion and adaptation of protein [14, 16, 20, 21, and 22].

## 3. Results and Discussion

In biotechnology deep way of growing producers including filamentous is economically expedient and technologically reasonable. That is why to receive filamentous biomass Blakeslea trispora and Pleurotus ostreatus with higher content of biologically active substances, beta-carotene and protein respectively, liquid nutrient media were used. The media of different compositions are offered. For example, extract-treacle medium contains such wastes of starch-treacle producing as corn extract (the source of nitrogen and factors of growth) and green treacle (the source of carbon and energy). For comparison there were learnt the nutrient media including other sources of the development of producers of biological active substances. Thus, Так, as a source of carbon and energy were used wastes containing starch or secondary products of milling production, which were put to the fermenting, hydrolyze in advance, but as a source of nitrogen, gluten and soya milk were used. Concentration of these components in the medium was determined by quantity of sugar in them (0.15%) and of nitrogen (0.22 – 0.24%) respectively. The received results on accumulation biomass and beta-carotene in mucoraceous mold Blakeslea trispora, are introduced in the table 1.

**Table 1**

**Dependence of outgoing biomass and beta carotene in Blakeslea trispora on the quality of nutrient media**

| Source of carbon | Gluten | | Soya milk | |
|---|---|---|---|---|
| | Dry biomass, g/100ml | Content of beta carotene, mg/100ml | Dry biomass, g/100ml | Content of beta carotene, mg/100ml |
| Hydrolyzer of corn flour | 2.98±0.13 | 67.67±3.37 | 2.77 ± 0.14 | 38.57 ± 1.92 |
| Hydrolyzer of buckwheat flour | 3.10±0.15 | 51.09±2.54 | 2.95 ± 0.15 | 30.40 ± 1.51 |
| Hydrolyzer of wheat flour | 2.26±0.13 | 21.26±1.95 | 2.03±0.13 | 16.53±1.65 |
| Hydrolyzer of wheat shorts | 2.07±0.11 | 18.20±1.73 | 1.83±0.10 | 15.24±1.38 |
| Hydrolyzer of oatmeal flour | 3.06±0.15 | 42.13±2.10 | 2.81 ± 0.14 | 33.06 ± 1.64 |
| Hydrolyzer of rie flour | 2.76±0.13 | 39.26±1.95 | 2.48 ± 0.14 | 25.89 ± 1.48 |

The data of the table 1 indicate that the highest outgoing of the biomass containing carotene is observed on the media containing gluten and hydrolyzers of buckwheat, oatmeal, and corn flour, but the highest activity (quantity of beta-carotene)

– on the medium with hydrolyzate of corn flour. Analogical situation is found on the media with determined sources of carbon in combination with another source of nitrogen – soya milk. But on all investigated hydrolyzer far more quantity of mucoraceous mass is provided by the presence of gluten from 2.07 through 3.10 g/100 ml CL against presence of soya milk from 1.83 through 2.95 g/100 ml CL. Content of beta-carotene is sure to be lower with presence of soya milk in hydrolyzer than with input of gluten. The difference between absolute amount of biomass on the media with gluten and hydrolyzer of buckwheat, oatmeal or corn flour is doubtful. Concentration beta-carotene on the medium with gluten and hydrolyzer of corn flour is 1.33 fold higher, than on the medium with gluten and hydrolyzer of buckwheat flour and 1.60 fold bigger, than on the media with gluten and hydrolyzer of oatmeal. Consequently, analysis of the table 1 lets reveal optimal composition of the nutrient media providing high outgoing of biomass (in fact 70 mg/100 ml) and the biggest outgoing beta-carotene (about 70 mg/100 ml) in it: hydrolyzer of corn flour (0.15% sugar) in combination with gluten (0.22–0.24% nitrogen). It is necessary to admit that content of biomass (2.68±0.130 g/100 ml) and beta-carotene (33.25±1.82 mg/100 ml) on the extract-treacle medium is lower than on the optimal one 1.1 fold and 2.06 fold respectively.

The results on accumulation biomass and protein in higher edible fungus Pleurotus ostreatus (oyster mushroom) strain НК-35 of fungus, received in the conditions of the given experiment are introduced in the table 2.

**Table 2**

**Dependence of outgoing of biomass and protein in Pleurotus ostreatus on the quality of nutrient media**

| Source of carbon | Gluten | | Soya milk | |
|---|---|---|---|---|
| | Dry biomass, g/100ml | Content of protein, % | Dry biomass, g/100ml | Content of protein, % |
| Hydrolyzer of corn flour | 2.49±0.13 | 57.89±2.80 | 1.99±0.10 | 41.40±2.35 |
| Hydrolyzer of buckwheat flour | 2.93±0.14 | 60.40±2.65 | 2.03±0.10 | 42.04±2.18 |
| Hydrolyzer of wheat flour | 2.12±0.12 | 58.52±2.73 | 1.69±0.07 | 40.97±2.075 |
| Hydrolyzer of oatmeal flour | 2.43±0.12 | 52.99±2.43 | 1.89±0.14 | 44.23±2.214 |
| Hydrolyzer of oatmeal flour | 2.19±0.109 | 56.05±2.80 | 1.95±0.087 | 38.87±1.89 |
| Hydrolyzer of rie flour | 2.28±0.114 | 55.16±2.75 | 2.00±0.100 | 43.55±2.17 |

Out of demonstrate table it is clear shown that higher quantity of the deep biomass of oyster mushroom is accumulated on the media containing hydrolyzers of buckwheat, corn and wheat flour in combination with gluten. On the same media one can see also higher content of protein in biomass. Combination of the pointed hydrolyzers with soya milk provides receiving of lower quantity of biomass than on gluten 1.4 fold; 1.2 fold and 1.2 fold respectively.

This regularity is observed also in analysis on the content of protein in the deep biomass of fungus. Thus, receiving of biomass Pleurotus ostreatus with high content of protein (till 60%) is possible. For it fermenting hydrolyzers of different starch-containing raw may be applied. Though, usage of of hydrolyzers of buckwheat, corn and wheat flour is

optimal. With the given experiment such source as nitrogen acquired a great importance as a component of the nutrient medium for accumulation of biomass and protein of the oyster mushroom. Thus, gluten provides big accumulation both biomass and protein in it against soya milk. But with presence of soya milk in the content of the nutrient medium, the very important property of floccus, namely, appearance of pleasant mushroom smell distinctive for oyster mushroom of natural growth.

Presence of proper smell and taste is necessary feature of the deep floccus as biotechnological product of food purpose. That was also found that growing of oyster mushroom on the extract-treacle medium doesn't provide the highest outgoing protein-containing biomass of fungus. Concentration of floccus in CL reaches 1.82±0.091 g/100ml, but content of protein is 45.99±2.01%, that is 1.5 fold and 1.3 fold lower than on the fermenting hydrolyzer of buckwheat flour in combination with gluten.

Efficiency of the frocess vermicultivation, except mentioned above is stipulated by biochemical high quality of the nutrient substrate, which is defined by many interconnected parameters, including physical condition of substrate. Thus, to a considerable degree, nutrional value of substrate depends on the degree of grinding of the original phytogenous material.

Grinding SH leads to dimension of the degree of order of its submicroscopic structure. As a result of it valuable components of nutrition meal relieve out of the structure of husks and become more accessible for recycle by vermiculture. One time size, form and structure of the particles of modified SH are adoptable for productive assimilation by population Eisenia foetida [23, 24, and 25]. The results of biochemical analyses showed that biomass Eisenia foetida, cultivated on the modified SH, contains in a well-balanced form necessary substances and vitamins– $B_1$–0,03 mg/kg, $B_2$–0,04 mg/kg, PP–0,9 mg/kg, D – 4–6 hundred ME, microelements of blood-making action, which respond to the requirements on the necessary substances for fattening of saplings of cattle and increase of immunity. There are also a set of enzymes producing by Eisenia foetida, lipids with components of higher fatty acids of both saturated and not saturated [25, 26]. Veterinarian-sanitarian evaluation of biomass Eisenia foetida was held in three variants–fresh, boiled for 30 min, dried for three days (with breaks for 8 hours at night time). In all cases common bacterial insemination did not increase 500 germ bodies in 1g of biomass.

At the same time pathogenic types of Escherichia coli, forming toxins, as well as salmonella in tests weren't found. Biotest for toxins of botulism was also negative [21].

Out of biomass Eisenia foetida, adapted on modified SH, dry powder could be received. Preparation of dry biomass is friable, dark-grey powder with strong smell of the liver of cattle. When it is stored in the condition 18–22°C, and with 0°C, it has distinctive hygroscopicity. When it is stored without oxygen for 30 days, friability of the prepared product is kept what lets mix dry biomass with other fodders [22, 27]. There was investigated amino-acid composition of the dry biomass of the vermculture population of Eisenia foetida, adapted on modified sunflower husks. Analisis showd that it consists of protein – 63.6%, lipids – 10.5–17.5%, nitrous extra active substances.

Amino-acid content of dry biomass of the culture Eisenia foetida is introduced in the table 3.

**Table 3**

**Amino-acid content of dry biomass of the culture Eisenia foetida adapted on modified sunflower husks**

| Amino-acid | From content of protein, g | From content in biomassEisenia foetida, g |
|---|---|---|
| lysine | 6.27 | 3.99 |
| histidine | 2.09 | 1.33 |
| arginine | 6.62 | 4.21 |
| asparagine acid | 8.09 | 5.15 |
| threonine | 4.58 | 2.91 |
| serine | 4.28 | 2.72 |
| glutamic acid | 17.33 | 11.02 |
| proline | 3.73 | 2.37 |
| glycine | 3.43 | 2.18 |
| cystine | 11.67 | 7.42 |
| valine | 4.56 | 2.90 |
| methionine | 1.97 | 1.25 |
| isoleucine | 4.54 | 2.89 |
| leucine | 6.15 | 3.91 |
| tyrosine | 3.74 | 2.38 |
| phenylalanine | 4.03 | 2.56 |
| alanine | 5.98 | 3.80 |

Biological adequacy of biomass Eisenia foetida was defined according to amino-acid score. There is (mg): isoleucine–71, leucine–97, lysine–63, methionine, cystine–214, phenylalanine and tyrosine–122, threonine–72, valine–72 in 1g of protein which was selected out of biomass Eisenia foetida. Against standard score scale content of the named amino-acids correspondingly consists (%): 177.5; 138.6; 114.5; 523; 203; 180; 144. The least quantity in biomass of Lysine (score 114.5%), the most is sum of phenylalanine and tyrosine (score 523%). Producer Eisenia foetida, cultivated on the modified subtract SH, is balanced according to amino-acid content and all not changeable amino acids, more saturated with microelements, vitamins, enzymes and other more active substances than the culture adapted on another types of substratum.

## 4. Conclusions

Thus, at present experimental work was shown the dependence of accumulation of biomass and synthesis of some biologically active substances (carotene, protein, not changeable amino acids) on quality on nutrient media and substrata of the proper producers. This regularity is typical for the producers of the biologically active substances from different taxonomical groups: lower mucoraceous molds, higher edible fungus, specimens of cattle – vermiculture Eisenia foetida. Consequently, the problem of receiving of biologically active preparations of different origin may be effectively solved even in the conditions of industrial production with the help of optimization of the composition of nutrient media and substrata.

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