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Biohumus Production Technology Using Effective Groups Of Microorganisms

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ABSTRACT

The article is devoted to development of scientific approaches in optimization of the standard technology for biohumus production and recycling of aquaculture waste using effective groups of microorganisms (EGM). By the example of microbiological subjects, enzyme systems investigation demonstrated possibility of using bacterial starter cultures in order to achieve the positive effects of technology – to accelerate the processes of organic waste fermentation and maintain stable biohumus microbiological parameters. According to the results of the chromatographic analysis of biosynthesis products of ammonifying, propionic acid bacteria and bifidobacteria possibility of technological processes intensification was determined for production of biohumus 4 - 6 times as more as compared with the native substrate.

Keywords: biotechnology, biohumus, biotransformation of organic waste, effective groups of microorganisms

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INTRODUCTION

To date, biotechnology is actively developing and according to the original sources [1, 2, 3] by 2020 will be one of the leading knowledge-intensive industries. One of the areas of modern biotechnology is associated with the biodegradation of different origin waste [4, 5]. A successful a successful solution to the industrial waste problem in trout production is directly related to the development of biotechnological methods for raw materials modification. They are associated with the creation of new technological solutions, based on effective use of specific enzyme systems of biological objects as well as effective microorganisms, which produce enzymes, proteins, essential amino acids and vitamins. The technological methods diversity in organic matter processing by microorganisms allows obtaining high quality products with new functional properties. Efficacy of bacterial starter cultures depends on their bioactivity, the composition and properties of microorganisms, cultivation conditions, composition of raw materials, modes of technological processing. Such methods of biotechnology have great potential from the point of view of modification of organic wastes for their further involvement in the efficient production of biological fertilizers [6, 7]. To develop production technology of ecologically clean organic fertilizer, a research objective was put forward: to optimize the standard technology for producing biohumus and recycling fishing-crib aquaculture waste by means of effective groups of microorganisms (EGM). Biohumus is a clumpy microgranular substance of brown-grayish color with earth smell. It contains all substances necessary for plants nutrition in a well-balanced and digestible form. The average content of dry organic matter in biohumus is about 50%, humus — 18%; medium reaction is favorable for plants and microorganisms – pH 6.8–7.4; average value of the general nitrogen content reaches 2.2%; phosphorus –2.6; potassium – 2.7% etc. Besides, in biohumus practically all necessary microelements and biologically active agents are present, among which there are enzymes, vitamins, phytohormones, etc. In 1 gram of the best samples of biohumus there are up to several billion cells of microorganisms that considerably exceeds the number of microbes in manure (about 150–350 million cells). Biohumus displays high enzyme activity, especially activity of oxidoreductases. It should be noted that significant amount of biohumus organic matter is presented by humic acids (31,7–41,2%) and fulvic acids (22,3–34,8%). Among humic acids the most valuable fraction – calcium humates (43,3–47,6%) prevails. Existence of fulvate-humate humus type promotes formation of agronomical valuable soil structure. Biohumus nutritional elements during interaction with soil mineral components form complex coordination compounds. Therefore they are reliably protected from washing away, are slowly dissolved in water, providing plants nutrition over a long period of time (not less than 2–3 years). It is considered [8] that 1 t of biohumus contains on average 45 kg of nutrition elements (NPK) and quite often biohumus surpasses organic fertilizers in their nutritional value. Valuable properties of biohumus at its application favorably affect formation of crops productivity, stimulate improvement of received production quality. It is established, for example, that thanks to biohumus the increase of cereals yield makes 30-40%, potatoes –30–70% and vegetables –35–70%. For development of environmentally friendly organic fertilizer technology the research objective was formulated: to optimize standard biohumus technology as well as to develop aquaculture waste recycling technology by means of the effective groups of microorganisms (EGM).

METHODS

For the development of EGM-based biohumus production technologies speed of the hydrolytic breakdown of proteins [9] in the processed materials was estimated be means of parameters proposed by Hamagaeva I.S. et al. [10].

EGM enzymatic activity was evaluated in four variants of the experiment:

1 – temperature range from 0 to 12° C and exposure time – 16 hours;
2 – temperature range from 30 to 45° C and exposure time – 3 hours;
3 – temperature 37° C and exposure time – 4 to 5 hours;
4 – temperature 40° C and exposure time – 4 to 24 hours.

The experiment was carried out on raw materials and EGM ratio of 4:1. In all experimental variants the ability of EGM to grow on meat extracts was evaluated as well as biochemical degradation by amino acids accumulation in the environment: lysine, glycine, alanine and valine. For quantitative content determination of sample components, chromatographic analysis of one of calibrating solutions and prepared sample was carried out. As a measuring result arithmetic mean value of amino acid (C_{ch}, g/l) content in the sample examined was taken into account. EGM are the part of microorganisms genetic collecton of Microbiology Department of Petrozavodsk State University and they represent following groups: ammonifying bacteria (AB) – *Bacillus, Pseudomonas, Micrococcus, Arthrobacter, Mycobacterium, Proteus* genera; propionic acid bacteria (PAB) of the genus *Propionibacterium*; lactic acid bacteria (LAB) of the genus *Lactobacillus*. Identification of microorganisms is executed by means of Bergey's Manual of Determinative Bacteriology [11].

RESULTS

To determine the degree of activity in relation to the formation of biohumus from raw fish material the ability of EGM to proteolytic enzymes synthesis was assessed. It is well known that percolating proteases are involved in cleavage of meat proteins, wherein nitrogenous compounds formed penetrate through the cell membrane and are involved in metabolic processes [12, 13]. During EGM metabolic process when proteins of tested extract (fish substrate) are affected by microbial proteolytic enzymes, free amino acids are produced. In this regard, we studied the accumulation of lysine, glycine, alanine and valine at different exposure conditions (Table 1).

Table 1:The content of free amino acids in tested extract (mg%) under different conditions of exposure

EGM	Amino acids			
	Lysine	Glycine	Alanine	Valine
temperature range from 0 to 12° C and exposure time – 16 hours				
AB	8,933	2,170	7,342	6,231
PAB	11,450	4,210	8,210	7,820
LAB	9,140	3,483	6,210	5,130
temperature range from 30 to 45° C and exposure time – 3 hours				
AB	10,320	4,560	8,134	7,541
PAB	13,120	6,712	9,540	9,324
LAB	9,348	5,210	8,320	6,270
temperature 37° C and exposure time – 4-5 hours				
AB	15,623	5,236	11,548	8,524
PAB	17,324	6,830	12, 410	13,280
LAB	12,230	6,249	10,542	11,232
temperature 40° C and exposure time – 4-24 hours				
AB	5,439	2,109	5,610	5,410
PAB	8,250	3,408	6,142	6,210
LAB	4,132	3,241	5,211	4,108

The maximum accumulation of amino acids examined was fixed in variant 3 of the experiment. The most effective at 37 C and exposure time 45 hours were proteolytic enzymes of propionic bacteria group (PBG). The concentration of lysine in given experimental conditions reached 17.324 mg%. It can be assumed that a significant increase in free amino acids in fish raw materials, enriched with EGM, is the result of protein hydrolysis under the action of proteolytic enzymes of bacteria, as well as the accumulation of free amino acids in the process of EGM vital activity. In the production of biohumus biochemical and microbiological processes that control its formation from fish waste play significant role. Therefore, in the series of experiments dynamics of EGM development and survival was explored during 10 hours (Figure 1). As basic cultures Bacillus ammonifiers – Bacillus subtilis, Pseudomonas – Pseudomonas alcaligenes, Micrococcus – Micrococcus luteus, Arthrobacter sp., Mycobacterium sp. were used, allocated from the soil on the medium with paraffin as the only source of carbon, Proteus – Proteus mirabilis and propionic bacteria of - Propionibacterium freudenreichii subsp. globosum. Propionic bacteria of Propionibacterium freudenreichii subsp. globosum caused hydrolysis of esculin and gelatine; formation of acid from mannitol, lactose, trehalose, adonite, erythrite, maltose, ribose; reduction of nitrate and formation of indole at ammonification. For Propionibacterium freudenreichii subsp. globosum growth stimulation twin-80 was used. The propionic acid bacteria treated by twin gave rapid growth on a nutrient medium with glucose and 6,5% NaCl at cultivation temperature 36,4°C and pH 6,7. As lactic bacteria homofermentative (typical) microorganisms Lactobacillus acidophilum were used capable to turn 90% of lactose into lactic acid on lactic fermentation mechanism that corresponds with Embden–Meyerhof–Parnas pathway. Lactobacillus acidophilum (acidophilous bacterium) has a temperature optimum − 37° C and it ferments glucose, sucrose and maltose. Up to 2,2 and more percent of lactic acid is accumulated in medium. At lactic acid fermentation, anaerobic process of lactate decomposition by lactic bacteria (LAB) enzymes was observed and accumulation of lactic acid and by-products of fermentation – pyruvate and hydrogen.

In the meat extract composition there was observed intensive growth of all groups of effective microorganisms. The maximum value was observed in propionic bacteria to total of 10^8 CFU/ml at 10-th hour of experiment. In activated suspension lactic acid bacteria were also at intensive development phase throughout the entire experiment that can result in significant reduction of raw materials transformation time in technological cycles of organic waste modifications.

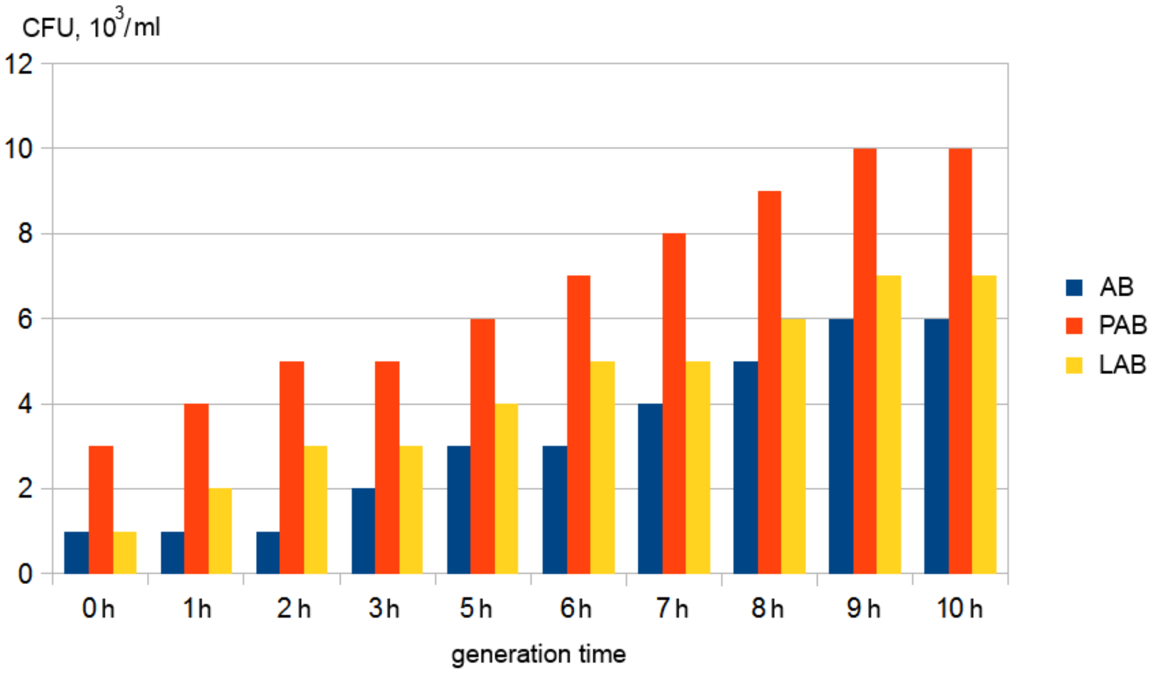

Figure 1. Dinamics of microflora growth in the fish meat extract composition (log CFU/ml): AB – ammonifying bacteria, PAB – propionic acid bacteria, LAB – lactic acid bacteria

As the results obtained show, meat extract is a favorable culture medium for the development of the EGM. Based on experimental data there were designed EGM parameters that form the basis of microbiological transformation of fish waste into biohumus (Table 2).

Table 2: Biochemical activity of allocated EGM for biohumus production

Indicator	Standard
Activity based on bioproduction cycle duration, h	15-17
Acidity, titratable, °T, active, pH	 60-63 4,95 -6,11
Cell number, CFU/ml AB PAB LAB	 10^9 10^8 10^7

The data in Table 2 indicate that ammonifying, propionic acid and lactic acid bacteria have a high biochemical activity and are promising cultures to optimize the standard technology for production of biohumus and recycling fishing-crib aquaculture waste.

CONCLUSION

As a result of the conducted researches theoretical and practical basics of biohumus production technology was worked out on the basis of fishing-crib aquaculture waste with the use of EGM. There were proposed conditions for waste biomodification in order to achieve positive effects of technology – to accelerate the processes of waste fermentation, maintaining stable microbial indicators of biohumus. Prospects of use are proved for organic waste transformation by ammonifying, propionic acid and lactic acid bacteria, which meet the specific requirements of modification processes of organic raw materials. It is found that using of specially selected bacteria biohumus production processes and feed may be intensified 4 to 6 times as compared with native substrate. Enzyme activity of effective microorganisms was experimentally confirmed. It was found that it depends on temperature conditions and combination of respective embodiments of bacterial flora. With increasing cultivation temperature up to 37° C, enzymatic activity of the EGM increases, which indicates the induction of biochemical properties of these cultures. The role of extracellular factors in EGM adaptation to change in environmental conditions was studied, which is of practical importance in biotechnology to control microbiological processes in the production of biohumus. Besides, the high survival of EGM that is of great importance for the guaranteed biohumus period of storage is established. The quantity of EGM viable cells which has to make not less than 10^7 CFU/g is regulated.

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