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A Selection of Conditions for the Biodegradation of Poultry Wastes Industry.

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ABSTRACT

The advantages of bioconversion processes are in the absence of a necessity for applying high temperatures and pressure during the implementation of enzymatic reactions, which testify to their greater efficiency in comparison with traditional methods of processing of raw materials, and their relative technical simplicity of production processes. Bioconversion allows achieving the environmental friendliness of produced products in comparison with chemical technologies, as raw materials for its implementation are renewable materials of animal origin, and the product itself is completely consumed in the processes of consumption. Therefore processes of this kind can not only accelerate the obtaining of the final product and reduce its cost, but also reduce costs for the protection of air, water and soil from contamination. Technology using strains of microorganisms have some several advantages in comparison with traditional technologies of processing organic waste: 1) low labor intensity and high economic efficiency; 2) ecological safety; 3) high operational stability and storage stability of the resulting products; 4) the minor duration of the process; 5) the maximum conservation of nutrients.

Keywords: pollution, poultry production, feather down raw materials.

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INTRODUCTION

An urgent task of our time is the need to extend import-substituting technology, converting low value waste into valuable products and components with high added value [1]. Among these components food and feed proteins are playing an important role. One of the world's problems with the ever-increasing population of the Earth is a lack of food, particularly of a protein component of human diet. According to the Institute of nutrition of RAMS for the last 20 years the deficit of dietary protein in Russia had exceeded 1 million tons per year [2]. A protein deficiency reduces the body's resistance to infections, as it reduces the level of antibody formation. A synthesis of the other protective factors – lysozyme and interferon, which exacerbates during the inflammatory processes, disturbs also. In addition, a protein deficiency is often accompanied by the deficiency of vitamins B12, A, D, K, and this also affects the status of health [3].

About the priority of this direction of food biotechnology testifies a "Comprehensive program of biotechnology development in the Russian Federation for the period till 2020", in which the selected targets are highlighted: "food protein" ("...modern methods of biotechnology in combination with the use of ultrafiltration and nanofiltration systems make economically feasible the extraction of dietary protein from a wide class of raw materials and wastes of food industry") and "deep processing of food raw materials" ("...modern technologies of deep processing of edible raw materials are based on the principles of non-waste production: the products are either returned to the production cycle or used in other industries") [4].

Based on the analysis of modern technical and economic level of existing development it is concluded that currently the use of recycled resources is solved in two ways: either use as non-recycled (feeding the cattle in their natural form), or entombing. Unfortunately, currently is often used the second method, which is not a safe one. For the first, we should consider the costs of shipping; secondly, we should consider the vast area occupied by waste. In addition, these areas are a source of environmental pollution [5].

Thus, we can see a clear necessity for the use of recycling, low-waste and non-waste technologies. They allow us to maximize the extract of all valuable components from raw materials, turning them into safe and useful products [6].

The most dynamic and knowledge-based industry of the global and domestic agro-industrial complex is poultry. Today in the global structure of meat of all types of animals bird occupies the second place after pork [7]. At the present time in connection with the intensive development of biotechnological methods a processing of waste of animal origin is a promising one, in particular - keratin-containing waste, with the use of biological methods. Among the variety of modern approaches available in this field there are the methods of disposal of the "new generation" pertaining to bioconversion. The processes carried out by microorganisms during bioconversion have the advantageous not only because a variety of raw materials are used in them (renewable animal materials and various waste), but also because the resulting products have potential application in various fields of human activity [8, 9].

The currently known technologies of bioconversion of feather down raw materials are characterized by a number of disadvantages: the low degree of bioconversion; low protein yield; laboriousness and multistage process, etc. In this regard, there was the need to improve bioconversion technologies, a more deep study of fundamental aspects of processes underlying the biocatalytic transformation of components of keratin-containing wastes and the use of new knowledge for developing new technological solutions [10].

OBJECTS AND METHODS

As the object of a research was used the feather raw material obtained from hens of different breeds:

French breed "F-15 Iza" OJSC "Plemptitsesovkhoz "Kolmogorovskiy" (Kemerovo region, Askinsky area, the village of Kolmogorovo);

– breed "Lohmann brown" CJSC "the Kuzbass integrated poultry farm" (the Kemerovo region, Novokuznetsk area, settlement Stepnoy);
– "Lomann LSL-classic" OJSC "Integrated poultry farm Inskaya" (Kemerovo region, settlement Yinskoy).

As the producers of enzymes with keratinase activity were used fifteen strains of microorganisms, provided by the All-Russian collection of industrial microorganisms Federal State Unitary Enterprise "Gosniigenetika" (http://www.genetika.ru/vkpm).

At different stages of work the following materials and reagents were used for the study:

– distilled water (State standard 6709-72);
– sodium chloride (State standard 4233-77, and 99.8%, chemically pure);
– peptone enzymatic dry (State standard 13805-76, 80,0%);
– agar-agar (State standard 16280-2002, 95,0%);
– starch soluble (State standard 10163-76, 98,0%, pure for analysis);
– acrylamide ("Sigma", USA);
– N,N'-methylene-bisacrylamide ("Sigma", USA);
– ethidiumbromide (Sigma, USA);

At different stages of work the following equipment was used for the study:

Spectrophotometer UV 1800 (Shimadzu, Japan).
A direct microscope AxioScope A1 (Carl Zeiss AG, Germany).
The AxioVert A1inverted microscope (Carl Zeiss AG, Germany).
Ultracentrifuge Beckman J2-HS (Beckman, USA).
The nitrogen analyzer Rapid N Cube (Elementar, Germany).
Liquid chromatograph LC-20 (Shimadzu, Japan).
ARACUS amino acid analyzer (Analytical Systems Gmb, Germany).
Camera for vertical electrophoresis and power supply PowerPack HC, (Bio-Rad, USA).
UV transilluminator TCP-20M (Vilber Lourmat, USA).
System of gel documentation Gel Doc XR Plus (Bio-Rad, USA).
Fermenter the Biostat A plus MO, 5 L, Sartorius (Sartorius, Germany).
Refractometer HI 96801 (HANNA, Romania).
Centrifuge CV-50 (ELMI, Latvia).
Analytical balance AND HR-202 i (A&D, Japan).
A pH meter - Sevew Compact (Mettler Toledo, USA).
Laboratory microbiological incubator ILM-170-01 "Laminar-C" (CJSC "Laminar systems", Russia).
Sublimation installation "Iney-6M" (Russia).

Theoretical and experimental investigations were carried out according to the modern methodology of study of complex phenomena using conventional, standard and original methods of biochemical, physico-chemical, structural-mechanical analysis.

RESULTS AND DISCUSSION

The next stage of research is aimed at selection of co-cultivation of a proposed combination of microorganisms with keratinase activity for their activation. To achieve this goal were varied the following parameters of the hydrolysis of the waste of the poultry industry consortium of microorganisms-destructors: composition of nutrient medium, temperature, pH and the ratio of cultivated material to the volume of processed keratin-containing raw materials.

For the first the keratin wastes of the poultry industry were transformed into a soluble form. According to the data of a scientifical literature it is known that keratin in its natural form is practically not degradable by proteolytic enzymes due to the presence of a large number of disulfide bonds of cysteine. Therefore, for the transformation of the protein into the denatured form due to the action of the proteolytic enzymes. The well-known method is used: the milled feather is exposed to an alkaline hydrolysis in the solution containing sodium hydroxide, hydrogen peroxide and sodium sulfite.

Alkaline hydrolysis of keratin-containing milled waste (feather down raw materials, obtained from hens of the breed "Lohmann brown", CJSC "Kuzbass integrated poultry farm") were with duration of 2,0; 4,0; 6,0 and 8.0 hours. The results showed that the maximum solubility of keratin-containing raw materials is

achieved within 8-hour of alkaline hydrolysis, and the content of a soluble protein in hydrolysate achieved the level of 92.6%. On the basis of the obtained results one of the methods of increasing the bioavailability of components of waste of the poultry industry for the enzymes produced by microorganisms belonging to the consortium, is an alkaline hydrolysis with duration of 8.0 h.

Other known methods of transformation of keratin into the denatured form are: acid hydrolysis, hydrothermal treatment, hydrolysis by urea under a pressure.

In this regard, there was a comparative assessment of different ways of transformation of keratin, which is a part of the waste of the poultry industry, into the denatured form.
The experimental results are presented in table 1.

Table 1 – The solubility of keratin-containing raw materials, depending on the duration of different types of hydrolysis

Duration of hydrolysis, h	Mass fraction of soluble protein in hydrolysate, %		
	Acid hydrolysis	Urea hydrolisis	Hydrothermal processing
2,0	18,0	20,3	27,6
4,0	23,5	28,7	38,8
6,0	37,8	41,5	57,5
8,0	43,4	51,6	71,3

Table 1 shows that the most effective ways of transformation of keratin into a soluble form are alkaline hydrolysis and hydrothermal processing providing a mass fraction of soluble protein in hydrolysate of 92,6% and 71.3 %, respectively, after 8 hours of hydrolysis.

The least effective were the acid hydrolysis and a processing of feather down raw materials by the urea (43,4% and 51.6%, respectively).

According to the obtained results in order to improve the bioavailability of the components of the poultry industry wastes for microorganisms – destructors, that are the part of the consortium, was selected a preliminary alkaline hydrolysis with a solution containing sodium hydroxide, hydrogen peroxide and sodium sulfite.

The choice of an optimal composition of the nutrient medium for the co-culturing of the four studied strains (in the ratio 1:1:1:1, the final concentration of microorganisms $1 \bullet 10^5$ CFU/g) was carried out by varying the ratio of the components and measuring the extent of biodegradation of keratin-containing raw materials. The composition of a culture media is presented in table 2.

Table 2 – Composition of a culture media for co-cultivation of the four strains with keratinase activity

Component	The content of the component, g/l, according to the number of the nutrient medium				
	1	2	3	4	5
Starch soluble	10,0	-	5,0	10,0	15,0
Peptone	-	10,0	5,0	-	-
NaCl	5,0	5,0	2,0	5,0	5,0
$(NH_4)_2SO_4$	2,0	1,0	-	1,0	2,0
$CaCO_3$	1,0	1,0	-	0,5	0,2
$NaNO_3$	2,0	2,0	2,0	1,0	1,0
KH_2PO_4	1,0	1,0	0,5	1,0	0,5
$MnCl_2$	0,001	-	-	0,001	0,002
$ZnSO_4$	0,002	0,001	0,001	-	-
$MgSO_4 \cdot 7H_2O$	0,5	0,5	0,5	0,5	0,5
KCl	0,5	0,5	0,2	0,5	0,5

$FeSO_4 \cdot 7H_2O$	0,01	0,01	-	-	-
Sucrose	20,0	20,0	15,0	-	25,0
Agar	20,0	20,0	15,0	20,0	15,0
Glucose	20,0	20,0	15,0	20,0	20,0

On this nutrient media was carried out a co-cultivation of the four studied strains at 30°C and pH 7,0.

From the obtained data it follows that the maximum degree of biodegradation of the waste of the poultry industry (71,5%) is achieved during processing by a consortium of microorganisms *Bacillus licheniformis* B-2986, *Streptomyces ornatus* S-1220, *Penicillium rubrum* F-601 and *Verticillium lateritum* F-626 (the ratio of strains 1:1:1:1), co-cultivated at 30°C and pH 7.0 in a period of 12.0 h on an nutrient medium composition (g/l): soluble starch – 15,0, sodium chloride – 5,0, ammonium sulfate – 2,0, calcium carbonate – 0.2, sodium nitrate – 1.0, potassium dihydrogen phosphate and 0.5, the chloride of manganese 0,002, magnesium sulfate – 0.5, KCl – 0.5, the sucrose – 25,0, agar – 15,0, glucose – 20,0.

This composition of the nutrient medium was selected for carrying out further studies aimed at determining of an optimal values of temperature and pH for the process of co-cultivation of studied microorganisms - destructors.

The co-cultivation of the studied strains was carried out on a nutrient medium composition No. 5 at pH 7.0 and various temperatures in the range of 25-40°C with a step of 5°C. This range of variation of temperatures was chosen due to the fact that the strains of microorganisms-destructors, members of the consortium have different optimum temperatures: between 24°C and 37°C.

An important parameter of hydrolysis is the ratio of the volume of a cultivated material to the volume of a processed raw material. In this regard, were conducted a series of experiments on the hydrolysis of waste of the poultry industry by a consortium of microorganisms - destructors (the ratio of strains 1:1:1:1), co-cultivated at pH 7.5 and temperature 37°C, by varying the ratio of a cultivated material to the volume of processed raw materials (1:2,0; 1: 5,0; 1:10,0).

From the obtained data it follows that when the ratio of the volume of a cultivated material to the volume of a processed raw material is 1: 5,0 the hydrolysis reaction of the waste of the poultry industry occurs with maximum efficiency (the degree of biodegradation of 83.5%).

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CONCLUSIONS

Thus, on the basis of the conducted research it can be concluded that the optimal conditions for the biodegradation of the waste of the poultry industry (feather down raw materials, obtained from hens of the breed "Lohmann brown", JSC "Kuzbass integrated poultry farm") by a consortium of microorganisms with keratinase activity of *Bacillus licheniformis* B-2986, *Streptomyces ornatus* S 1220, *Penicillium rubrum* F-601 and *Verticillium lateritum* F-626 (the ratio of strains 1:1:1:1) are the following: temperature 37°C; pH 7.5; duration of cultivation is 12.0 hours; the ratio of inoculum to the volume of processed raw material 1:5,0.

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