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OPTIMIZATION OF CULTIVATION CONDITIONS OF *BACILLUS* GENUS BACTERIA CULTIVATION FOR PROTEOLYTIC ENZYMES PRODUCTION

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The spectrum of economically viable protein substrates that can be used in production of proteolytic enzymes was determined. Based on the experimental data component's composition of the nutrient medium was optimized. The maximum proteolytic activity of the genus Bacillus bacteria was determined in the case of their incubation for 72 hours at temperature 37 °C on a medium with concentrations of wheat bran protein – 1,42 % and wheat flour protein – 1,58 %.

Key words: *bacteria Bacillus, substrates, wheat bran, whole-wheat flour, proteolytic enzymes, optimization.*

Introduction. Poteolytic enzyme, also called Proteinase, any of a group of enzymes that break the long chainlike molecules of proteins into shorter fragments (peptides) and eventually into their components, amino acids. Proteolytic enzymes are present in bacteria and plants but are most abundant in animals [1].

Protease production depends on the producer strain and culture medium. To get productive enzyme is necessary to optimize the culture medium in many ways. Environments for receiving protease typically contains a source of carbohydrates – starch, lactose, and a nitrogen source – soybean meal, casein, corn steep liquor. Carbohydrates are essential for the duration of cultivation. It is important to maintain a constant, but a low concentration of carbohydrates, because the high concentration represses the production of the enzyme. Free amino acids repress production of the

enzyme, while the presence of peptides and proteins in the medium induce. To obtain a protease suggested using deep and shallow cultivation [2].

Simple and economical methods of cultivation of the surface can be used to produce proteases (acidic, alkaline and neutral). The main producers – *Aspergillus*, *Mucor*, *Penicillium*, and *Rhizopus*. As substrates in addressing various agroothody, but most research suggests that the best solution of the enzyme is achieved by using wheat bran. Comparative studies show that in obtaining the alkaline protease (*Bacillus amyloliquefaciens* ATCC 23844), the amount of enzyme with 1 gram bran with surface cultivation is equivalent to 100 ml of liquid medium in submerged cultivation. Protease synthesis is also repressed by a high concentration of carbohydrates [4].

The important parameters that influence the large-scale production using surface cultivation protease, is the density and thickness of the substrate. When cultured in the medium output trays protease low. This is associated with dry substrate. This can be avoided by using rotary drum fermenters. Most preferred fermentors with aeration and filtration sensors monitoring temperature and humidity. The maximum yield of protease was recorded using *Bacillus amyloliquefaciens* [5].

The same method is described submerged culture on the membrane surface. The essence of the method consists in growing mycelium on a membrane carrier, which is in contact with the liquid medium. This method allows you to control the pH and the concentration of the enzyme facilitates extraction methods. In applying this method, using *Aspergillus oryzae* IAM 2704, was received protease yield up to 2-fold compared with the surface, and 10 times higher than in submerged culture [6].

Extracellular proteases produced, so their selection using distilled water or buffer solutions. To get clean of drugs used deposition methods salts or solvents to further purification by chromatographic methods or electrophoresis.

Offered a lot in detection of protease activity. The most common are the various modifications of the classical method of Anson [3].

Table 1

Basic technologies of protease production on agrosubstrates

Substrate	**Strain-producer**	**Activity**
Wheat bran	*Aspergillus awamori* MTCC 6652	1930 units/g
	Aspergillus flavus MI 327634	10,5 units/g
	Aspergillus oryzae NRRL 1808	31 units/g
	Aspergillus oryzae	1500 units/g
	Bacillus amyloliquefaciens	560 units/g
	Beauveria felina	200 00 units/g
	Bacillus sp.	429 units/g
	Penicillum sp.	11000 units/g
	Rhizopus oryzae	58,7 units/g
	Rhizopus oryzae	358 units/g
	Streptomysec sp. CN 902	90,5 units/g
Rice bran	*Aspergillus oryzae* NRRL 1808	3 units/g
Rice husks + rice bran (7:3)	*Aspergillus oryzae* NRRL 2160	0,986 units/g
Threshing rice waste	*Aspergillus niger* MTCC 281	67 units/g
Wheat and soy flour (1:1)	*Aspergillus oryzae*	18 units/g
Brewer's grain	*Bacillus subtilis* DL-1	528 units/g
Rice bran + wheat bran (3:1)	*Aspergillus oryzae* (Ozykat-1)	1200 units/g
Whey	*Aspergillus terreus*	2,24 units/g
Sweet potato waste	*Aspergillus niger* NTU	837 units/g
	Aspergillus oryzae NTU	809 units/g
	Rhizopus sp. NRRL	900 units/g

Most publications 1999-2009 period is considering the production of proteases on agrosubstrates using shallow cultivation as a promising direction. Research centers that address the topic, are concentrated in India, Egypt, Thailand, Taiwan, Saudi Arabia and Tunisia. Also, there are publications of Russian researchers. However, most of these technologies is at the stage of laboratory tests. Higher yield of proteases have been reported with wheat bran, and waste processing legumes as a substrate for solid cultivation. As well as a potential raw material for the production of amylase treated agroothody: whey, rice bran, sweet potato processing waste, molasses etc [7].

The main task of research is to optimize the protein composition of nutrient medium for cultivation of *Bacillus* spp. bacteria for the production of proteolytic enzymes.

Materials and methods of researches. For the research minimal salt medium was prepared according the Table 2.

Table 2

Minimal Salt Medium (Modified from E. Rosenberg) [2]

Component	**Mass, g**
NaCl	1,25
K_2HPO_4	2,37
KH_2PO_4	0,28
$MgSO_4*7H_2O$	0,25
$CaCl_2*H_2O$	0,05
Tap water	500

The characteristics of agrosubstrates used as protein source of nutrient medium in our research is summarized in Table 3.

Table 3

Objects of the research were protein sources

Name of the protein source	**Fats, %**	**Protein, %**	**Carbohydrates, %**
whole-wheat flour	2	9,5	71
pea flour	0,4	5,4	14,5
phasoleous flour	2	21	47
soy flour	1	48,9	21,7
wheat bran	3,2	15,5	64,5

Bacillus genus bacteria were used as a producers. *Bacillus subtilis* – Gram-positive, rod shaped bacteria, commonly found in soil. *Bacillus licheniformis* is a Gram-positive, mesophilic bacterium. Its optimal growth temperature is around 30 °C, though it can survive at much higher temperatures.

For our experiment we used surface cultivation. We poured 50 ml of prepared cultural medium into the flasks, then add the different protein sources with different protein concentration which we calculated earlier. The maximum concentration of

protein was 1 %, minimal – 0,1 %. The flasks with nutrient media were autoclaved and inoculated with *Bacillus* spp. bacteria (Fig. 1). The cultivation was conducted at 37 °C during 48, 72, 96 hours.

Fig. 1. Flasks with different protein concentration cultural media

Method for enzyme activity determination – spectrophotometric with Folin-Ciocalteu reagent.

For mathematical methods we used such programs as: Microsoft Excel, Statistica and MathCad.

Results and discussions. We identified the most effective substrates. The data presented in Table 4.

Table 4

Dependence of enzyme activity on the concentration and kind of substrate

Protein sources		**Enzyme activity et different time of cultivation, s.u.**		
		48 hours	**72 hours**	**96 hours**
phasoleous flour	0,1 %	49,850	33,424	22,617
	1 %	70,513	30,833	37,574
pea flour	0,1 %	83,654	69,303	53,154
	1 %	41,205	60,484	94,186
whole-wheat flour	0,1 %	66,882	80,455	92,214
	1 %	33,856	20,110	14,749
soy flour	0,1 %	35,499	53,049	70,859
	1 %	23,049	29,533	45,008
wheat bran	0,1 %	27,891	70,254	20,110
	1 %	53,827	45, 700	40,599

From these results it was determined that pea flour, wheat bran and whole-wheat flour have the greatest impact on proteolytic activity of *Bacillus* genus bacteria.

Using the Plakett-Bruman method and full factorial analysis we identify two the most profitable substrates – whole-wheat flour and wheat bran by comparing the enzyme activity in the samples with different protein concentrations (1 %, 1,5 % and 2 %). Experimental data are shown in the Fig. 2.

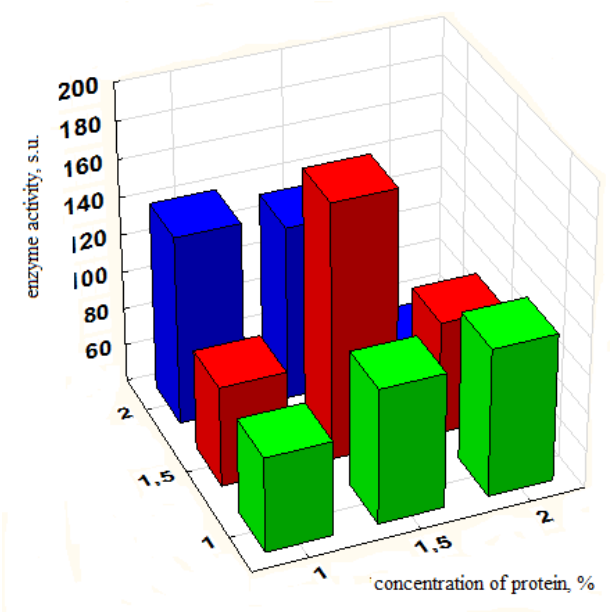

Fig. 2. Dependence of bacteria enzyme activity on protein concentration of whole-wheat flour and wheat bran

After that the mathematical model was built and its shown on the Fig. 3. The equation that shows the dependence of enzyme activity on different protein concentrations was obtained. Model adequacy was checked with the help of F test.

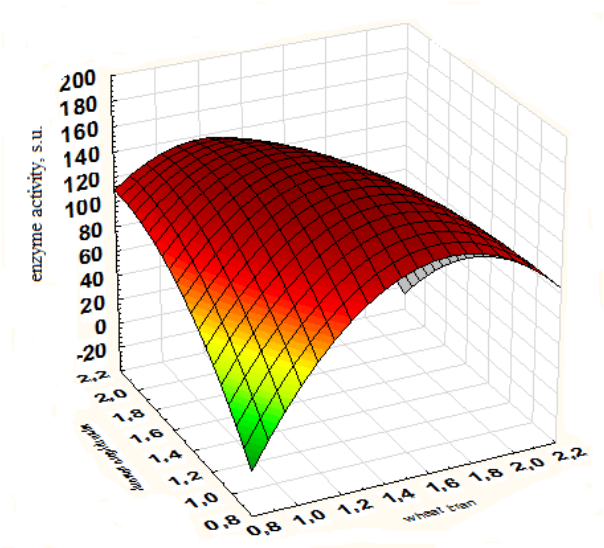

Fig. 3. Mathematic model of the research

Based on the obtained results using Math Cad we calculated the optimal values of protein concentrations – 1,424 % of wheat bran and 1,529 % of whole-meal flour and got the results shown in Fig. 4.

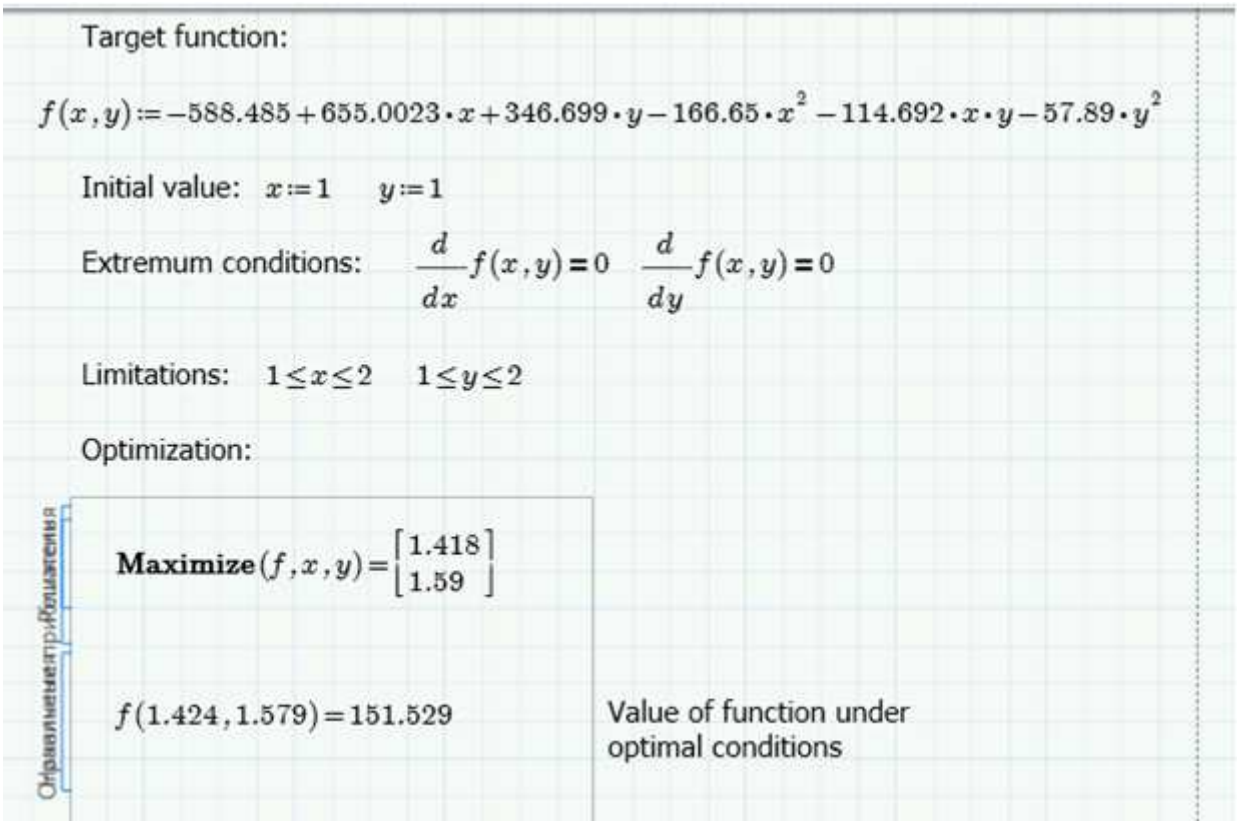

Fig. 4. Calculations of optimal protein sources concentrations in program MathCad

We found that the highest active proteins observed during 72 hours and with the concentration of protein in the wheat bran 1,4 % and 1,6 % in whole-meal flour.

CONCLUSIONS

1. The range of economically viable for Ukraine protein substrates was identified that can be used in biotechnology production of proteolytic enzymes, such as whole-meal flour, wheat bran, pea flour phasoleus flour, soy flour.

2. Substrates that most affect the proteolytic activity of the *Bacillus* spp. were identified. They are whole-meal flour and wheat bran.

3. A full factorial experiment was performed to determine the optimal ratio of protein components in a nutrient medium.

4. The optimization of microorganisms cultivation parameters was conducted. It was established that the optimum concentrations of wheat bran protein – 1,4 % and whole-meal flour protein – 1,6 % as the main protein source of nutrients medium.

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ОПТИМІЗАЦІЯ УМОВ КУЛЬТИВУВАННЯ БАКТЕРІЙ РОДУ BACILLUS З МЕТОЮ ОТРИМАННЯ ПРОТЕОЛІТИЧНИХ ФЕРМЕНТІВ

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Визначено спектр економічно доцільних білкових субстратів, що можуть бути використані у біотехнології отримання протеолітичних ферментів та

на основі отриманих даних проведено оптимізацію компонентного складу поживного середовища, встановлено максимальну протеолітичну активність бактерій роду Bacillus *при їх культивуванні впродовж 72 годин при температурі 37 °С на поживному середовищі з концентраціями білка висівок – 1,42 % та білка муки грубого помолу – 1,58 %.*

Ключові слова: *бактерії роду* Bacillus*, субстрати, пшеничні висівки, мука грубого помолу, протеолітичні ферменти, оптимізація.*

ОПТИМИЗАЦИЯ ПАРАМЕТРОВ КУЛЬТИВИРОВАНИЯ БАКТЕРИЙ РОДА BACILLUS ДЛЯ ПОЛУЧЕНИЯ ПРОТЕОЛИТИЧЕСКИХ ФЕРМЕНТОВ

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Определен спектр экономически целесообразных белковых субстратов, которые могут быть использованы в биотехнологии получения протеолитических ферментов и на основе полученных данных была проведена оптимизация компонентного состава среды, установлена максимальная протеолитическая активность бактерий рода Bacillus *при их культивировании в течении 72 часов при температуре 37 °С на питательной среде с концентрациями белка пшеничных отрубей – 1,42 % и белка муки грубого помола – 1,58 %.*

Ключевые слова*: бактерии рода* Bacillus*, субстраты, пшеничные отруби, мука грубого помола, протеолитические ферменты, оптимизация.*