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Biosynthesis of Enzymes by *Aspergillus Niger IBT-90* and an Evaluation of Their Application in Textile Technologies

Abstract
Enzymes including pectinolytes, cellulolytes and hemicellulases have found their place in textile industry. One modern trend of their application is the usage of a mixture of enzymes in the pre-treatment of woven fabrics made of cellulose fibres. The proportion of individual enzymes in this mixture is very important. The work described in this paper determined the conditions of the biosynthesis of these enzymes by the filamentous fungus Aspergillus niger IBT-90 by a mathematical method of factorial planning and gradient optimisation, considering especially the maximum activity of pectinolytic enzymes. The composition of cultivation culture for enzyme biosynthesis (the type and amount of macro- and micro-elements) was defined, the effect of the type and amount of inductors on this process (microcrystalline cellulose, wheat dietary fibre, oat dietary fibre or apple dietary fibre, evening primrose cake, carrot pomace, sesame hulls, cotton dust) was evaluated together with the cultivation method (stationary or dynamic) and cultivation conditions (cultivation time, the pH of the medium). The process optimisation led to a threefold increase in the activity of pectinolytic enzymes and double the activity of cellulolytic enzymes and xylanase. The complete suitability of the enzymes produced has been confirmed in the pre-treatment of cotton woven fabric (higher values of water sorption as compared to traditional alkali treatment).

Key words: *cellulolytic, pectinolytic enzymes, xylanase, biosynthesis, woven fabric biotreatment.*

Introduction

The continued degradation of the natural environment forces us to find new, environment-friendly technologies. Biotechnology offers such technologies for different branches of industry. Furthermore, the textile industry has for many years been successfully benefiting from biotechnologies, applying a wide range of enzymes mainly in textile finishing processes [10].

Cotton, flax and hemp are among the natural fibres used in the textile industry. Cellulose accompanied by pectins, hemicellulose, lignin, waxes, fats and mineral compounds, are the basic components of these natural fibres. Pectins and hemicelluloses contained in the outer layer of raw cotton fibre are to a high degree soluble in alkaline baths, and hence they can be removed from the fibre by a process of traditional pre-treatment. One unfavourable aspect of boiling off the alkaline used for cellulose textile fabrics is the generation of highly loaded wastewaters. Thus many research workers analyse the possibilities of substituting the alkali scouring of cotton textiles by enzymatic treatment. An enzyme mixture applied in the pre-treatment of natural cellulose fibres should contain, apart from cellulolytic enzymes, hemicelluloses and pectinolytic enzymes as well.

The basic problem in enzyme biosynthesis by micro-organisms is the selection of proper producer. Among micro-organisms producing cellulolytic enzymes, filamentous fungi predominate; in this group the most interesting are strains of *Trichoderma, Aspergillus, Penicillium, Fusarium, Myrothecium* and *Chaetomium* [4]. As cellulose does not occur in a crude form in natural raw materials, then the micro-organisms which degrade cellulose must also degrade the accompanying polymers (hemicellulose and pectins). The fungi strains mentioned above are numbered among good producers of enzymes which degrade these polymers [7]. The filamentous fungus *Aspergillus niger* is used for industrial production of pectinolytic enzymes [1,11]. *Aspergillus niger*, which is generally regarded as safe [GRAS] allows its metabolites to be applied even in the grocery industry [14].

Regulation of enzyme synthesis (which can treat natural cellulose fibres) is a complex phenomenon depending on many environmental factors [4]. The most important methods of enzyme biosynthesis regulation are induction and catabolic repression, while the main mechanism regulating the activity of newly produced enzymes is the inhibition through the final product. Cellulose, cellobiose, lactose, sophorose, thiocellobiose and carboxymethylcellulose are the inductors of cellulolytic enzyme synthesis [4]. Pectinolytic enzymes are induced by many substances. Pectin and pectic acid are most often used for this purpose [19]. Frequently natural products rich in pectins are used, e.g. apple pomace or citrus peels [12]. Fructose, mannose, saccharose and cellobiose are also inductors of pectinolytic enzymes [11].

Cellulolytic, pectinolytic enzymes and hemicellulases are subject to catabolic repression. Generally, the addition of substrates easily metabolised by glucose, inhibits enzyme biosynthesis [4], although the repression mechanism is reversible [9].

The complex structure of cellulose, pectins and hemicelluloses, the substrates onto which the discussed enzymes react, requires the necessary co-activity of a complex enzymatic system for effective enzyme action. Hence, these enzymes should be found in suitable proportions. Cellulolytic enzymes encompass 4 types of glycoside hydrolases. The hydrolysis of hemicelluloses requires the co-action of hydrolases (acting on glycoside bonds) and esterases, while pectin hydrolysis is carried out by glycoside hydrolases, ester hydrolases and liazes [2,6,8]. Hence, the biosynthesis of the above-mentioned enzymes for

cellulose textiles should aim at the production of a wide range of such enzymes.

The Institute of Technical Biochemistry of the Technical University of Łódź has for many years been involved in research on the optimisation of enzyme biosynthesis by *Aspergillus niger IBT-90*. The usefulness of these enzymes in textile processes has been confirmed by the Textile Research Institute in Łódź [17].

This work presents the results of the optimisation of conditions for enzyme biosynthesis. The enzymes are produced by the filamentous fungus *Aspergillus niger IBT-90*. Special emphasis has been put on pectinolytic enzymes. The scope of the research covered the selection of cultivation culture, the type and amount of biosynthesis inductors, cultivation conditions and the evaluation of the possibilities of applying such enzymes in the pretreatment of textiles made of natural cellulosic fibres.

Experimental

Micro-organisms

The fungal strain *Aspergillus niger IBT-90* from the culture collection of the Institute of Technical Biochemistry was used for the production of pectinolytic and cellulolytic enzymes and hemicellulases. This strain was kept on agar slants containing malt broth (8°Bx) and 2% of agar.

Cultivation culture

The selection of cultivation culture for the *Aspergillus niger IBT-90* biosynthesis of pectinolytic, cellulolytic enzymes and hemicellulases was performed using the method of mathematical optimisation. The applied cultivation cultures contained varied amounts of mineral salts (e.g. KH_2PO_4, $CaCl_2$, $MgSO_4$, $FeSO_4$, $MnSO_4$, $ZnSO_4$ $CuSO_4$, $CoSO_4$ and $CO(NH_2)_2$ which are most often used in enzyme biosynthesis and which are well known from the literature and the research carried out by the Institute of Technical Biochemistry.

The source of carbon for the *Aspergillus niger IBT-90* cultivation was apple pectin (Jasło, Poland) and AVICEL - microcrystalline cellulose or other cellulose materials such as oat, wheat and apple dietary fibre, carrot pomace, sesame hulls, evening primrose cake and cotton dust. Malt sprouts were used as the substance enriching cultivation culture. The cultivation cultures were sterilised at the temperature of 120°C for 20 minutes.

Cultivation conditions

The cultivation of the applied strain was performed in a submerged culture at 30°C in a 300-ml conical flask with 50 ml of the medium in stationary conditions or on a shaker at the frequency of 200 rpm. The cultivation culture was inoculated with 2% of neutral dispersion of fungi spores washed out from the agar slants by 10 ml of a physiological sodium chloride solution. Optimisation of cultivation time was the subject of this research work.

Analytical methods

The activities of the cellulolytic enzymes endo-1.4-β-glucanase, β-glucosidase, exocellobiohydrolase, FPA and endo-1.4-β-xylanase were determined by defining (according to the Somogyi-Nelson method [13]) the amount of reducing sugars released by these enzymes (μmol/min = J) in optimal conditions from a suitable substrate: sodium salt of carboxymethylcellulose, salicin, AVICEL - microcrystalline cellulose, Whatman no.1 chromatographic paper and birchwood xylan [18].

The activity of polygalacturonase was determined by defining (according to the DNS method [16]) the amount of reducing sugars (μmol/min = J) - in relation to the amount of D-galacturonic acid - released in optimal conditions from apple pectin. Total pectinolytic activity (°PM) was determined by the measurement of the drop in apple pectin viscosity due to the effect of pectinolytic enzymes [5].

The enzymes' activity on the tested woven fabrics was determined by evaluating the change in the textile samples' mass and the change in the water absorbency of the textiles subjected to bio-pretreatment [15]. Sorption properties were also evaluated for woven fabrics made of cotton after enzymatic and alkali pretreatment. The water sorption properties of the cotton fabrics were tested using a SORP-3 sorptionmeter, an apparatus developed and constructed at the Textile Research Institute in Łódź. The evaluation of the sorption properties of the fabrics tested was based on the sorption coefficients determined by analysis of the sorption curve.

Results and Discussion

Optimisation of parameters of enzymes biosynthesis by *Aspergillus niger IBT-90*

The biosynthesis of enzymes by micro-organisms requires the elaboration of the optimal conditions for this process each time. The efficiency of enzyme biosynthesis depends on the selection of a suitable enzyme producer and process conditions. The environment in which the micro-organism grows, namely the chemical composition of cultivation culture together with cultivation process parameters such as the pH of the medium, growth time and temperature, considerably influence the results of the cultivation process. The results of the cultivation process are evaluated on the basis of the biomass efficiency or the type of biosynthesis product - namely, the enzyme and its efficiency. Mathematical methods of optimisation using multidimensional plans provide quick and effective development of the optimum values.

Table 1. *List of parameters subjected to mathematical optimisation.*

Symbol	Optimised parameters	Unit	x_o	Δx	x_g	x_d
x_1	Microcrystalline cellulose	%	1.00	0.50	1.50	0.50
x_2	Pectin	%	3.00	2.00	5.00	1.00
x_3	Malt sprouts	%	4.00	2.00	6.00	2.00
x_4	Initial pH of the medium	unit	4.5	1.0	5.5	3.5
x_5	Cultivation time	days	7	3	10	4
x_6	KH_2PO_4	g/l	6.44	3.20	9.64	3.24
x_7	$CO(NH_2)_2$	g/l	2.86	1.40	4.26	1.46
x_8	$CaCl_2 \cdot 6H_2O$	g/l	0.60	0.30	0.90	0.30
x_9	$MgSO_4 \cdot 7H_2O$	g/l	0.36	0.20	0.56	0.16
x_{10}	$FeSO_4$	mg/l	6.57	3.20	9.77	3.37
x_{11}	$CoSO_4$	mg/l	1.03	0.50	1.53	0.53
x_{12}	$MnSO_4$	mg/l	1.02	0.50	1.52	0.52
x_{13}	$ZnSO_4 \cdot 7H_2O$	mg/l	0.28	0.10	0.38	0.18
x_{14}	$CuSO_4 \cdot 5H_2O$	mg/l	1.65	0.80	2.15	0.85

The strain of the filamentous fungus *Aspergillus niger IBT-90* which up to now has been used in the research of the Institute of Technical Biochemistry for production of cellulolytic enzymes and hemicellulases was also used in this work to produce pectinolytic enzymes. It turned out that, by changing the composition of cultivation culture, this strain can be directed to the biosynthesis of pectinolytic enzymes as well. To determine the optimum conditions for this biosynthesis, the method of factorial experiments matched with the gradient optimisation of Box and Wilson [3] was applied. So, a two-level experimental plan was prepared in which each variable occurs at both levels, the top and the bottom. Twelve constituents of cultivation culture were subjected to the optimisation procedure, together with the pH of the medium and cultivation time. Table 1 presents these values. The central part of the plan shows the cultivation culture composition as used by the Institute of Technical Biochemistry for cellulolytic enzyme biosynthesis, yet with the addition of pectin which is the inducer of pectinolytic enzyme biosynthesis.

Table 2. *Plan of factorial experiment - two-layer ('-1' - parameter value, bottom level; '+1' - parameter value, top level).*

Variant number	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}	x_{11}	x_{12}	x_{13}	x_{14}
1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
2	+1	-1	-1	-1	-1	-1	+1	-1	+1	+1	-1	+1	+1	+1
3	-1	+1	-1	-1	-1	-1	+1	-1	+1	+1	+1	+1	+1	-1
4	+1	+1	-1	-1	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1
5	-1	-1	+1	-1	-1	+1	-1	-1	+1	+1	+1	+1	-1	+1
6	+1	-1	+1	-1	-1	+1	-1	+1	+1	+1	-1	-1	+1	-1
7	-1	+1	+1	-1	-1	-1	-1	+1	-1	+1	-1	+1	+1	+1
8	+1	+1	+1	-1	-1	-1	+1	+1	-1	-1	-1	+1	-1	+1
9	-1	-1	-1	+1	-1	-1	+1	+1	-1	-1	+1	+1	+1	+1
10	+1	-1	-1	+1	-1	+1	+1	+1	-1	-1	+1	-1	+1	-1
11	-1	+1	-1	+1	-1	+1	-1	-1	-1	-1	+1	+1	+1	+1
12	+1	+1	-1	+1	-1	+1	-1	-1	+1	-1	-1	-1	+1	+1
13	-1	-1	+1	+1	-1	-1	-1	-1	+1	-1	+1	+1	+1	+1
14	+1	-1	+1	+1	-1	-1	+1	-1	+1	+1	-1	-1	+1	-1
15	-1	+1	+1	+1	-1	-1	+1	-1	+1	+1	+1	-1	-1	-1
16	+1	+1	+1	+1	-1	+1	+1	+1	-1	-1	-1	-1	-1	-1
17	-1	-1	-1	-1	+1	-1	-1	-1	+1	+1	+1	+1	+1	+1
18	+1	-1	-1	-1	+1	+1	-1	+1	-1	-1	-1	+1	+1	+1
19	-1	+1	-1	-1	+1	+1	-1	+1	-1	-1	+1	+1	-1	+1
20	+1	+1	-1	-1	+1	+1	+1	+1	-1	+1	-1	-1	-1	-1
21	-1	-1	+1	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1
22	+1	-1	+1	-1	+1	-1	+1	+1	+1	+1	-1	-1	-1	-1
23	-1	+1	+1	-1	+1	-1	-1	-1	+1	+1	-1	+1	-1	+1
24	+1	+1	+1	-1	+1	+1	-1	-1	+1	+1	-1	-1	-1	-1
25	-1	-1	-1	+1	+1	+1	-1	-1	+1	+1	+1	-1	+1	-1
26	+1	-1	-1	+1	+1	+1	+1	-1	+1	-1	+1	-1	-1	-1
27	-1	+1	-1	+1	+1	-1	+1	-1	-1	-1	+1	+1	-1	+1
28	+1	+1	-1	+1	+1	-1	+1	+1	-1	-1	-1	-1	+1	-1
29	-1	-1	+1	+1	+1	-1	-1	+1	-1	-1	-1	+1	+1	+1
30	+1	-1	+1	+1	+1	+1	-1	+1	-1	-1	-1	-1	-1	+1
31	-1	+1	+1	+1	+1	+1	-1	+1	-1	-1	+1	-1	-1	-1
32	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1

After the cultivation process in stationary conditions of the selected fungus strain (according to the experiment plan presented in Table 2), the activity of the pectinolytic and cellulolytic enzymes and xylanase was determined in the culture broth produced. The results are presented in Table 3.

In variants 17 and 18, higher activities were obtained respectively for galacturonase and for total pectinolytic activity when compared to variant 33, where the parameters applied were the same as those presented in the central part of the experiment plan. Especially high polygalacturonase activities of 21.5, 22.2, 21.4, 21.2 J/ml were obtained in variants no. 29, 7, 21 and 24 respectively. The highest values of total pectinolytic activity were obtained in experiments no. 7 (778.4°PM) and no. 24 (733.8°PM).

A higher concentration than that assumed in the central part of the experiment plan (5% of pectins and 6% of malt sprouts) allows large amounts of pectinolytic enzymes to accumulate in the cultivation culture. In the cultivation culture without cellulose (no. 34) the activities of these enzymes were higher than in the cultivation culture with the same composition but with cellulose addition (no. 33). In experiment no. 35 (no pectin added), a drastic inhibition of the biosynthesis of pectinolytic enzymes was observed.

An activity analysis of cellulolytic enzymes and xylanase obtained in the factorial experiment of mathematic optimisation showed that in 6 variants of cultivation, the activities of FPA, exocellobiohydrolase and glucosidase were higher than in variant no. 33, applying the parameters as presented in the central part of the experiment plan. Now, taking into the consideration the activity of endo-1.4-β-xylanase this referred to 8 cultivation variants, and in the case of endo-1.4-β-glucanase activity this referred to 12 cultivation variants. High values of individual enzyme activities were achieved in the following cultivation variants: FPA - no. 24, 29, 32, exocellobiohydrolase - no. 23, 24, 30, β-glucosidase - no. 20, 23, 31, endo-1.4-β-glucanase - no. 24, 28, 32 and endo-1.4-β-xylanase - no. 24, 29, 30.

The longer cultivation time and the higher contents of malt sprouts in the cultivation culture result in the increased production of cellulolytic enzymes and xylanase. The highest values of optimised parameters (variant no. 31) facilitate the accumulation of enzymes with the highest values of FPA (1.15 J/ml) and endo-1.4-β-glucanase (11.8 J/ml).

The highest activities of exocellobiohydrolase (1.13 J/ml) and endo-1.4-β-xylanase (84.8 J/ml) were achieved in the cultivation culture which also has the highest concentration of cellulose, pectins, malt sprouts, potassium dihydrophosphate (V) and magnesium and iron (II) sulphates as well as the longest cultivation time. The remaining optimised parameter values should reach the lower limits of the factorial plan applied.

The highest activity of β-glucosidase (5.77 J/ml) was obtained in cultivation culture no. 20 which contained the highest (of those applied) concentrations of cellulose, pectins, potassium dihyrdophosphate, urea, calcium chloride, iron and copper sulphates which had the longest cultivation time. The remaining optimised parameters in this experiment had lower values.

Gradient optimisation of biosynthesis parameters of enzymes with high pectinolytic activity

On the basis of the results of the factorial experiment presented in Table 3, the regression coefficients (k_1) defining the linear influence of the individually tested parameters on the cultivation results were calculated. These coefficients allowed us to identify the directions and variation

ranges for each of the optimised parameters. These calculations were made to maximise the value of total pectinolytic activity. For example, the k_1 coefficient was calculated according to the formula:

$$k_1 = 1/32\ [-y_1 + \ldots\ldots + y_{14}]$$

where: y - enzyme activity value; symbol '-' or '+' - according to the matrix of planning.

Table 4 presents the values of k_1 coefficients and variation ranges of the tested parameters in gradient optimisation of the biosynthesis parameters of those enzymes with high pectinolytic activity, calculated according to the Box and Wilson method [3].

The calculated values of variation ranges, for gradient optimisation of the biosynthesis parameters of enzymes with high pectinolytic activity, indicate that in order to increase the activity of these enzymes in cultivation culture, the concentration of pectins, malt sprouts, KH_2PO_4, $CaCl_2$, $FeSO_4$, $MnSO_4$, $CuSO_4$ should be increased ('+'), the cultivation time should be prolonged, the pH of the medium should be lowered, but the concentration of microcrystalline cellulose, $CO(NH_2)_2$, $MgSO_4$, $CoSO_4$, and $ZnSO_4$ should be decreased in relation to the values of these parameters as presented in the central part of the experiment plan.

On the basis of the calculated values of variation steps, a 10-experiment plan was prepared. In these experiments, the values of individual optimised parameters in the cultivation culture composition were increased or decreased by the values of variation steps in relation to the central part of the plan. Thus, the subsequent cultivation cultures contained, e.g. 3.2%, 3.4%, etc. of pectin or 0.999%, 0.998%, etc of cellulose.

For the purposes of comparison, the cultivation was carried out in the culture parameters matching the central part of the experiment plan (variant no. 0). Variants no. 11 and 12 were also prepared in which cultivation parameters reaching the highest pectinolytic activities in factorial experiments were applied (respectively, variants no. 7 and 24 in Table 2). The activities of individual enzymes which were obtained in these variants are presented in Table 5.

The effects of gradient optimisation were as follows: pectinolytic activity increase (1.6 times) and polygalacturonase activity increase (4.2 times). Increases in cellulolytic enzymes and hemicellulases was also observed: FPA - 1.8 times, exocellobiohydrolase - 1.6 times, β-glucosidase - 2.1 times, endo-1.4-β-glucanase - 3.1 times and endo-1.4-βxylanase - 1.7 times. The highest values of all the evaluated enzymes (except for polygalacturonase) were obtained in cultivation variant no.12.

Table 3. *Enzyme activities obtained in factorial experiment.*

Cultivation variant	Total pectinolytic activity, °PM	Enzyme activity, J/ml					
		Polygalacturonase	FPA	Exo-cellobiohydrolase	β-glucosidase	Endo-1.4-β-glucanase	Endo-1.4-β-xylanase
1	380.7	10.3	0.22	0.20	1.25	1.51	16.5
2	125.1	3.2	0.14	0.02	0.92	1.69	5.6
3	383.9	8.5	0.00	0.05	1.24	2.72	7.5
4	396.4	9.0	0.17	0.08	1.53	3.54	11.8
5	243.0	14.0	0.29	0.18	1.73	3.13	21.9
6	273.3	6.4	0.21	0.17	1.73	3.03	25.4
7	778.4	22.2	0.13	0.10	1.18	4.53	28.7
8	612.8	20.9	0.16	0.09	1.31	6.56	27.5
9	113.6	0.8	0.11	0.07	1.16	2.66	5.3
10	70.8	2.4	0.04	0.07	1.06	2.38	4.3
11	400.6	14.1	0.12	0.14	1.34	1.96	12.0
12	495.4	11.3	0.20	0.17	1.75	4.81	11.2
13	449.9	10.6	0.17	0.11	1.57	3.45	16.5
14	238.4	8.2	0.18	0.19	1.84	4.75	23.8
15	238.3	5.7	0.19	0.10	1.23	4.63	8.3
16	330.2	6.8	0.20	0.00	0.84	2.55	6.2
17	284.1	15.1	0.50	0.20	2.26	3.71	18.9
18	178.2	8.6	0.54	0.24	3.14	3.41	22.5
19	454.1	11.4	0.50	0.57	4.90	7.79	27.3
20	481.1	10.2	0.60	0.42	5.77	6.09	20.2
21	294.4	11.6	0.66	0.57	2.82	6.32	64.0
22	308.4	11.1	0.84	0.53	3.65	5.58	74.5
23	612.8	13.1	0.63	0.90	5.71	7.07	60.2
24	733.8	21.2	1.04	1.13	5.42	10.66	84.8
25	208.2	9.7	0.30	0.23	2.56	3.66	25.8
26	55.5	4.4	0.18	0.28	2.79	3.81	19.7
27	242.3	12.0	0.52	0.40	4.60	6.97	29.2
28	267.9	12.3	0.50	0.45	5.04	6.85	25.6
29	438.1	24.5	1.26	0.83	3.46	9.88	75.9
30	314.9	14.1	0.90	1.00	3.91	7.47	84.4
31	650.3	19.2	0.99	0.78	5.45	7.97	64.3
32	587.9	21.4	1.15	0.56	4.18	11.18	72.4
33	314.9	11.1	0.70	0.65	4.68	5.95	52.2
34	390.6	14.7	0.53	0.67	3.75	3.99	44.3
35	12.2	5.5	0.17	0.26	1.32	1.52	33.0

The influence of the type of inductors in cultivation culture on enzyme biosynthesis by *Aspergillus niger IBT-90*

Pectinolytic, cellulolytic enzymes and hemicellulases belong to a group of induced enzymes. The presence of their inductors in cultivation cultures is thus very important. Table 6 presents the inductors of the tested enzyme biosynthesis applied in this work. Table 7 presents the enzyme activities achieved on cultivation culture compositions as described above, containing changing amounts of these inductors. This cultivation had a longer biosynthesis time (10 days), as this helps to accumulate greater amounts of cellulases and hemicellulases. The increased addition of evening primrose cake, carrot pomace and sesame hulls results from the lower contents of cellulose in these leftovers as compared to cotton dust and the microcrystalline cellulose AVICEL; sesame hulls were used as a product containing 50% of water.

Sesame hulls turned out to be the best inductor of enzymes produced by *Aspergillus niger IBT-90*. This inductor allowed pectinolytic activity to reach 715.1°PM, which was higher than the ac-

tivity obtained for the cultivation culture with microcrystalline cellulose (as used in the mathematical optimisation). The presence of sesame hulls in cultivation culture is beneficial for the biosynthesis of cellulases and hemicellulases. The activities of these enzymes increased by the following amounts when compared to the cultivation culture with cellulose: FPA - 1.7 times, exocellobiohydrolase - 5.2 times, β-glucosidase - 2.3 times, endo-1.4-β-glucanase - 1.7 times and endo-1.4-β-xylanase - 2.4 times. However, for the same cultivation culture lower polygalacturonase activity was obtained (16.9 J/ml) when compared to the cultivation culture with cellulose. Moreover, it was demonstrated that the elimination of pectin from the cultivation culture (variant no. 2, 4 and 6) clearly inhibits the biosynthesis of pectinolytic enzymes.

Table 4. *Values of variation steps of tested parameters in gradient optimisation of enzyme biosynthesis by Aspergillus niger IBT-90.*

Symbol	k_i	Δx	$k_i\Delta x$	Steps
x_1	-2.270	0.5%	-1.135	-0.001
x_2	+115.291	2%	+230.582	+0.200
x_3	+80.218	2%	+160.436	+0.139
x_4	-44.941	1	-44.941	-0.039
x_5	+18.153	4 dni	+72.612	+1.500
x_6	+3.273	3.2 g/l	+10.474	+0.009
x_7	-67.158	1.4 g/l	-94.021	-0.082
x_8	+20.809	0.3 g/l	+6.243	+0.005
x_9	-11.684	0.2 g/l	-2.337	-0.002
x_{10}	+22.881	3.2 mg/l	+73.219	+0.064
x_{11}	-46.750	0.5 mg/l	-23.375	-0.020
x_{12}	+23.608	0.5 mg/l	+11.804	+0.010
x_{13}	-32.973	0.1 mg/l	-3.297	-0.003
x_{14}	+31.860	0.8 mg/l	+25.488	+0.002

Table 5. *Enzyme activities obtained in gradient experiment.*

Cultivation variant	Total pectinolytic activity, °PM	Enzyme activity, J/ml					
		Polygalacturonase	FPA	Exo-cellobiohydrolase	β-glucosidase	Endo-1.4-β-glucanase	Endo-1.4-β-xylanase
0	386.5	3.9	0.41	0.72	3.56	3.40	36.5
1	409.1	4.2	0.66	0.59	4.31	4.35	36.4
2	457.3	4.9	0.56	0.47	4.01	3.55	34.5
3	534.1	3.8	0.45	0.54	3.45	3.97	30.7
4	558.9	4.2	0.52	0.55	3.98	4.04	21.3
5	381.8	4.5	0.44	0.58	3.98	5.04	36.3
6	451.0	4.4	0.62	0.79	4.37	4.85	35.3
7	389.8	5.6	0.49	0.70	4.25	5.35	37.1
8	441.7	6.2	0.53	0.81	4.61	5.80	43.4
9	558.9	6.7	0.36	0.69	4.03	6.12	39.3
10	387.4	7.8	0.39	0.81	5.06	7.97	45.0
11	536.8	16.3	0.34	0.30	1.28	2.92	11.5
12	619.4	5.0	0.74	1.17	7.62	10.45	62.3
13	8.23	1.5	0.17	0.12	1.44	1.50	13.6

Table 6. *List of inductors applied in enzyme biosynthesis by Aspergillus niger IBT-90.*

Cultivation variant	Inductor type and its amount, %			
1	Microcrystalline cellulose	1.5	Apple pectin	5
2	Wheat dietary fibre	6.5	-	-
3	Wheat dietary fibre	1.5	Apple pectin	5
4	Oat dietary fibre	6.5	-	-
5	Oat dietary fibre	1.5	Apple pectin	5
6	Apple dietary fibre	6.5	-	-
7	Apple dietary fibre	1.5	Apple pectin	5
8	Evening primrose cake	3.0	Apple pectin	5
9	Carrot pomace	3.0	Apple pectin	5
10	Sesame hulls	6.0	Apple pectin	5
11	Cotton dust	1.5	Apple pectin	5

The influence of *Aspergillus niger IBT-90* cultivation on enzyme biosynthesis

One of the crucial factors determining the process of enzyme biosynthesis by micro-organisms is the very method of this biosynthesis. Hence, the influence of cultivation conditions - stationary or dynamic - on the intensity of enzymes biosynthesis by applied fungus was evaluated during a 10-day cultivation on a selected culture containing 5% of apple pectin and various sources of carbon (see Table 8).

The results presented in Table 8 demonstrate the predominant role of the stationary method of *Aspergillus niger IBT-90* cultivation over the dynamic method in enzyme biosynthesis. Furthermore, the change of cultivation conditions from stationary to dynamic decreased the activities of the pectinolytic enzymes produced (by 24.5 times) and polygalacturonase (by 4.5 times). However, the choice of cultivation method to accumulate cellulolytic enzymes in cultivation culture should depend on the carbon source in this culture. Most applied carbon sources turned out to be more efficient in cellulolytic enzyme biosynthesis performed in the stationary cultivation of the selected fungus (up to three times more activity as compared to the dynamic method). The application of carrot pomace requires cultivation to be performed on a shaker.

The influence of the concentration of sesame hulls in cultivation culture on enzyme biosynthesis by *Aspergillus niger IBT-90*

A detailed evaluation of the influence of sesame hulls on the tested enzyme biosynthesis required a suitable selection of their quantity in the applied fungus cultivation culture. Table 9 presents the quantities of used sesame hulls in cultivation culture and the activities of enzymes produced in these cultures.

The presence of 4-6% of sesame hulls in the cultivation culture facilitates the accumulation of pectinolytic enzymes. However, lower concentrations of sesame hulls (2%)

facilitate the biosynthesis of most cellulolytic enzymes and xylanase. Only high FPA enzymes are especially accumulated in the cultivation culture which contains the highest concentrations of sesame hulls (10%).

Evaluation of the application of *Aspergillus niger IBT-90* enzymes in the pretreatment of woven fabrics made of cotton fibres

The research performed demonstrates that the optimum activity of applied system of pectinolytic and cellulolytic enzymes from *Aspergillus niger IBT-90* in the pre-treatment of cotton fabrics is obtained in the range of pH 4.6-4.8. The ability of liquid sorption is an essential parameter of flat textile fabrics, not only in evaluating wearing properties but also in the process of textile finishing. The analysis of the sorption coefficients obtained for cotton woven fabric allows us to state that the fabric after enzymatic treatment is characterised by higher sorption values when compared to the fabric after traditional alkali treatment. At the same time, water sorption rate is lower. The biotreatment of cotton woven fabrics using varied amounts of pectinolytic enzymes (from 1518 to 3163°PM/g woven fabric) did not result in differing sorption properties of the variants tested (Figure 1).

Figure 1. *H_2O sorption kinetics curve - cotton fabrics after pectinolytic enzymes and alkali treatment.*

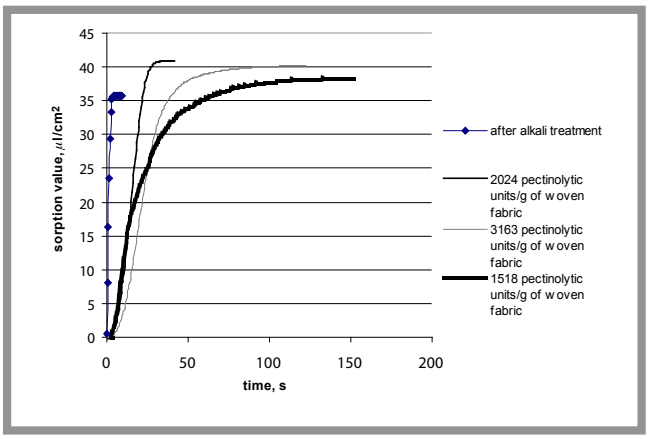

Table 7. *The influence of inductor type in cultivation culture on enzyme biosynthesis by Aspergillus niger IBT-90.*

Cultivation variant acc. to Table 6	Total pectinolytic activity, °PM	Enzymes activities, J/ml					
		Polygalactu-ronase	FPA	Exo-cellobio-hydrolase	β-glucosi-dase	Endo-1.4-β-glucanase	Endo-1.4-β-xylanase
1	683.9	24.8	0.47	0.26	4.99	5.87	33.9
2	63.2	31.5	0.82	0.65	3.88	7.56	62.0
3	584.9	29.8	0.37	0.27	5.63	5.35	39.2
4	115.4	23.1	0.52	0.64	7.05	6.83	79.6
5	247.3	25.6	0.53	0.93	9.80	9.85	70.5
6	50.0	35.1	0.34	0.67	3.93	6.54	78.8
7	584.9	31.1	0.17	0.43	6.78	5.22	32.0
8	658.3	23.4	0.47	1.15	8.28	6.85	54.1
9	588.7	24.5	0.24	0.52	4.97	5.73	25.7
10	715.1	16.9	0.81	1.03	9.91	8.54	82.3
11	523.8	15.9	0.38	0.51	7.28	6.45	51.4

Table 8. *The influence of Aspergillus niger IBT-90 cultivation method on enzyme biosynthesis.*

Type and amount of carbon source, %	Cultivation method	Total pectinolytic activity, °PM	Enzyme activity, J/ml					
			Polygalactu-ronase	FPA	Exo-cellobio-hydrolase	β-glucosi-dase	Endo-1.4-β-gluca-nase	Endo-1.4-β-xyla-nase
Micro-crystalline cellulose - 1.5	stationary	683.9	24.8	0.47	0.26	4.99	5.87	33.9
	dynamic	28.2	9.9	0.32	0.35	5.13	2.87	44.1
Evening primrose cake - 3.0	stationary	658.3	23.4	0.47	1.15	8.28	6.85	54.1
	dynamic	51.7	11.5	0.32	0.65	6.49	5.87	34.9
Carrot pomace - 3.0	stationary	588.7	24.5	0.24	0.52	4.97	5.73	25.7
	dynamic	111.9	6.7	0.71	0.84	7.02	5.78	31.4
Sesame hulls - 6.0	stationary	715.1	16.9	0.81	1.03	9.91	8.54	82.3
	dynamic	41.0	3.8	0.38	0.68	6.38	5.59	35.4

Table 9. *The influence of sesame hull concentration in cultivation culture on enzyme biosynthesis by Aspergillus niger IBT-90.*

Sesame hulls concentration, %	Total pectinolytic activity, °PM	Enzyme activity, J/ml					
		Polygalactu-ronase	FPA	Exo-cellobio-hydrolase	β-glucosi-dase	Endo-1.4-β-glucanase	Endo-1.4-β-xylanase
2	669.9	27.0	0.77	0.68	4.19	6.31	45.4
4	783.5	28.2	0.72	0.76	3.85	4.91	33.2
6	600.4	37.9	0.73	0.72	4.02	5.12	35.8
8	669.9	26.3	0.70	0.58	3.35	5.49	31.5
10	699.2	17.1	0.90	0.48	3.65	4.18	35.0

Conclusions

- As the result of mathematical optimisation, the best conditions for directed pectinolytic biosynthesis by the filamentous fungus *Aspergillus niger IBT-90* are as follows: apple pectin - 5%, malt sprouts - 6%, sesame hulls - 4-6%; pH value before cultivation process - 3.5, cultivation time - 10 days; concentration in g/l: KH_2PO_4 - 9.64, $CO(NH_2)_2$ - 1.46, $CaCl_2 \cdot 6H_2O$ - 0.30, $MgSO_4$ - 0.56, and concentration in mg/l: FeSO4 - 9.78, $CoSO_4$ - 0.54, $MnSO_4$ - 0.52, $ZnSO_4 \cdot 7H_2O$ - 1.8, $CuSO_4 \cdot 5H_2O$ - 0.86.
- Of all the carbon sources applied in pectinolytic enzyme biosynthesis, namely microcrystalline cellulose AVICEL, evening primrose cake, carrot pomace, sesame hulls and cotton dust, the best were sesame hulls. The defined amount in cultivation culture should reach 4-6%.
- Of the two methods of *Aspergillus niger IBT-90* cultivation - stationary or dynamic - better results were obtained by fungus surface cultivation, with a

pectinolytic activity 5.3 to 24.3 times higher.

- As the result of optimisation, the highest enzyme activities in *Aspergillus niger IBT-90* culture broth medium reached total pectinolytic activity 817.7°PM and in J/ml polygalcturonase - 37.9, FPA - 1.15, exocellobiohydrolase - 1.36, β-glucosidase - 11.28, endo-1.4-β-glucanase - 11.8 and endo-1.4-β-xylanase - 84.8.
- As the result of mathematical optimisation of tested enzyme biosynthesis parameters, the following increases were observed: pectinolytic activity - 2.6 times, polygalacturonase activity - 3.4 times, FPA - 1.6 times, exocellobiohydrolase - 2.1 times, β-glucosidase – 2.4 times, endo-1.4-β-glucanase - 1.9 times and endo-1.4-β-xylanase - 1.6 times.

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