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Optimization of the simultaneous production of cellulase and xylanase by submerged and solid-state fermentation of wheat chaff

MIRJANA JOVANOVIĆ[1], DAMJAN VUČUROVIĆ[1]*, BOJANA BAJIĆ[1], SINIŠA DODIĆ[1], VANJA VLAJKOV[1] and RADA JEVTIĆ-MUČIBABIĆ[2]

[1]*University of Novi Sad, Faculty of Technology Novi Sad, Department of Biotechnology and Pharmaceutical Engineering, Bulevar cara Lazara 1, Novi Sad, Serbia and* [2]*University of Novi Sad, Institute of Food Technology Novi Sad, Bulevar cara Lazara 1, Novi Sad, Serbia*

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Abstract: Wheat chaff as an agricultural waste represents a cheap raw material for biotechnological processes. With its lignocellulosic composition, it is suitable for producing hydrolytic enzymes for second generation renewable fuel production technologies. The aim of this work was to optimize the process parameters (cultivation temperature 25–35 °C, pH value 4–6 and cultivation time 3–7 days) of the cultivating fungi (*Trichoderma reesei* QM 9414) on a media based on wheat chaff by submerged and solid-state techniques, in order to enhance and compare the two types of simultaneous cellulase and xylanase production. Optimal conditions for the submerged fermentation were 29.65 °C for temperature, pH 4.27 and 7 days of cultivation, while for the solid-state fermentation, the optimal conditions were 28.01 °C, pH 6.00 and 7 days. The cellulolytic and xylanolytic activities of the obtained cultivation broth filtrates were 0.0535 and 0.1676 U mL^{-1} for the submerged fermentation, and 0.0407 and 0.1401 U mL^{-1} for the solid-state fermentation, respectively, and with a 26.77 and 13.39 % enhancement of enzyme activity for submerged fermentation, and a 22.96 and 42.66 % enhancement for solid-state fermentation, respectively, compared to the results obtained before optimization.

Keywords: agricultural waste; lignocellulosic feedstock; fungi; hydrolytic enzymes; statistical analysis; enzyme activity.

INTRODUCTION

Progress in science and medicine, together with the developments in the industry sector and the advancement of agricultural production has enabled a better and longer life of people. On the other hand, the explosion in the human population and the conscienceless behaviour of the majority has led to problems

* Corresponding author. E-mail: dvdamjan@uns.ac.rs
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such as the increased need for food, energy and space, altered or destroyed numerous ecosystems, increased quantities of accumulated waste and consequently, increased health and safety risks.

In order to overcome these problems, the world today is striving towards sustainable development, which implies the recycling of waste materials and the use of renewable resources - water, air and biomass, alternative energy sources and cleaner, environmentally friendly production.[1] The necessary chemicals can be obtained from biomass by fermenting the sugar substrate or by chemical synthesis of fermentation products, which makes every material that contains sugars usable in biotechnological production.[2]

Efficient production of fermentable biomass hydrolysates is one of the main conditions for the economically competitive production of numerous biotechnology products from renewable raw materials.[3] Intensive research is currently focused on improving the hydrolytic degradation of biomass.[4] These efforts include improvements in the technology of biomass pre-treatment and the production of hydrolytic enzymes that catalyse the conversion of complex sugars to free and fermentable ones.[5,6]

There is no production of hydrolytic enzymes in Serbia but they are imported from abroad for appropriate purposes, although the region is abundant with raw materials for enzyme production.[7] Wheat chaff, as a by-product of wheat processing, is obtained at the very beginning of this process and, accordingly, the cost of its production is small.[8] With its composition,[9] wheat chaff represents a very attractive raw material for the production of enzymes. On the other hand, the previous use of this by-product of wheat processing was only as a food for livestock.[10] Therefore, the question arises of the possibility of obtaining greater economic and environmental profit by using the raw material for the production of high-value products, such as enzymes, with the valorisation of other process outputs in order to achieve a concept of cleaner production, *i.e.*, the zero-emission concept.[11]

The implementation of the technology for the production of enzymes from wheat chaff on industrial scales requires that this process primarily be optimised at the laboratory level.[12] In order to optimize the process, it is necessary to study the enzyme production in detail by cultivating fungi on the by-products of wheat processing under different process parameters.[13] Process optimization can contribute to understanding the different operating conditions and the interactions of the examined variables on the bioprocess of cultivation on the given biomass.

Since the process of enzyme production from wheat chaff is insufficiently explored, there is a need to determine the optimal process parameters for obtaining enzymes by cultivating fungi on nutrient media based on wheat chaff, with the aim of producing enzymes from the given by-products.

EXPERIMENTAL

Producing microorganism

To test the production of cellulolytic and xylanolytic enzymes, the producing strains used were *Trichoderma reesei* QM 9414, *Aspergillus* spp. and *Penicillium* spp., which are kept in the collection of cultures at the Faculty of Technology Novi Sad, Serbia. Refreshing of the fungi was realised on potatoes dextrose agar (PDA) by incubating them for 3–4 days at 28 °C.

The inoculation of the nutrient media was performed with a pre-prepared spore suspension in a sterile saline solution containing 10^6 spores g^{-1}. For the purpose of initial testing or selection of the producing strain, 10 % of the inoculum was added to the liquid substrates, and for the solid-state substrates, the same volume of the spore suspension was sprayed over their surface.

Media preparation

For the purpose of the research, the by-product of wheat processing (wheat chaff) was used to prepare nutrient media. The raw material was obtained from a local wheat processing plant (mill) "Žitopromet-Mlin" a.d., Senta, Serbia.

The composition of liquid substrates for the submerged cultivation technique (SmF) on wheat chaff with the aim of selecting the producing strain was 3 g of wheat chaff, 0.5 % $(NH_4)_2SO_4$ and 1.36 % K_2HPO_4 in 100 mL of distilled water.

For cultivation on solid substrates (SSF) with the aim of selecting the producing strain, the same amount of raw material (3 g) was suspended in the same amount (100 mL) of an aqueous solution containing 0.5 % $(NH_4)_2SO_4$ and 1.36 % K_2HPO_4, as for the liquid media. After 15 min of mixing, the pH value was checked and corrected to 4.5±0.1 by adding 1 % NaOH or 1 % H_2SO_4. After an additional 15 min of stirring, the suspension was allowed to stand so that the solid phase could settle in the gravitational field. The liquid phase was decanted and the residue used as a solid substrate for the production of enzymes. In this way, enzymes were produced from the same amount of used raw material, *i.e.*, 3 g of wheat chaff, as well as the same preparation method (100 mL of prepared salt solution), so that the obtained results were comparable.[14]

Sterilization of the prepared media was performed in an autoclave at a temperature of 121 °C and a pressure of 2.1 bar for 20 min.

Cultivation conditions

Production of enzymes by fungi cultivation for both submerged and solid-state techniques was realised in 300 ml Erlenmeyer flasks. Initial trials on choosing the fungi strain were performed for 7 days at a temperature of 28±1 °C. For optimization purposes, the examined process factors were varied in the following ranges: temperature 25–30 °C, pH 4–6 and cultivation time 3–7 days.

Sample preparation and analysis

After cultivation on solid media, the products of strain metabolism were extracted with 100 mL 0.1 M acetate buffer (pH 5.0) with constant mixing at 200 rpm during 30 min at a constant temperature, in order to equalise the liquid volume with the submerged cultivation broths.

Separation of solid and liquid phase after extraction of solid media as well as the submerged cultures was performed by filtration through a qualitative filter paper. The obtained filtrates were subjected to standard analysis of the cultivation media.

The intensity of hydrolytic action of the cultivation liquids and solid extracts towards cellulose and xylanase were assayed separately for each substrate by measuring the release of reducing sugars using 3,5-dinitrosalicylic acid (DNS) method.[15]

Enzyme assay for cellulase activity

The substrate for cellulase activity estimation was a 1 % solution of a soluble carboxymethylcellulose (CMC) in a 0.1 M acetate buffer with a pH value of 5.5. Full dissolution of carboxymethylcellulose was achieved by cooking this solution in a boiling water bath. The obtained carboxymethylcellulose solution was mixed with a liquid phase of submerged fermentation or a solid-state fermentation extract in a volume ratio of 3:1. A 0.1 M acetate buffer with a pH value of 5.5 was used for a blank test. After homogenization, the hydrolysis was performed for 30 min at 45 °C in a tempered water bath. Finally, after hydrolysis and the DNS method, one unit (U) of cellulase activity was defined as the amount the enzyme that liberated 1 μmol of reducing sugar as glucose mL^{-1} min^{-1} under the assay conditions.

Enzyme assay for xylanase activity

The substrate for assessing the xylanase activity was a 1 % solution of xylan in 0.05 M acetate buffer at a pH value of 4.5. Complete dissolution of xylan was achieved by cooking this solution in a boiling water bath. Then the solution was mixed with a liquid phase from submerged fermentation or a solid-state fermentation extract in a ratio of 3:1. For a blank test, 0.05 M acetate buffer with a pH value of 4.5 was used. After homogenization, the hydrolysis was performed for 60 minutes at 45 °C in a tempered water bath. Finally, after hydrolysis and the DNS method, one unit (U) of xylanase activity was defined as the amount the enzyme that liberated 1 μmol of reducing sugar as xylose mL^{-1} min^{-1} under the assay conditions.

Statistical optimization

All the results presented in this paper represent the mean values from three experiments repeated under the same conditions. The results were statistically analysed by variance analysis in the degree of significance $\alpha = 0.05$. The adequacy of the model was estimated based on the determination coefficient (R^2) and the *p*-value of the model. The response surface methodology (RSM) method is a modelling method suitable for studying the impact of multiple factors (temperature, pH and cultivation time) on responses (cellulase and xylanase activity) by their simultaneous variation with the implementation of a limited number of experiments. This is precisely the reason why this method was used to optimize the conditions of production of enzymes by submerged and solid-state cultivation techniques from wheat chaff.

Statistical processing of experimental data was performed using the software package Statistica 13.5 (StatSoft, USA). The significance of the influence of each of the factors and their interactions were determined by comparing the *p*-values for each of the coefficients in the regression equation. To optimize the factors, the desired (required) function method was applied in the software package Design-Expert 7.0 (StatEase, Inc., USA).

RESULTS AND DISCUSSION

Strain selection

The results of enzyme activity in the filtrates obtained after cultivating *Trichoderma reesei* QM 9414, *Penicillium* spp., and *Aspergillus* spp. on wheat chaff based media by SmF and SSF are given in Table I. Based on the results for enzyme activity in the filtrates after cultivating the three types of fungi on wheat chaff, it could be concluded that higher values could be obtained by the SmF

compared to the SSF method. In terms of cellulose activity, the highest value was obtained for *Trichoderma reesei* QM 9414, followed by *Aspergillus* spp. and finally *Penicillium* spp. for both SmF and SSF. On the other hand, the highest xylanase activity was obtained using *Trichoderma reesei* QM 9414, followed by *Penicillium* spp. and finally *Aspergillus* spp. for both SmF and SSF. Since *Trichoderma reesei* QM 9414 gave the highest results for both examined enzymes activities and cultivation techniques, this fungus was chosen as the producing strain for the optimization process.

TABLE I. Cellulase and xylanase activity of the filtrates after cultivating three different types of fungi on wheat chaff-based media by the submerged and solid-state technique

Producing strain	Cellulase activity, U mL^{-1}		Xylanase activity, U mL^{-1}	
	SmF	SSF	SmF	SSF
Penicillium spp.	0.0118	0.0031	0.1413	0.0772
Trichoderma reesei QM 9414	0.0422	0.0331	0.1478	0.0982
Aspergillus spp.	0.0351	0.0227	0.1259	0.0580

Analysing Table I, it can be seen that the xylanase activity had higher values compared to the cellulase activity for the observed producing strain and cultivation technique. This fact is in accordance with previously published results of Hirasawa.[17] The obtained results in Table I are comparable to literature data since Mihajlovski[13] reported a cellulase activity of 0.405 U mL^{-1} after cultivating *Paenobacillus chitinolyticus* CKS1 in a barley bran liquid medium. As much higher levels of enzyme (cellulase and xylanase) activity were previously obtained,[18,19] there is a need for process optimization.

Optimization of the process parameters

In the case of enzyme biosynthesis by any cultivation technique, the composition of the nutrient medium, the method of preparation and inoculum dosing, as well as the process parameters must be optimal for the selected produced microorganism.[20] When optimizing bioprocesses, the application of RSM is of great importance.[21] For this reason, Box–Behnken design (BBD) within RSM was used to determine the maximum cellulase and xylanase activity that could be obtained from cultivating *Trichoderma reesei* on wheat chaff based media by SmF and SSF by analysing the effect of the three different process parameters (Table II).

In order to describe the response functions for cellulase and xylanase activities, the results in Table II were fitted into second order polynomial equations of multiple regression analysis and the obtained equations are presented together with the analysis of variance ANOVA in Table III. The software suggested a quadratic model as the most suitable for describing all the responses:

$$Y = x + aA + bB + cC + a^2A^2 + b^2B^2 + c^2C^2 + abAB + acAC + bcBC \quad (1)$$

where Y is the response, x is the intercept, while A (cultivation temperature), B (pH value) and C (cultivation time) are independent variables with their appropriate coefficients a, b and c (linear: a, b and c; quadratic: a^2, b^2 and c^2; interaction: ab, ac and bc), respectively.

TABLE II. Experimental plan derived from BBD for the examined process variables and responses of SmF and SSF of wheat chaff

Run	Variable			Activity, U mL^{-1}			
				Responses			
				SmF		SSF	
	A t / °C	B pH	C Cultivation time, day	Y_1 cellulase	Y_2 xylanase	Y_1 cellulase	Y_2 xylanase
1	30	6	3	0.0222	0.1214	0.0204	0.0916
2	30	5	5	0.0416	0.1341	0.0104	0.1074
3	30	4	7	0.0514	0.1720	0.0241	0.1312
4	35	4	5	0.0149	0.0171	0.0099	0.0858
5	30	5	5	0.0414	0.1570	0.0107	0.1224
6	25	5	7	0.0458	0.0617	0.0158	0.1316
7	25	6	5	0.0137	0.0219	0.0047	0.0920
8	30	4	3	0.0165	0.1278	0.0168	0.0858
9	35	5	3	0.0077	0.0126	0.0497	0.0450
10	35	6	5	0.0092	0.0118	0.0134	0.0689
11	25	5	3	0.0096	0.0096	0.0124	0.0275
12	25	4	5	0.0147	0.0189	0.0068	0.0812
13	30	6	7	0.0398	0.1491	0.0378	0.1089
14	35	5	7	0.0195	0.0164	0.0127	0.1126
15	30	5	5	0.0383	0.1466	0.0119	0.1153

TABLE III. Regression equation coefficients and their p-values for the modelled responses of hydrolytic enzymes production from wheat chaff; Y_1: cellulase activity, U mL^{-1}; Y_2: xylanase activity, U mL^{-1}; A: cultivation temperature, °C; B: pH value, and C: cultivation time, days

Effect	Regression coefficients				p-value			
	SmF		SSF		SmF		SSF	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Intercept	0.0400	0.1500	0.0110	0.1200	–	–	–	–
Linear								
A	–0.0041	–0.0067	0.0057	–0.0025	0.0351	0.1140	0.1715	0.7133
B	–0.0015	–0.0039	0.0023	–0.0028	0.3156	0.3129	0.5464	0.6799
C	0.0130	0.0160	–0.0011	0.0290	0.0003	0.0063	0.7693	0.0060
Quadratic								
A^2	–0.0200	–0.0120	–0.0022	–0.0290	0.0002	<0.0001	0.6940	0.0274
B^2	–0.0077	–0.0055	–0.00005	–0.0039	0.0138	0.3396	0.9928	0.6936
C^2	–0.0002	0.0021	0.014.	–0.0067	0.9173	0.6926	0.0475	0.5067
Interaction								
AB	–0.0012	–0.0021	0.0014	–0.0069	0.5787	0.6946	0.7946	0.4804
AC	–0.0061	–0.0120	–0.0100	–0.0091	0.0288	0.0606	0.1053	0.3620

TABLE III. Continued

Effect	Regression coefficients				*p*-value			
	SmF		SSF		SmF		SSF	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
	Interaction							
BC	–0.0043	–0.0041	0.0025	–0.0070	0.0822	0.4486	0.6425	0.4742
Model					0.0016	0.0001	0.0034	0.0082
Lack of fit					0.1221	0.6729	0.3112	0.1005

According to ANOVA, the significant model terms are those whose *p*-value is lower than 0.05.

For the cellulase activity in the media filtrates obtained after cultivating *Trichoderma reesei* on wheat chaff *via* SmF, the significant model terms are *A*, *C*, *AC*, A^2 and B^2, while for the xylanase activity of the same samples, the significant model terms are *C* and A^2. The significant model term for the response cellulase activity for SSF enzyme production is only the term C^2, while for the response xylanase activity significant terms are *C* and A^2. From this analysis it could be concluded that the most dominant factor is *C* (cultivation time), then *A* (cultivation temperature) and finally *B* (pH value). In addition, the significant terms for the response xylanase activity are the same for both SmF and SSF. A graphical display of the effects of examined parameters and their significance on the responses can be seen in Fig. 1.

The types of lines in a perturbation graph are directly related to the type of effect the parameter has on the response. For example, if the line is linear (straight line), then the linear form of this parameter has a significant influence on the response. This can be observed for parameter *C* in Fig. 1A, B and D. In other words, by increasing the value of parameter *C* (cultivation time), the enzyme activity of cellulase of both SmF and SSF and xylanase activity of SmF increase. Secondly, if a line has the form of a parable, then the quadratic term of this parameter has a significant effect on the response. Observing Fig. 1, this could be seen for parameter *A* in Fig. 1A, B and D, and also for parameter *C* in Fig. 1C. Practically this means that the hydrolytic activities of the examined enzymes have a maximum value somewhere in the examined range of the mentioned parameters. *T. reesei* enzymes are thermo-labile compounds,[22] *i.e.*, their structure and activity depend on the temperature of their surroundings,[23] it is not surprising that this parameter has such an effect on the responses. Likewise, the producing strain enhances its enzyme synthesis depending on its phase of growth (lag, exponential, *etc.*)[24] and for this reason, there is an optimum cultivation time when the enzyme activity is at its top. Finally, if the lines of two parameters intersect twice at different point of the observed interval, these two parameters have a joint influence on the response. This interaction of parameters was affirmed only for parameters *A* and *C* in Fig. 1A.

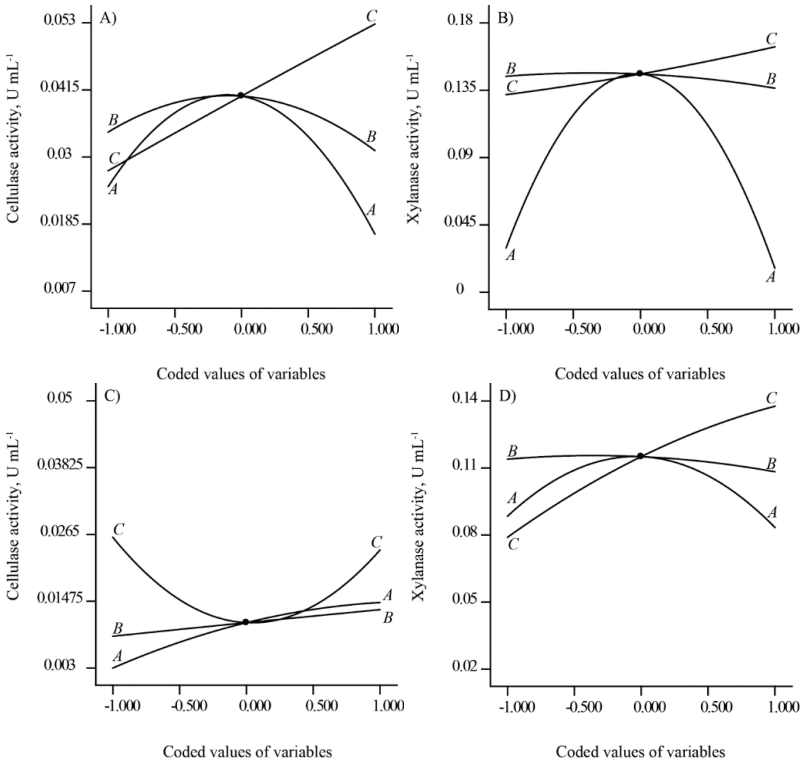

Fig. 1. Perturbation graphs for analysing the effect of the examined parameters (*A*: cultivation temperature, °C; *B*: pH value, 1 and *C*: cultivation time, days) on responses for: A) cellulase activity after SmF; B) xylanase activity after SmF; C) cellulase activity after SSF; D) xylanase activity after SSF.

According to Table III, the *p*-values ($p < 0.05$) of the models and the lack of fit values ($p > 0.05$) showed that the models are significant and in the observed form (quadratic) are suitable for predicting the production of enzymes by *Trichoderma reesei* from wheat chaff for both SmF and SSF. The regression coefficients (R^2) of 0.9756, 0.9917, 0.9438 and 0.9687 for cellulase activity after SmF, xylanase activity after SmF, cellulase activity after SSF and xylanase activity after SSF, respectively, indicate the good correlation between the experimentally obtained data and values predicted by the models (Fig. 2). Likewise, the correlation of the examined responses (cellulase and xylanase activity) was obtained from modelling software and their values are 0.897 and 0.884 for SmF and SSF, respectively.

The general approach in the desirability function method consists in converting individual responses into desired functions the values of which range from 0 to 1 (Fig. 3). The value of the individual desired function "0" is the worst value, while the value "1" represents the best value of the observed response.[25] For the

SmF, the maximal value of the desirability function of 0.987 can be obtained if the temperature is ranged between 28.43 and 30.82 °C and the pH value between 4.15 and 4.32. In order to obtain a maximal enzyme activity by SSF, the cultivation temperature should be between 27.56 and 28.42 °C, while the pH value should be kept at 6. Under these conditions, the value of the desirability function is 1.000.

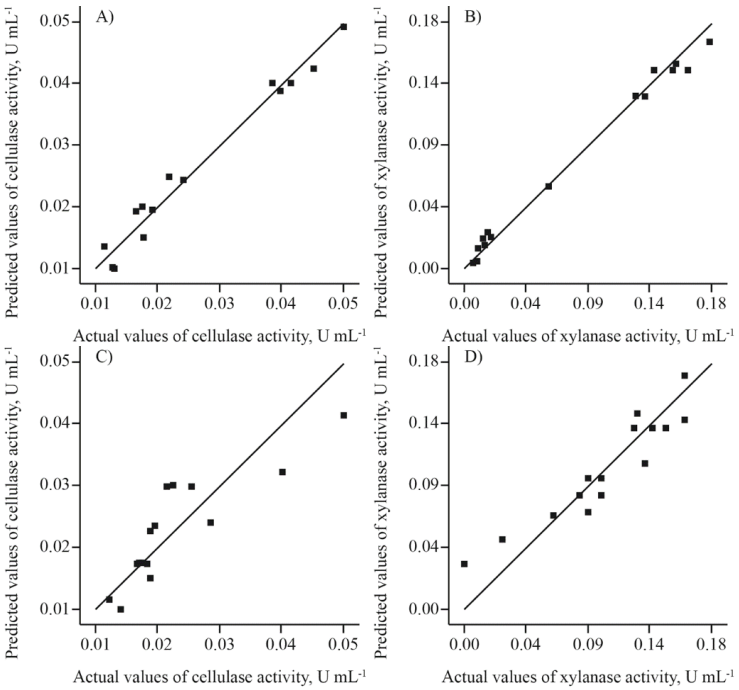

Fig. 2. Plots of model predicted *vs*. actual experimental results of the responses: A) cellulase activity after SmF; B) xylanase activity after SmF; C) cellulase activity after SSF;D) xylanase activity after SSF.

In order to determine the optimal setting of examined parameters for obtaining the highest values of the responses, the desirability function was used. Initial criteria were that the variables stay in the examined range, while the activities of cellulase and xylanase should reach their maximum value. The results of this desirability function analysis are given in Table IV and shown in Fig. 3. Comparing the determined enzyme activities from Tables I and IV, it can be concluded that there is an increase, *i.e.*, enhancement of 26.77, 13.39, 22.96 and 42.66 % in terms of cellulase activity after SmF, xylanase activity after SmF, cellulase activity after SSF and xylanase activity after SSF, respectively, when comparing the results before and after the optimisation process.

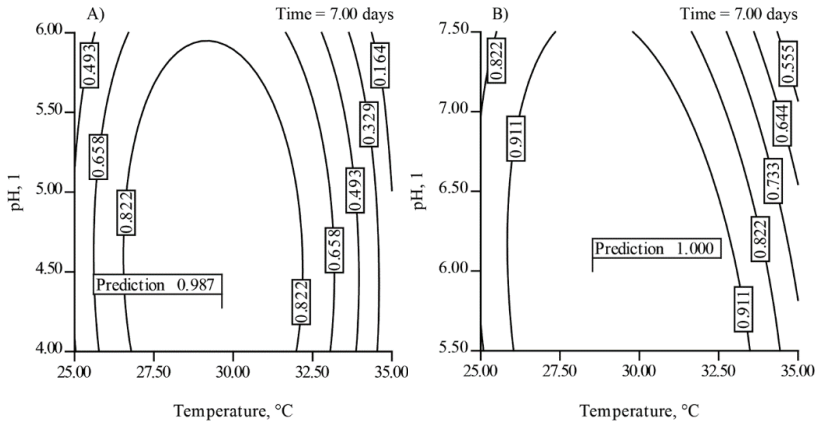

Fig. 3. Desirability function graph for cellulase and xylanase activity as a function cultivation temperature, pH value and cultivation time for A) SmF and B) SSF of wheat chaff.

TABLE IV. Optimum point prediction of the parameters *A*: cultivation temperature, °C; *B*: pH value, 1 and *C*: cultivation time, days by the desirability function method for Y_1: cellulase activity, U mL^{-1} and Y_2: xylanase activity, U mL^{-1} after SmF and SSF of wheat chaff; optimization model validation

Factor	SmF			SSF		
	Point	Y_1 / U mL^{-1}	Y_2 / U mL^{-1}	Point	Y_1 / U mL^{-1}	Y_2 / U mL^{-1}
A / °C	29.65	0.0535	0.1676	28.01	0.0407	0.1401
B	4.27			6.00		
C / day	7.00			7.00		
Validation		0.0549	0.1703		0.0455	0.1398

As can be seen from Table IV, the optimal temperature and pH values differ for SmF and SSF, namely because the conditions of the two cultivation techniques are quite different. The water content (water/solid ratio) is high for SmF and very low for SSF, which influences the acidity level (pH value) of the media during the primary metabolism of the producing strain when mostly organic acids are being secreted into the media. Thus, SmF can handle a more acid surrounding, while SSF cannot. On the other hand, there are some heat and mass transfer limitations in SSF compared to SmF, which is also the consequence of lower water content in the SSF, and for this reason, there is a difference in the optimal temperature.

Running an additional set of experiments under optimal conditions suggested by the model showed that the obtained data for examined enzyme activities are in a good agreement with the values predicted by the optimization model (Table IV).

Previous studies on enzyme production did include the examined strains *Pennicilum*,[26] *Aspergillus*[27] and *Trichoderma*[17] but all of them used some other types of substrate or agricultural waste for their media preparation: wheat bran,[28]

sugar bagasse,[29] paper pulp,[30] wheat straw,[31] rice straw,[28] rice bran,[32] *etc*. Nevertheless, all of the aforementioned authors obtained higher enzyme activities due to higher substrate loading,[21] genetically engineered producing strain,[17] by adding enzyme production enhancers (for example, lactose[33]) or by better enzyme extraction and separation techniques.[13] In addition, wheat chaff became interesting only recently due to it being a good source of (xylo)oligosaccharides,[10] which can be obtained after the action of certain enzymes, thus supporting the fact that in terms of cultivating microorganisms on wheat chaff based media, the producing strain would need to synthesize hydrolytic enzymes in order to utilize the substrate, *i.e.*, enzyme production.

Similarly, a previous study by Saqib[27] compared SmF and SSF for the production of enzymes and showed that higher enzyme activity is obtained by SSF. However, the initial amount of substrate (agricultural waste – wheat straw) was ten times lower in SmF. The results reported here were obtained from the same amount of raw material (3 g) as suggested by Hansen[14] in order to be able to compare the two cultivation techniques and the results showed that a slight advantage should be given to SmF. Furthermore., the production of enzymes on the industrial level leans towards SmF due to the better parameter control, ease of scalability and simpler product recovery, which is the opposite for SSF together with mass and heat transfer limitations.[34] Still, further research should be performed in laboratory bioreactors on SmF and SSF in order to compare the two techniques in terms of medium composition, operating costs and purification techniques. Only then can a final conclusion be drawn on which of the two production techniques would be the best to produce hydrolytic enzymes from wheat chaff.

CONCLUSIONS

Using cheap agricultural waste for obtaining high-value products is very attractive nowadays. Wheat chaff as a by-product of milling facilities and which is currently being used only as animal fodder has the potential to become a raw material for the production of hydrolytic enzymes. Different fungi strains (*Penicillium*, *Aspergillus* and *Trichoderma*) have been investigated, with the aim of producing cellulases and xylanases from wheat chaff by submerged and solid-state techniques. As *Trichoderma* showed the highest potential in enzymes production, an optimisation procedure of process conditions (cultivation temperature, pH value and cultivation time) has been performed in order to enhance the observed bioprocess. Still, further research should be directed towards optimizing the cultivation medium, bioreactor style, enzyme separation and purification techniques.

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ИЗВОД

ОПТИМИЗАЦИЈА СИМУЛТАНЕ ПРОИЗВОДЊЕ ЦЕЛУЛАЗА И КСИЛАНАЗА СУБМЕРЗНОМ ТЕХНИКОМ И ТЕХНИКОМ КУЛТИВАЦИЈЕ НА ЧВРСТИМ ХРАНЉИВИМ ПОДЛОГАМА НА БАЗИ ПШЕНИЧНЕ ПЛЕВИЦЕ

МИРЈАНА ЈОВАНОВИЋ[1], ДАМЈАН вучуровић[1], бојана бајић[1], синиша додић[1], вања влајков[1] и РАДА ЈЕВТИЋ-МУЧИБАБИЋ[2]

[1]Универзитет у Новом Саду, Технолошки факултет Нови Сад, Катедра за биотехнологију и фармацеутско инжењерство, Булевар цара Лазара 1, 21 000 Нови Сад и [2]Универзитет у Новом Саду, Научни институт за прехрамбене технологије у Новом Саду, Булевар цара Лазара 1, 21 000 Нови Сад

Пшенична плевица као пољопривредни отпад представља јефтину сировину за биотехнолошке процесе. Својим лигноцелулозним саставом она је погодна за производњу хидролитичких ензима за примену у технологијама обновљивих горива друге генерације. Циљ овог рада је био оптимизација процесних параметара (температуре култивације 25–35 °C, вредности pH 4–6 и времена култивације 3–7 дана) култивације плесни (*Trichoderma reesei*) на хранљивој подлози чија је основа пшенична плевица, и то субмерзном техником култивације и култивацијом на чврстим хранљивим подлогама, како би се побољшале и упоредиле ове две врсте симултане производње целулаза и ксиланаза. Оптимални услови субмерзне култивације су били 29,65 °C за температуру, 4,27 за вредности pH и 7 дана за време култивације, док су за култивацију на чврстим подлогама оптимални услови били 28,01 °C, 6 и 7 дана, редом. Добијене целулолитичке и ксиланолитичке активности филтрата култивационих подлога су износиле 0,0536 и 0,1676 U mL^{-1} за субмерзну ферментацију, а 0,0407 и 0,1401 U mL^{-1} за ферментацију на чврстим подлогама, редом, што је допринело повећању ензимске активности од 26,77 и 13,39 % за субмерзну ферментацију, а 22,96 и 42,66 % за ферментацију на чврстим подлогама, редом, у поређењу са резултатима добијеним пре оптимизације.

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