Optimizing Enzymatic Biodegradation of Skins Waste Using Response Surface Methodology

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Response surface methodology (RSM) was employed to evaluate the optimum biodegradation of pelt skin wastes (BPSW). The parameters investigated include the pH, temperature, and enzymes mixture ratio of hydrolases, proteases and oxide - reductases on biodegradation degree for Pelt skin waste. The pH varied between 4.00 ± 0.2 and 7.00± 0.2 and temperature between 30-50°C. Was used three mixtures tested having the following composition expressed as ratio between E1:E2:E3. M1- (1:1:1); M2-(2:1:1) and M3-(3:1:1) expressed in mg/L. Response surface methodology (RSM) was used to evaluate the influence of each independent variable on the overall biodegradation efficiency of waste skin. Optimum biodegradation efficiency was achieved at pH=8.4, E1 concentration in the enzyme mixture 2 mg/L and temperature 42.4 ^{0}C. Biodegradation degree is 0.996. Very similar results could be obtained for optimal extraction conditions using the desirability profiles available also in STATISTICA software.

Keywords: biodegradation; Pelt skin, response surface methodology, ANOVA test, optimal working parameters, STATISTICA software,

Leather manufacturing capitalize only 25% of the raw material (raw hides) the rest are waste (hair, fat, skin etc), 99% of leather waste is stored at the landfill. The capitalization of these wastes represents an area of interest in economic and environmental terms for obtaining the biocompozites, biocompost and biofertilisers [1-3].

An effective method of skin waste capitalization is protein composites obtaining by biochemical treatments with microorganisms/enzymes and obtaining of the proteinase and binders with different applications [2, 4]. Raw hide waste resulting from fleshing, splits and pelt trimming, proteins from the solution exhausted from liming can be used for biochemical processing in the form of proteins [4-6]. Collagen, keratin and fat, contained in hide can be biodegraded of enzymes, such as lipases, oxide - reductase and protease that can be used to obtaining of hydrolisates with many possibilities of capitalization, for example obtaining biofertilisers with complex composition, biostimulators [7-9].

Biodegradation of pelt skin by biochemical processes is strongly influenced by many factors such as nature of pelt waste, concentration of enzymes type in mixture, pH, and temperature and contact time of pelt with enzymes. Conventional technique *one-factor-at-a time* is extremely laborious and time and reactive consuming and the optimization of process by synergistic interactions between two or more factor is unable.

For optimize the process considering all work parameters was used the statistical experimental design using response surface methodology (RMS). This methods have more application for optimizing machining techniques for chemical and biochemical process in water and wastewater treatment [9-18].

In this study we used a response surface methods (RSM) for optimizing the influence of the following variables: solution *p*H, temperature and one component concentration in the enzyme mixture upon biodegradation efficiency of Pelt skin.

Experimental part

Skin samples preparation

Groups of seven numbered pieces of bovine hides wastes, cut in pieces that was about 2x2 cm, untreated, provided by tannery SC Pielorex Jilava, county Ilfov, were added into Erlenmayer flasks (250 mL capacity), in 100 mL of mixture of enzymes and distilled water. Erlenmayer flasks with the skin samples were inserted provided with stoppers and thermostated in a *Thermoshake* with 12 plates (Incubating Shaker) at work temperature. Initial, each piece were dried at 50°C until reaching constant mass, and were then cooled and weighed and at various time intervals waste skin samples were taken, washed using distilled water, dried at 50 °C until reaching constant mass and then weighed. The experiment took between 60 and 170 h. The most representative moment for the comparative study was considered the enzymatic activity after 60 h, in relation to which the influence of all parameters was analysed.

A thermostat shake installation has been used permitting the contact of enzymes with wastes at constant temperature and constant stirring. The *p*H values were kept constant using tampon solution acetate and ammonia and varied in accordance with a factorial design.

Enzymatic mixture preparation

In this study were have prepared 3 mixture of enzymes, using three enzymatic preparation named: E1 - mainly containing hydrolases, E2 - mainly containing deaminizes and decarboxylases and E3 -mainly containing oxide-reductases. In the three mixtures we have maintained constant the concentration of E2 and E3 at 1g/L and, respectively, 1mL/L. The concentration of E1 in the mixture was varied between 1 and 3 mg/L. The three mixtures tested have the following composition expressed as ratio between E1:E2:E3. M1- (1:1:1); M2-(2:1:1) and M3-(3:1:1).

Biodegradation degree

The biodegradation degree, B_d, was expressed as a ratio between the biodegraded waste mass, expressed in mg,

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and the initial mass of the waste, expressed in grams, expressed in percent:

$$B_d = \frac{m_o - m_b}{m_o} x100 \quad (1)$$

where B_d is the degree of enzymatic degradation, (%)

m_o the weight of initial Pelt skin sample dried at 50°C to constant weight;

m_b the weight of Pelt skin sample after enzymatic biodegradation process, washing with distilled waterand dried at 50°C to constant weight.

Experimental design

Response surface methodology (RSM) was used to evaluate the influence of each independent variable on the overall biodegradation efficiency of waste skin. A Box-Behnken factorial design with three factors and three levels including three replicates at the center point was used to develop a correlation between initial enzymes concentration, temperature and *p*H and the response variable which is the overall biodegradation efficiency. This methodology allows the formulation of a second-order polynomial model to describe the process, expressed according the following equation:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ii} x_i x_j \quad (2)$$

where Yi is the response variable, β_0 is an intercept, βi is the linear coefficient, β_{ii} and β_{ij} are quadratic and interactive coefficients, respectively. The efficiency of the model, generated after the regression analysis of the response, was tested by ANOVA and Fisher's F-test. The interactions between variables were also represented using three-dimensional response surface plots.

Results and discussions

Table 1 gives the independent factors and their values.

Table 2 describes the experimental design. The STATISTICA statistical package software trial version (Stat Soft Inc., Tulsa, USA) was used for experimental design analysis and data processing.

From table 3 one could see that the F-value model is 38.40 and corresponds to a p value of 0.000436 which implies that the model is significant. Also, the model coefficient of determination has a very high value (0.985), indicating that 98.5% of the total variation is explained by this quadratic regression model. Besides, the value of the adjusted determination coefficient (Adj. R^2) is very high (0.960), which means that the model is highly significant. The values of p less than 0.05 indicate that the terms significant in model equation are linear terms X1 and X2, quadratic terms: $X2^2$ and $X3^2$ and interacting term X1·X3. All the other initial variables and interacting factors were not found to be statistically significant at 95% confidence limits. From Pareto chart, presented in figure 1, one could see these aspects well illustrated. So, the most significant

Table 1
VARIABLES AND EXPERIMENTAL DESIGN LEVELS FOR RESPONSE SURFACE

Independent variable	Coded symbol	Level		
		-1	0	1
pH	X_1	4	7	10
Temperature (°C)	X_2	30	40	50
Concentration of E1in the enzyme mixture (mg/L)	X_3	1	2	3

Table 2
BOX-BEHNKEN EXPERIMENTAL DESIGN MATRIX OF INDEPENDENT VARIABLES AND THE CORRESPONDING EXPERIMENTAL AND PREDICTED VALUES FOR BIODEGRADATION EFFICIENCY (Y1)

Run	X1	X2	X3	Y1(exp.)	Y1 predicted
1	0	0	0	0.987	0.994
2	-1	-1	0	0.710	0.707
3	-1	1	0	0.850	0.874
4	1	1	0	0.904	0.917
5	0	-1	-1	0.671	0.698
6	0	-1	1	0.662	0.672
7	-1	0	-1	0.894	0.885
8	0	0	0	0.987	0.994
9	-1	0	1	0.819	0.827
10	1	0	1	0.921	0.940
11	0	0	0	0.992	0.994
12	0	1	-1	0.827	0.827
13	0	1	1	0.867	0.850
14	1	-1	0	0.791	0.777
15	1	0	-1	0.883	0.885

Table 3
ANOVA TEST FOR RESPONSE FUNCTION Y1 (BIODEGRADATION EFFICIENCY)

Term	Sum of squares	DF	Mean square	F	p
Model	0.158619	9	0.017624	38.40290	0.000436[a]
X_1	0.006384	1	0.006384	13.9116	0.013573[a]
X_2	0.047125	1	0.047125	102.6827	0.000160[a]
X_3	0.000004	1	0.000004	0.0098	0.924968
X_1^2	0.002536	1	0.002536	5.5262	0.065496
X_2^2	0.081652	1	0.081652	177.9176	0.000042[a]
X_3^2	0.025564	1	0.025564	55.7034	0.000682[a]
X_1X_2	0.000182	1	0.000182	0.3971	0.556256
X_1X_3	0.003192	1	0.003192	6.9558	0.046121[a]
X_2X_3	0.000600	1	0.000600	1.3079	0.304553
Error	0.002295	5	0.000459		
Total SS	0.160914	14			
R^2=0.985740	Adj R^2=0.960071				

[a]P<0.05 is considered significant

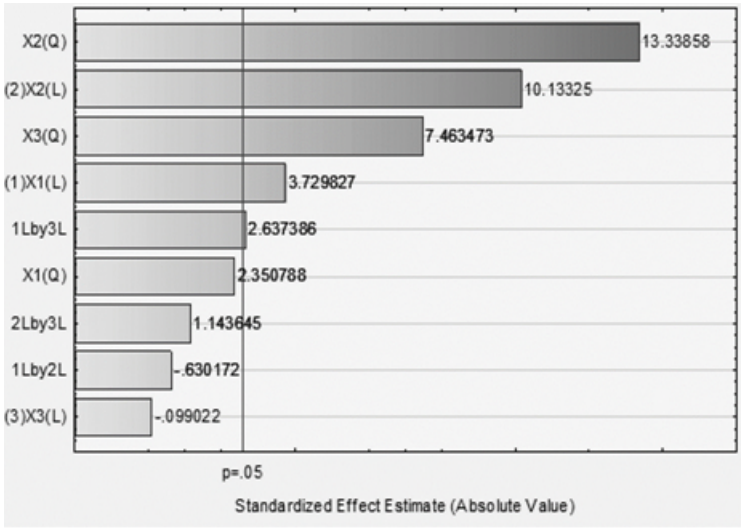

Fig. 1. Paretto chart of main effects obtained from Box-Behnken factorial design

factors which are influencing the process are: solution *p*H (only linear term), operating temperature (linear and quadratic terms), concentration of E1 in the enzyme mixture (only quadratic term) and the interacting term between solution pH and concentration of E1 in the enzyme mixture. The final equation obtained in terms of coded variables for biodegradation efficiency (Y1) is given bellow:

$$Y_1 = 0.98866 + 0.0282X_1 - 0.02620X_1^2 + 0.07675X_2 - \\ -0.14870X_2^2 - 0.000750X_3 - 0.08320X_3^2 - 0.00675X_1X_2 + \\ +0.02825X_1X_3 + 0.01225X_2X_3 \quad (3)$$

The fitted quadratic model simplified obtained (terms statistically insignificant, based on p-values smaller than 0.05, were omitted) is described by equation 4.

$$Y_1 = 0.98866 + 0.0282X_1 + 0.07675X_2 - 0.14870X_2^2 - \\ -0.08320X_3^2 + 0.02825X_1X_3 \quad (4)$$

Response surface plot from figure 2 represents the interaction between temperature and solution *p*H (expressed as coded variables) when the third variable (concentration of E1in the enzyme mixture) is maintained at level 0. Figure 2 shows a significant influence on biodegradation efficiency especially of work temperature. In figure 3 is presented the response surface plot for the interaction between *p*H and E1 concentration in the enzyme mixture, when temperature is maintained at level 0. In this case one could observe a significant influence of E1 concentration in the enzyme mixture upon biodegradation efficiency. In figure 4 one could observe that both variables temperature and E1 concentration in the enzyme mixture have a significant influence upon biodegradation efficiency of Pelt skin. Optimum biodegradation efficiency was achieved at *p*H=8.4, E1 concentration in the enzyme mixture 2 mg/L and temperature 42.4 ^{0}C. Very similar results could be obtained for optimal extraction conditions using the desirability profiles available also in STATISTICA software. In figure 5 are presented the desirability profiles obtained for biodegradation of Pelt skin.

The experiments were carried out to validate the model prediction. Under the above conditions, the value obtained for biodegradation efficiency was 0.996 in a good agreement with theoretical predictions.

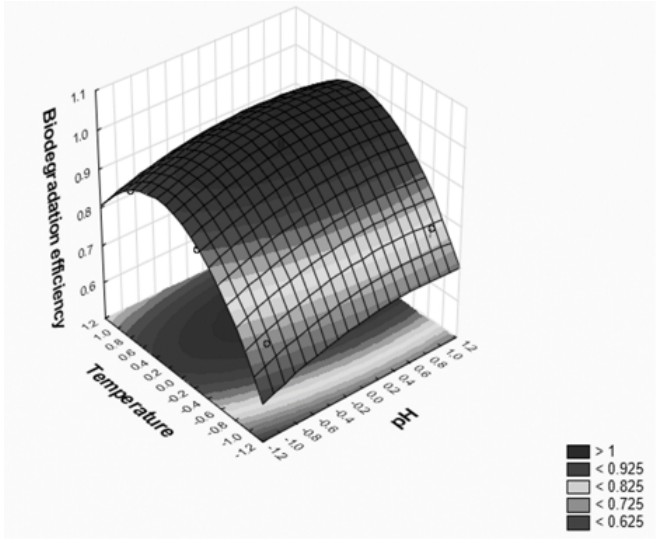

Fig. 2. Response surface plot showing the interactive effects of pH and temperature on biodegradation efficiency of Pelt skin.

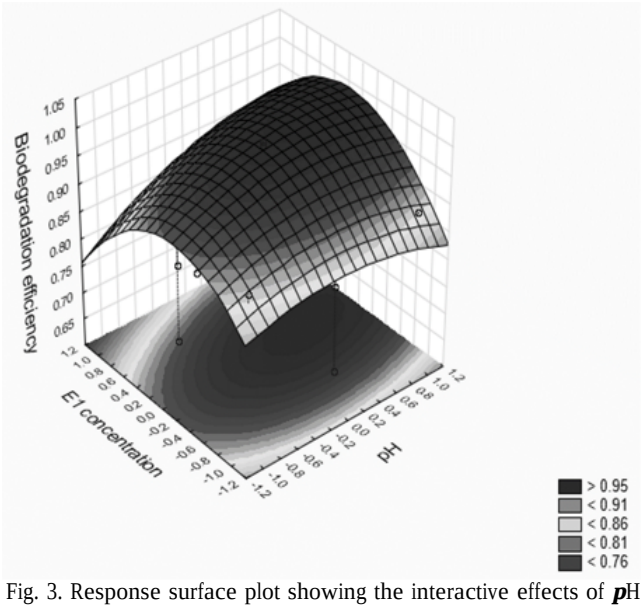

Fig. 3. Response surface plot showing the interactive effects of ***p***H and E1 concentration in the enzyme mixture on biodegradation efficiency of Pelt skin.

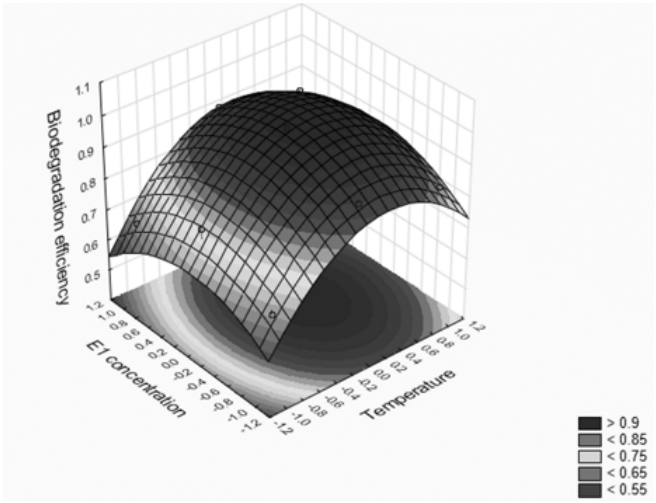

Fig. 4. Response surface plot showing the interactive effects of temperature and E1 concentration in the enzyme mixture on biodegradation efficiency of Pelt skin.

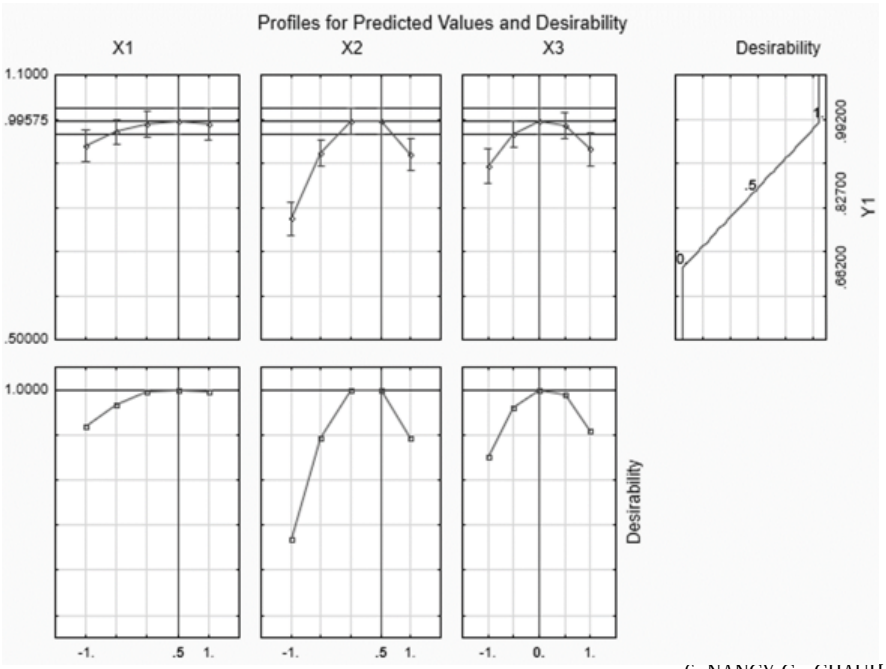

Fig. 5. Profiles for predicted biodegradation efficiency and the desirability level for different influencing factors for optimum biodegradation of Pelt skin.

Conclusions

An experimental study was conducted for in order to determine the biodegradation efficiency of Pelt skin in different conditions. A Box-Behnken factorial design was applied for optimization of biodegradation efficiency. A second order polynomial model was proposed and the regression coefficients were calculated. Among terms of the model the most significant contribution is due to are linear terms for *p*H and temperature, quadratic terms of temperature and E1 concentration in the enzyme mixture and the interacting term between pH and E1 concentration in the enzyme mixture. The optimum process parameters were determined as: *p*H=8.4, E1 concentration in the enzyme mixture 2 mg/L and temperature 42.4^{0}C. The experimental values obtained under these conditions were in good agreement with the predicted values obtained using RSM.

References

1. BAJZA, Z, VRUCEK V., Waste Manag., **21**, no. 1, 2001, p. 79.
2. EL-SHAMY, E.N., Int. J. of Current Research, **10**, no 2, 2018, p. 65197
3. ZAINESCU G., DESELNICU D.C., IOANNIDIS I., CONSTANTINESCU R., SIRBU C., J. Int Sci. Publ. Ecol. Saf., **7**, no 2, 2013, p. 345.
4. PALANISAMY T., RAO J.R., NAIR B.U., RAMASAMI T., Trends Biotechnol, **22**, no 4, 2004, p. 181.
5. PANTAZI, M., STEFAN, D. M., CONSTANTINESCU, R.,VASILESCU, A. M., Rev Chim(Bucharest), **65**, 2014, p. 233.
6. NANCY G.**,** CHAUHAN P.S., KUMAR V., PURI N., GUPTA N. J., Clean Prod., 79, 2014, p. 249.
7. SARAN S, MAHAJAN R.V., KAUSHIK R., ISAR J., SAXENA R.K., J. Clean Prod., **54**, 2013, p. 315.
8. STEFAN, D.S., CONSTANTINESCU, R.R., MEGHEA, A., ANGHEL, R., STEFAN, M., TUDOSIE, M.S., Rev. Chim.(Bucharest), **67**, no 7, 2016, p. 1401.
9.ADINARAYANAK K., ELLAIAH P., SRINIVASULU B., DEVI L., TSENG DH, HUANG S.L., Biotechnol. Lett., **23**, no. 20, 2001, p. 1653-1657.
10. AHMAD A.L.**,** ISMSIL S., BHATIA S., Environ Sci Technol., **39**, no. 8, 2005, p. 2828.
11. STOICA-GUZUN A., STROESCU M., JINGA. S.I., MIHALACHE N., BOTEZ A., CRISTIAN M., BERGER D., DAMIAN C.M., VALENTIN I., Int. J. Bio.l Macromol., **91**, 2016, p. 1062.
12. GAN C.Y., MANAF N.H.A., LATIFF A.A., Carbohydr. Polym., **79**, no 4, 2010, p. 825.
13. PINGRET D., FABIANO-TIXIER A.S., LE BOURVELLEC C., RENARD M.G.C., CHEMAT F., J. Food. Eng., **111**, no. 1, 2012, p. 73.
14. STROESCU M.**,** STOICA-GUZUN A., GHERGU S., CHIRA N., JIPA I., Ind., Crop. Prod., **43**, 2013, p. 405.
15. BOUCHEZ M., BLANCHET D., VANDECASTLE J.P., Appl. Microbiol. Biotechnol., **43**, no 1, 1995, p. 156.
16. MURTHY M.S.R.C.**,** SWAMINATHAN T., RAKSHIT K.Y., Bioprocess Eng., **22**, no 1, 2000, p. 35.
17. ORLITA A., Microbial biodeterioration of leather and its control: A review. Int. Biodeterior. Biodegrad., **53**, no 3, 2004. p. 157.
18. WU S., YU X., HU Z., ZHANG L., CHEN J., J. Environ, Sci., **21**, no 9, 2009, p. 1276.

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