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Artificial Neural Network (ANN) and Response Surface Methodology (RSM) of Extraction of Pectin from Sweet Lemon Peels by Microbial Protopectinase

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Abstract - Pectin is an important byproduct of fruit and vegetable processing industry. Conventional process for solubilization of protopectin involves hot acid extraction, which generates acid effluent and causes degradation of pectin in the process. Application of microbial protopectinases (*PPase*) for solubilization of protopectin is expected to enhance the quality and recovery of pectin.
Standardization of process parameters including to achieve maximum recovery needs extensive experimentation. Various parameters including enzyme source, enzyme concentration, treatment time, and composition waste influence the recovery. Artificial Neural Network (ANN) and Response surface methodology (RSM) are innovative tools for modeling and prediction of such complex of biotechnology processes.
In the present work, experiments were carried out to standardize the optimum conditions for pectin extraction using microbial protopectinase (*PPase*) from *Kluveromyces marxianus-MTCC 188* and *Kluveromyces wickerhamii –MTCC 455* (pH 5.0 at 30^0C). ANN and RSM models was developed using elite-ANN© and Minitab 511 software to predict pectin yield as a function of enzyme concentration and time of treatment. Optimum showed excellent predictability with $R^2 > 0.95$ for both training and test data sets of ANN and $R^2 > 0.90$ for RSM.

Key words- pectin; protopectinase; pectin yield; artificial neural network; response surface methodology.

I. INTRODUCTION

Pectin is an important byproduct of fruit and vegetable processing industry. Pectin exist as water insoluble protopectin between the middle lamella, linking primary cell wall made up of cellulose and hemicelluloses [1].Commercially, pectin has broad application in both the food and pharmaceutical industries, where it acts as gelling and thickening agent [2,3]. In addition, pectin is prove to have beneficial effects on human health [4]. Application of microbial protopectinase for solubilization of protopectin is expected to enhance the quality and recovery of pectin. *Tricosporon penicillatum*, yeast is reported to liberate water-soluble highly polymerized pectin from protopectin [5]. Subsequently, Sakai and Okushima [6] isolated *PPase* from culture filtrate of *Trichosporon Penicillatum SNO-3 Sakai et al.*, [7] used *Galactomyces reessii* for proptopectinase production.

Standardization of process parameters of enzyme treatment to achieve maximum recovery needs extensive experimentation. Various parameters of enzyme source, enzyme concentration, treatment time, and composition of waste influence the recovery. Artificial Neural Network and Response Surface Methodology are being suggested as tools for modeling and prediction of biotechnological process. Present investigations report application of ANN and RSM to predict yield of pectin as a function.

II. METHODOLOGY

A. Enzyme source

Protopectinase was obtained from two different organisms *Kluveromyces marxianus-MTCC 188* and *Kluveromyces wickerhamii –MTCC 455* on liquid medium [8] at pH 5.0, 30 ^{0}C for 24 hours. Crude enzyme extract was dialyzed against sucrose and used for treatment. Enzyme activity was standardized by carbazol-sulphuric acid method [9].

B. Treatment

Dried sweet lemon peels were used as substrate. Different experiments were carried out to standardize the best suited condition for extraction. Treatment was carried out at pH 5.0, 30 ^{0}C for different time intervals parameters (Table 1). Optimum recovery of pectin under experimental condition is recorded with 15 units of enzyme and 9 hours.

C. ANN and RSM application for prediction of pectin yield

Suitability of application of ANN and RSM methodology software to predict pectin yield as a function of enzyme concentration and time of treatment was studied.

ANN software elite-ANN© [10] was used with three hidden layers and complex level. Experimental data was divided into two data series. First set, consisting of about 75-80% of the data points was named as 'Training data set' and remaining data set was termed as test set. It was used for training of ANN to develop model for yield of pectin with two inputs (U/ml of enzyme and time (hours) of incubation), and one output (pectin yield in %). The architecture of ANN consists of three hidden layers with 10 nodes each with complex learning process and final error 0.00945. Adequacy and predictability of the developed ANN model is judged by the actual and the predicted values for the test data (Figure 1) which show a satisfactory match as indicated by the correlation coefficient (0.953) and root mean square error (0.522). Minor variations ANN model showed excellent predictability with R^2> 0.95 for both training and test data sets.

Table 1: Yield of pectin (%) using different treatment conditions with microbial protopectinase where *1,*2,*3,*4,*5 were used test data

Time of incubation (hrs)	Yield of pectin					
	PPase(10 U)		PPase(15 U)		PPase(20 U)	
	K.marxianus	*K.wickerhamii*	*K.marxianus*	*K.wickerhamii*	*K.marxianus*	*K.wickerhamii*
6	2.03±0.52	0.85±0.24	3.65±0.38	2.60±0.43	4.87±0.50	3.10±0.15
9	4.97±0.66^{*5}	2.98±0.37	6.64±0.11	4.44±0.31^{*1}	6.44±0.40	3.86±0.11
12	3.19±0.13	2.22±0.15	4.12±0.10^{*4}	3.17±0.14	3.55±0.32	2.81±0.20
18	2.78±0.10	1.91±0.19	3.10±0.18^{*3}	2.5±0.44^{*2}	2.48±0.20	2.63±0.16
24	1.97±0.08	1.37±0.08	2.52+0.08	2.53±0.08	1.87±0.08	1.25±0.23

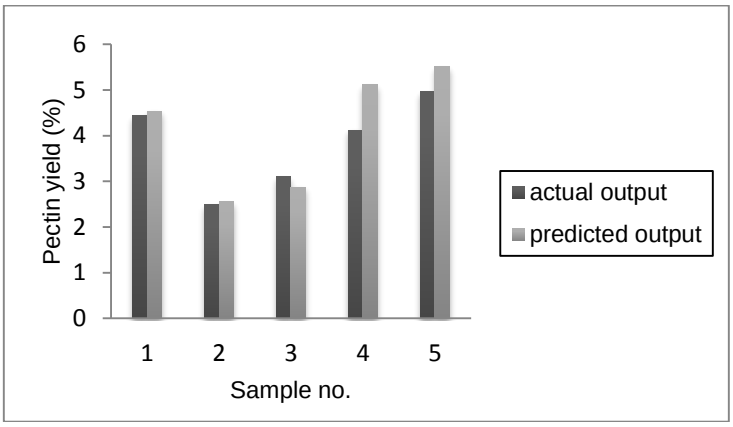


Figure 1: Comparision between actual and predicted values for test data set (*) of protopectinase where, R^2=0.953271; RMSE=0.522381

RSM methodology consists of a group of empirical techniques devoted to the evaluation of relation existing between a cluster of controlled experimental factors and the measured responses. For the available data Box-Behnken model was used to select the factors that influence enzyme production [11]. The range and levels of the variables are given in table 2. The Statistical analysis was done using Minitab1511. The regression equation was developed for pectin yield as function of enzyme time of treatment in hours(x_1) and concentration of enzyme (x_2). Regression coefficient for the system indicated positive effects of both variables at lower limit (table 3). Analysis of variance (ANOVA) for the two systems showed that F value was > P, this was suggestive of a good fit of the model (table 4). The error functions for all the regression terms were within acceptable range, with correlation coefficient > 0.9 and root mean square error in the range of 0.13 - 0.22. Individual effects were significant because p value is < 0.001 for recovered pectin using both the protopectinase enzyme.The surface graphs were developed for the recovery of pectin at different combination of parameters namely hours of incubation and enzyme concentrations (figure 2).

Table 2: Range of variables for study of pectin yield

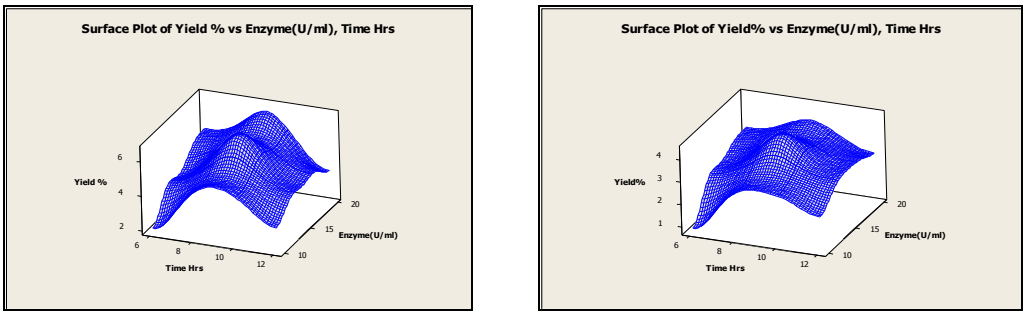
Variable	**Range**		
	-1	**0**	**1**
Time of treatment (Hours) x_1	6	9	12
Protopectinase concentration (Units) x_2	10	15	20

Table 3: Estimated regression coefficients using data in uncoded units

Terms	Term	*K.marxianus* coefficient	*K.wickerhamii* coefficient
	Constant	6.4322	4.2419
Time	β_1	0.0517	0.2745
Units	β_2	0.7783	0.6162
Time ^{2}	β_{11}	-2.448	-1.288
Units^2	β_{22}	-0.628	-0.753
Time * Units	B_{12}	-0.618	-0.414

Table 4: Analysis of Variance (ANOVA) for protopectinase activity

K.marxianus						
Source	Degree of freedom (DF)	Seq SS	Adj SS	Adj MS	F	P
Regression	5	17.954	17.954	3.5909	51.16	0.004
Linear	2	3.6508	3.6508	1.8254	26.01	0.013
Square	2	12.778	12.778	6.3891	91.03	0.002
Interaction	1	1.5252	1.5252	1.5252	21.73	0.019
Residual Error	3	0.2106	0.2106	0.0702		
Total	8	18.165				
K.wickerhamii						
Source	Degree of freedom (DF)	Seq SS	Adj SS	Adj MS	F	P
Regression	5	7.867	7.867	1.5734	31.53	0.009
Linear	2	2.7301	2.7301	1.365	27.36	0.012
Square	2	4.4506	4.4506	2.2253	44.6	0.006
Interaction	1	0.6864	0.6864	0.6864	13.76	0.034
Residual Error	3	0.1497	0.1497	0.0499		
Total	8	8.0167				



F***igure 2: Pectin yield % Vs Enzyme concentration (U/ml) for protopectinases from K.marxianus and K.winkerhamii respectively***

III. CONCLUSION

The modeling of biological process is more complex because of the involvement of microorganisms. The complexity is further multiplied due to unsteady state nature of the process. Present investigations indicate that ANN and RSM model can be applied for the predictions of biotechnological processes such as enzyme applications effectively and with high accuracy.

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