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OPTIMIZATION OF CULTURAL PARAMETERS FOR PECTIN METHYLESTRASE AND POLYGALACTURONASE PRODUCTION FROM *SCHIZOPHYLLUM COMMUNE* IN SOLID STATE FERMENTATION

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Abstract

The aim of this study was optimization of pectinolytic enzymes i.e. pectin methylesterase and polygalacturonase from *Schizophyllum commune* using the mosambi peels as substrate in a solid state fermentation process. Results revealed that maximum pectin methylesterase activity of 394.82 U/ml was observed at pH 6, temperature 35°C, time period of 3 days, substrate concentration of 3 g and 3 ml inoculum size. While polygalacturonase showed maximum activity of 381.69 U/ml at pH 4, temperature 45°C, time period of 1 day, substrate concentration 25 g and 5 ml inoculum size. Statistical analysis revealed that the proposed model was found very significant as indicated by *p*-values.

Introduction

The reactions involved in the preparation of different food products are catalyzed by many enzymes. During these reactions, many intermediate processes are shortened due to use of enzymes so it is one of the significant tools in modern food processes. A huge amount of industrial enzymes are classified into different groups. Out of these groups, pectinases are the most significant enzymes used in vegetable and fruit industries (Tapre and Jain 2014). The pectinases or pectinolytic enzymes are the group of enzymes which are involved in pectin degradation (Murudula and Anitharaj 2011). Pectic substances or pectin are the complex polysaccharides present in the middle lamella of plant cell wall (Sandhya and Kurup 2013). In its structure pectin is homopolymeric, made up of a partially methylated poly-a- (1,4) -galacturonic acid (Torres *et al.* 2006).

Pectin methylesterase (PME, EC 3.1.1.11) is an abundant enzyme in plants. This enzyme de-esterifies the methoxylated pectin in the plant cell wall (Demirdoven and Baysal 2014). During its reaction, PME has the capacity to release methoxyl group from pectin chain to form carboxylate groups (polygalacturonic acid). In addition to this, methanol and H_3O^+ are released (Kushani *et al.* 2014). Pectin methylesterase plays important role in plants as well as in food industry (Poturcu *et al* 2017). The effect of PME in food industry has been widely considered. It produces required effects in quality enhancement as well as unwanted effects in some drinks (Jesus *et al*. 2017).

Polygalacturonases or hydrolytic depolymerases are enzymes which are involved in the degradation of pectin or pectic substances. They have widespread applications in textile and food processing, management of pectic waste waters and degumming of plant fibres (Torres *et al.* 2006). Polygalacturonase (PG) degrades pectin into D-galacturonic acid monomers and is used broadly in food industry particularly for juice clarification (Yadav *et al.* 2015).

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Existence of pectinolytic enzymes has been described in many fungi and bacteria species but majority of industrial preparations of pectic enzymes are derived from fungal source (Kushani *et al.* 2014). *Schizophyllum commune* is a wood-rotting basidiomycetes. *S. commune* is spread all over the world and is easily identifiable due to its fan-shaped basidiocarp and a split gill on the base. Its fruiting bodies are collected from the wild and have been utilized as food, particularly in the southern part of Thailand. Various fibrinolytic enzymes are produced from *S. commune*. Furthermore, its metabolites, enzymes and polysaccharides have been used for several products (Patcharaporn *et al.* 2008).

In the process of fermentation, microorganisms like bacteria and fungi are used to produce enzymes. There are two techniques of fermentation for the production of enzymes i.e. submerged fermentation and solid-state fermentation. Submerged fermentation comprises the manufacturing of enzymes by microbes in a liquid nutrient media (Renge and Khedkar 2012). Solid-state fermentation (SSF) is a process that takes place on insoluble material that as support as well as a source of nutrients. This process uses low amount of water, under the action of fermenting material (Alcantara *et al.* 2010). As compared to other microorganisms, fungi usually grow on solid substrate. That's why solid state fermentation suggests highest opportunities when fungi are used (Bhargav *et al.* 2008).

Optimization means to improve the activity of a procedure, a system, or a product with the aim of obtaining the extreme advantage from it. Optimization has been usually used in analytical chemistry as a source of determining situations at which a process gives the best probable output (Marcos *et al.* 2008). Response Surface Methodology (RSM) is a collection of mathematical and statistical tools suitable for improving, developing, and optimizing methods (Carley *et al.* 2004). Response surface methodology (RSM) is a most popular method which is utilized in optimization of cultural conditions and other important parameters that are useful in synthesis of various biomolecule (Mehmood *at el*. 2018, Naz *et al.* 2017). The present study aims at optimizing production of pectin methylesterase (PME) and polygalacturonase (PG) through solid state fermentation from *Schizophyllum commune* by utilizing agro-industrial waste (peels of mosambi).

Materials and Methods

Five variables i.e. pH, temperature, time period, substrate concentration and inoculum size were optimized through central composite design (CCD) of response surface methodology.

Agro-industrial waste mosambi peels were used as substrate for pectinolytic enzyme i.e PME and PG production. The substrate was collected from the local fruit market of Gujrat, Pakistan. Peels were washed with distilled water to remove impurities and dried at 50 ^{0}C for 2 - 3 days. The dried peels were milled into fine powder for further use.

Schizophyllum commune a fungal strain was used for the production of pectinolytic enzymes. The fungal strain was grown on potato dextrose agar (PDA) slants and stored at 30 ^{0}C. Inoculum media were prepared in 250 Erlenmeyer flask. The inoculum media comprised of (%): glucose 2; $MgSO_4.7H_2O$ 0.05; $KH_2PO_4.7H_2O$ 0.02; $(NH_4)_2SO_4$ 0.05; $CaCl_2$ 0.05 and autoclaved at 120^{0}C for 30 minutes. Then a loop full of sporulation culture of *Shizophyllum commune* was transferred aseptically to inoculum media. The flask was kept in water bath shaker at 35^{0}C with shaking speed of 120 rpm for 3 - 4 days.

The specific amount of substrate was moistened with medium of different pH and sterilized at 121^{o}C for 15 min. After that it was inoculated with different volumes of inoculum size and incubated at different temperatures for various time periods as per statistical design. After termination of the fermentation period, enzyme was extracted and enzyme activity was measured.

Solid state fermentation (SSF) parameters were optimized through response surface methodology for PME and PG production. Twenty-six trials for 5 parameters namely pH (4 - 10), temperature (15 - 58^0C), time period (1 - 7 days), substrate concentration (3 - 35 g) and inoculum size (1 - 7 ml) were applied.

Enzyme was extracted after 3 days by adding 50 ml distilled water in each flask followed by shaking at 120 rpm for 30 min. The experimental mass was filtered through Whatman filter paper No.1 and filtrate obtained was collected in 50 ml falcon tubes. The filtrate was then centrifuged for 10 min at 4000 rpm and 4^0C. Clear supernatant containing crude enzyme was saved and further analyzed for pectin lyase activity by spectrophotometrically (Ali *et al.* 2016).

For PME activity 2 ml pectin (0.5 g/ml of pectin), 100 µl bromocresol green (0.01 g bromocresol green in 100 ml acetate buffer) and 0.5 ml of crude enzyme was taken in test tubes. The reaction mixture was incubated at 60ºC for 2 min. Then absorbance was taken spectrophotometrically at 620 nm. For PG activity, 1 ml crude enzyme and 1 ml polygalacturonic acid substrate was taken and the reaction mixture was incubated at 35ºC for 20 min. After that 3 ml of DNS was added and boiled for 15 minutes. By adding distilled water volume was made up to 10 ml. cooled it at room temperature and absorbance was taken spectrophotometrically at 550 nm (Hubert *et al.* 2017).

All the data were statistically analyzed and analysis of variance was performed using Statistica software 99th version.

Results and Discussion

In the present study central composite design of response surface methodology was applied to investigate the effect of 5 parameters on pectin methylesterase and polygalacturonase production. Response surface methodology has been effectively used to optimize biotechnological and biochemical procedures linked to food systems (Murudulla and Anitharaj 2011). Experiments were conducted and the response was calculated through second order polynomial regression equation (Eq. 1, 2). The parameters used were pH (A), temperature (B), substrate concentration (C), time period (D) and inoculum size (E). Table 1 shows that PME activity ranged from 114.0 U/ml to 394.82 U/ml. The maximum activity of PME 394.82 U/ml was observed at pH 6, temperature 35°C, time period of 3 days, substrate concentration of 3g and 3 ml inoculum size, while polygalacturonase showed maximum activity of 381.69 U/ml at pH 4, temperature 45°C, time period of 5 days, substrate concentration 25 g and 1 ml inoculum size (Table 1). Shweta *et al.* (2006) studied the use of response surface methodology (RSM) to optimize ecological factors for pectinase production from *Rhizopus* species. Palaniyappan *et al.* (2009) also studied optimization method using RSM to monitor the effect of different parameters on pectinase production.

Pectin methylestrase activity (U/ml) = $-1110.25 - 0.60\,A + 69.88\,B + 45.54\,C + 16.69\,D + 193.05\,E + 6.89\,A^2 - 0.76B^2 + 8.04C^2 + 0.32D^2 - 5.74E^2 - 1.27AB + 0.61\,AC - 2.27\,BC - 2.77\,AD - 0.17\,BD - 1.30\,CD - 9.26\,AE - 1.76\,BE - 8.72\,CE - 2.28\,DE$ **Eq. 1.**

Polygalacturonase activity (U/ml) = $16.5738 + 25.7745\,A - 11.8943\,B + 45.3915\,C + 11.8493\,D + 89.3141\,E + 2.4818\,A^2 + 0.1747B^2 + 45.3915C^2 + 11.8493D^2 - 5.7094E^2 - 0.2558AB - 6.9252\,AC + 0.5716\,BC - 1.5472\,AD + 0.1650\,BD - 0.0244\,CD - 3.7113\,AE + 0.5054\,BE - 12.7427\,CE - 0.8278\,DE$ **Eq. 2.**

Statistical analysis shows that the proposed model was highly significant as revealed by F-value and p-value of 41.4483, 20.6878 and 0.000304, 0.001637for PME and PG, respectively (Table 2). The goodness of fit of the model was determined by coefficient of determination (R^2) having value of 0.994 and 0.988 for PME and PG indicating the accuracy of the model (Fig. 1). The authenticity of the model was further supported by adjusted R^2 having value of 0.97 and

0.9403 which indicated that only 3 and 6% of variation was not predicted by the model for PME and PG, respectively.

Table 1. Central composite design for PME and PG by *Schizophyllum commune.*

Run No.	A	B	C	D	E	PME (U/ml)	PG (U/ml)
1	8	20	5	5	5	376.9200	96.8500
2	6	35	3	15	3	292.5500	204.2800
3	4	20	5	25	5	254.2000	104.4300
4	6	35	1	15	3	374.0800	220.6400
5	6	45	3	15	3	152.2200	297.4000
6	4	45	5	25	1	263.1500	381.6900
7	6	35	3	15	7	114.3800	123.4900
8	6	20	3	15	3	201.4700	175.3400
9	6	35	3	35	3	285.8800	125.9100
10	8	20	5	25	1	234.7300	136.2700
11	8	20	1	5	1	246.0300	235.1910
12	4	45	5	5	5	243.6300	246.6910
13	4	20	1	5	5	375.6600	238.8830
14	6	35	7	15	3	303.9800	226.1300
15	6	35	3	3	3	394.8200	194.5800
16	8	45	5	5	1	311.4200	260.8500
17	4	20	1	25	1	271.3700	140.7600
18	4	35	3	15	3	379.9800	268.5500
19	8	45	1	25	1	242.3700	211.2640
20	8	45	1	5	5	331.3100	342.5900
21	8	20	1	25	5	124.1800	158.4080
22	4	20	5	5	1	250.3300	203.8900
23	4	45	1	5	1	376.8800	107.4800
24	4	45	1	25	5	362.8800	347.7300
25	10	35	3	15	3	275.9900	218.5900
26	6	35	3	15	0.5	310.1900	191.3790

A = pH, B = temperature (^{o}C), **C**. time period (days), D. Substrate concentration (g) and E. Inoculum size (ml).

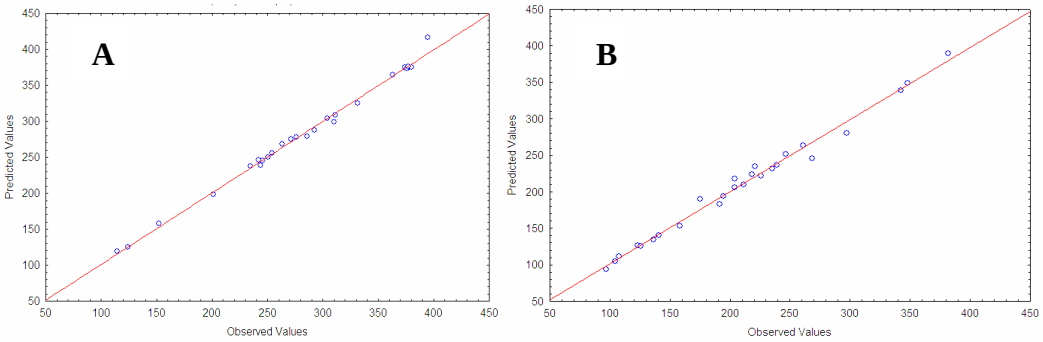

Fig. 1. Graph between observed and predicted values of PME (A) and PG (B).

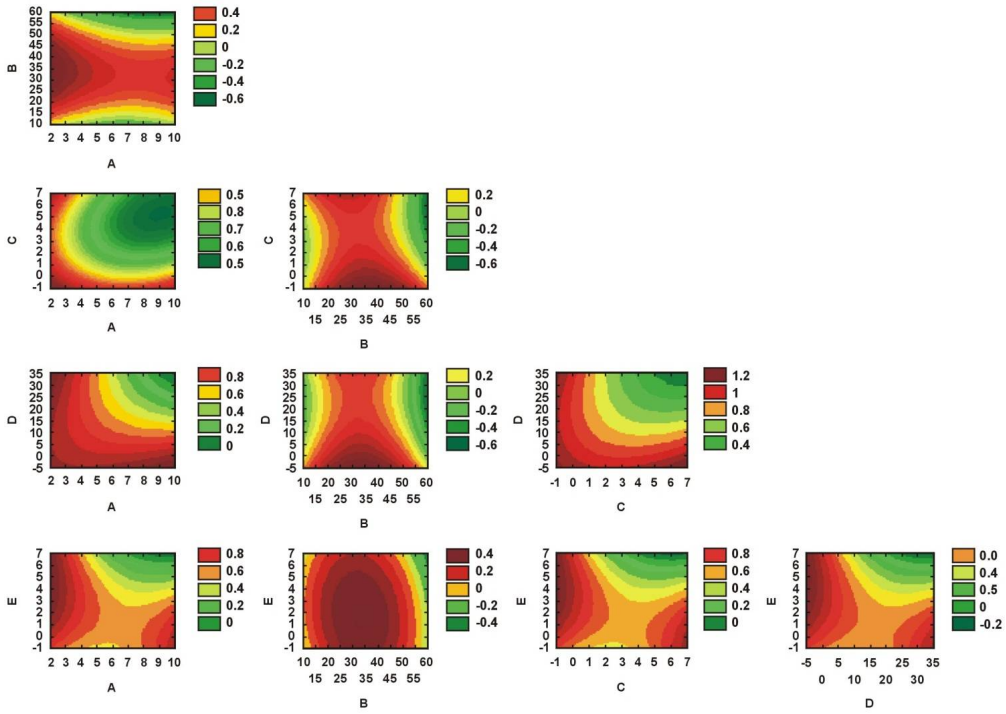

Fig. 2. Contour plots for PME indicating the interaction of different variables.

Figs 2 and 3 illustrated the contour plots for PME production by *Schizophyllum commune* in solid state fermentation. These plots were made from two selected independent variables and the value of third variable was kept persistent because it is the central value to get optimum conditions for maximum PME production. The different colors in these plots indicated different levels of PME production between two independent parameters and keeping third parameter at constant value. These graphs showed that each parameter had significant effect on PME production by *Schizophyllum commune* in solid state fermentation.

Table 2. Analysis of variance for PME (U/ml) and PG (U/ml) production by *S. commune.*

Enzyme	Effect	Degree of freedom	Sum of squares	Mean square	F-value	p-value
PME	Model	20	153080.3	7654.016	41.44483	0.000304
	A	1	0.3200	0.32	0.0017	0.968629
	A^2	1	10079.40	10079.40	54.5778	0.000715
	B	1	43248.36	43248.36	234.1804	0.000022
	B^2	1	38583.37	38583.37	208.9206	0.000029
	C	1	1993.53	1993.53	10.7946	0.021821
	C^2	1	13707.29	13707.29	74.2220	0.000348
	D	1	6549.44	6549.44	35.4638	0.001909
	D^2	1	14946.38	14946.38	80.9314	0.000283
	E	1	34879.77	34879.77	188.8664	0.000037
	E^2	1	7757.52	7757.52	42.0053	0.001304
	AB	1	9916.93	9916.93	53.6980	0.000742
	AC	1	55.450	55.46	0.3003	0.607287
	BC	1	31642.28	31642.28	171.3361	0.000046
	AD	1	28873.27	28873.27	156.3424	0.000058
	BD	1	4409.07	4409.07	23.8742	0.004529
	CD	1	6405.14	6405.14	34.6824	0.002006
	AE	1	12937.14	12937.14	70.0518	0.00039
	BE	1	19217.41	19217.41	104.0581	0.000155
	CE	1	11473.91	11473.91	62.1287	0.000528
	DE	1	19579.72	19579.72	106.0199	0.000149
	Error	5	923.40	184.68		
PG	Model	20	3.10	3.10	0.00886	0.928658
	A	1	573.55	573.55	1.63716	0.256871
	A^2	1	1306.87	1306.87	3.73035	0.111289
	B	1	1253.08	1253.08	3.57683	0.117172
	B^2	1	2054.45	2054.45	5.86426	0.059997
	C	1	1980.22	1980.22	5.65239	0.063363
	C^2	1	521.26	521.26	1.28791	0.276933
	D	1	3302.18	3302.18	9.42582	0.027779
	D^2	1	6222.30	6222.30	17.76108	0.008373
	E	1	7465.50	7465.50	21.30970	0.005755
	E^2	1	7663.63	7663.63	21.87525	0.005448
	AB	1	400.21	400.21	1.14236	0.334016
	AC	1	7235.80	7235.80	20.65403	0.006142
	BC	1	1998.32	1998.32	5.70405	0.062518
	AD	1	9028.67	9028.67	25.77165	0.003846
	BD	1	4200.57	4200.57	11.99020	0.017990
	CD	1	2.24	2.24	0.00639	0.939403
	AE	1	2078.15	2078.15	5.93193	0.0058976
	BE	1	1579.78	1579.78	4.50936	0.087116
	CE	1	24498.27	24498.27	69.92845	0.000400
	DE	1	2584.83	2584.83	7.37821	0.041960
	Error	5	1751.67	350.33		

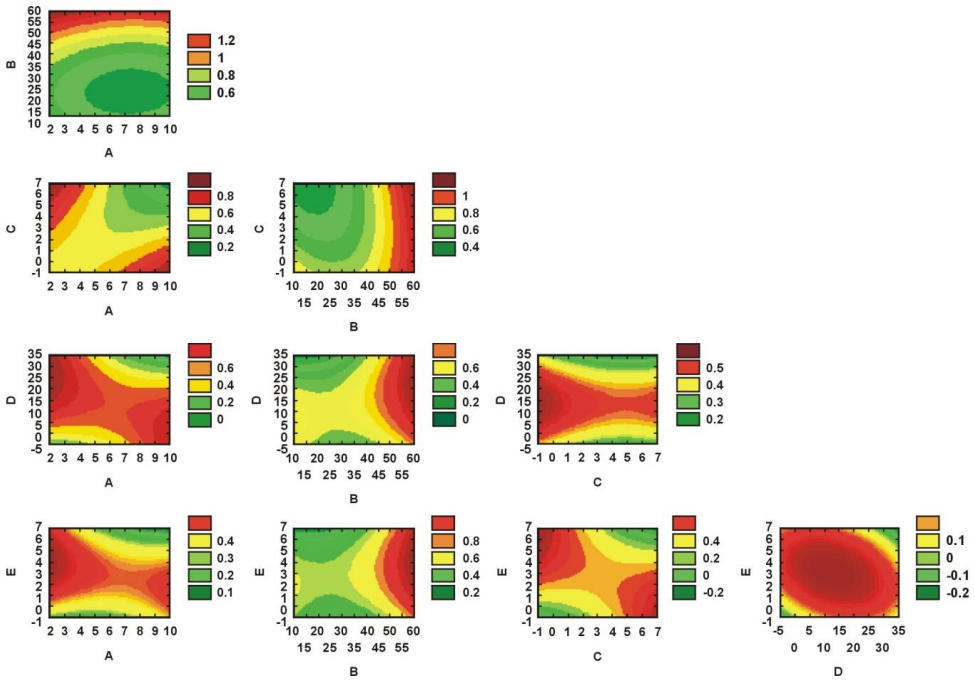

Fig. 3. Contour plots for PG indicating the interaction of different variables.

Fig. 4 explained the desirability chart for PME production by *S. commune* in solid state fermentation. This chart depicted that if value of parameter A is 6, parameter B is 32.8, parameter C is 3, parameter D is 14.92 and parameter E is 2.98 then the maximum predicted response will be 439.69 U/ml and minimum response will be 125.74 U/ml. Using these values of different paramters, the response was very close to the predicted values which validated the model prediction.

Enzyme manufacturing from microbes has grown a lot of attention at the industrial scale (Renge *et al.* 2012). The biotechnological potential of pectinolytic enzymes from microorganisms has drawn much of attention from various researchers worldwide as likely biological catalysts in a variety of industrial processes (Hossam and Hamdy 2011). Agro-industrial wastes are potential source for pectinolytic enzyme manufacturing for utilization in industry because of their higher stability under extreme environments and extensive substrate specificity (Siumara *et al.* 2010).

In this study *Schizophyllum commune* was preferred as microorganism for the production of pectinolytic enzymes through solid state fermentation utilizing S*weet limetta* (mosambi peels) as an agro-industrial waste. The production of pectinolytic enzymes i.e. pectin methylesterase and polygalacturonase was optimized through response surface methodology. Response surface methodology has been effectively used to optimize biotechnological and biochemical procedures linked to food systems (Murudula and Anitharaj 2011). As described above in the results that PME showed maximum activity of 394.82 U/ml at pH 6, temperature 35°C, substrate concentration 3 g, 3 ml inoculum size and 3 days of fermentation period while PG showed maximum activity of 381.69 U/ml at pH 4, temperature 45°C, time period of 5 day, substrate concentration 25g and 1 ml inoculum size. The utilization of mosambi peels as substrate in SSF provide a cheaper way of enzyme production. The composition of medium is very important for

enzyme production. Enzyme production is greatly affected by change in pH and temperature of the fermented medium. If pH or temperature rises above or below the optimum, it will greatly affect

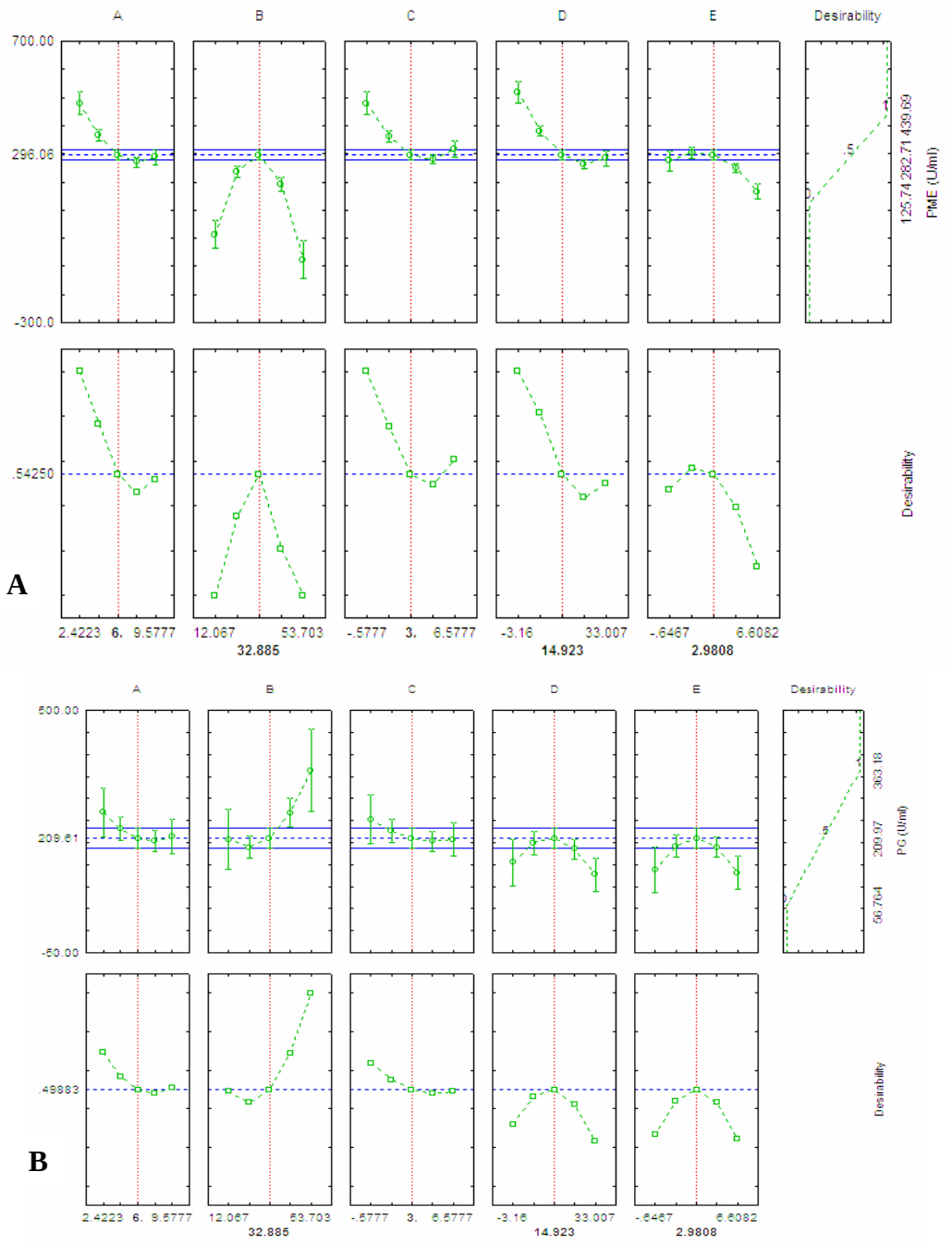

Fig. 4. Desirability chart for PME (A) and PG (B) production by *S. commune.*

the growth of microorganisms in solid state fermentation thus reducing the production of pectinolytic enzymes. Shweta *et al.* (2016) studied the use of response surface methodology (RSM) to optimize ecological factors for pectinase production from *Rhizopus* sp. Palaniyappan *et al.* (2009) also studied optimization method using RSM to monitor the effect of different parameters on pectinase production.

From the results of the present study it may also be concluded that mosambi peels which are inexpensive can produce maximum activity of PME and PG. Pure commercial pectin is too expensive to be used as substrate so mosambi peels are best alternative as a source of pectin substrate. So it is suggested that mosambi peels may be a good alternative for PME and PG production from industrial point of view. So the optimum conditions for maximum PME activity were pH 6, temperature 35°C, time period 3 day, substrate concentration 3 g and 3 ml inoculum size, which on validation produced PME activity of 394.82 U/ml. Whereas, PG gave maximum activity at pH 4, temperature 45°C, time period of 5 day, substrate concentration 25 g and 1 ml inoculum size. And these results was in good confirmation with the predicted values thus proving the accuracy of the model.

The production of PME and PG by mosambi peels is a suitable way of converting agro-industrial wastes into useful products. The results of the present study revealed that *Schizophyllum commune* can produce PME enzyme using agricultural residue like mosambi peels as a substrate in solid state fermentation. Results of this study showed that nutrients and cultural properties played a crucial role in enzyme production. The optimizations of all the parameters are being considered as pre-requisites to make the process of enzyme production inexpensive at industrial scale. The projected model is effective to be used for fruit juice industry and detergent industry.

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References

Alcantara SR, Francisco AC, Almeida D, Flavio LH and Silva D 2010. Pectinases production by solid state fermentation with cashew apple bagasse: Water activity and influence of nitrogen source. Chem. Engine. Tran. **36**: 121-126.

Ali MB, Irshad M, Anwar Z, Zafar M and Imran M 2016. Screening and statistical optimization of physiochemical parameters for the production of xylanases from agroindustrial wastes. Adv. Enzyme Res. **4**: 20-33.

Bhargav S, Panda BP, Ali M, Javed S, Bhargav 2008. Solid State Fermentation: An Overview. Chem. Biochem. Eng. Q. **22**: 49-70.

Carley KM, Kamneva NY and Reminga J. Response Surface Methodology. CASOS Technical Report. October 2004; CMU-ISRI 04-136.

Demirdoven A and Baysal T 2014. Optimization of Ohmic heating applications for pectin methylesterase inactivation in orange juice. J. Food Sci. Technol. **51**: 1817-1826.

Hossam S and Hamdy 2011. Purification and characterization of pectin lyase produced by *Rhizopus oryzae* grown on orange peels. Annl. Microbiol. **55**: 205-211.

Hubert KKD, Parfait KEJ, Constant YJ and Kablan T 2017. Partial purification and Characterization of Two Pectinases (Polygalacturonase and Pectin lyase) from Papaya Pericarp (*Carica papaya* cv. solo 8) Int. J. Curr. Microbiol. App. Sci. **6**: 2729-2739

Jesus A. Tovar S, Adriana C, Gallegos F, Juan C, Esquivel S, Garcia E, Jesus A, Chavez M and Herrerar RR 2017. Analytical Methods for Pectin Methylesterase Activity Determination: A Review. Food Anal. Methods. **10**: 3634-3646.

Kushani R, Ziaee E, Niakosri M and Golmakani T 2014. Optimization of thermal and thermosonication treatment on pectin methyl esterase inactivation of sour orange juice (*Citrus Aurantium*). J. Food Process. Preserv. **39**: 567-573.

Marcos AB, Ricardo ES, Eliane PO and Villar LS 2008. Response surface methodology as a tool for optimization in analytical chemistry. Talanta. **76**: 965-977.

Mehmood T, Saman T, Irfan M, Anwar F, Ikram MS, Tabassam Q 2018. Pectinase production from schizophyllum commune through central composite design using citrus waste and its immobilization for industrial exploitation. Waste Biomass Valor. https://doi.org/10.1007/s12649-018-0279-9

Murudula S and Anitharaj R 2011. Pectinase production in solid state fermentation by *Aspergillus niger* using orange peels as substrate. Global. J. Biochem. Biotehnol. **6**: 64-71.

Naz S, Irfan M and Farooq MU 2017. Xylanase production from *Bacillus subtilis* in submerged fermentation using Box- Behenken Design. Pak. J. Biotechnol. **14**: 151-156.

Palaniyappan M, Vijayagopal V, Renuka V and Viruthagiri T 2009. Statistical optimization of substrate, carbon and nitrogen source by response surface methodology for pectinase production using *Aspergillus fumigatus* MTCC 870 in submerged fermentation. Afr. J. Biotechonl. **8**: 6355-6363.

Patcharaporn P, Aran HK, Ohsugi, Masahir and Yaowaluk D 2008. Production and Properties of a Fibrinolytic enzyme by *Schizophyllum commune* BL23. J. Sci. Technol. **30**: 447-453.

Poturcu K, Ozmen I, Biyik HH 2017. Characterization of an alkaline thermostable pectin lyase from newly isolated *Aspergillus niger* WHAK1 and its application on fruit juice clarification. Arab. J. Sci. Eng. **42**, 19-29

Renge VC, Khedkar SV and Nikita AR 2012. Enzyme synthesis by fermentation method: A review. Sci. Revs. Chem. Commun. **2**: 585-590.

Sandhya R and Kurup G 2013. Screening and isolation of pectinase from fruit and vegetable wastes and the use of orange waste as a substrate for pectinase production. Int. Res. J. Biological. Sci. **2**: 34-39.

Shweta H, Nivedita S and Shruti P 2016. Multiple parameter optimization for maximization of pectinase production by *Rhizopus* sp. C4 under solid state fermentation. Fermentation. **2**: 1-9.

Siumara R, Alcantara, Francisco AC, Almeida D and Flavio LH 2010. Pectinases production by solid state fermentation with cashew apple bagasse: water activity and influence of nitrogen source. Chem. Eng. Trans.**20**: 121-126.

Tapre AR and Jain RK 2014. Pectinases: Enzymes for fruit processing industry. Int. Food Res. J. **21**: 447-453.

Torres EF, Sepulveda TV and Gonzalez GV 2006. Production of hydrolytic depolymerising pectinases. Food Technol. Biotechnol. **44**: 221-227.

Yadav KK, Garg N, Kumar D, Kumar S, Singh A and Muthukumar M 2015. Application of response surface methodology for optimization of polygalacturonase production by *Aspergillus niger*. J. Environ Biol. **36**: 255-9.

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