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Statistical optimisation of xylanase production by estuarine *Streptomyces* sp. and its application in clarification of fruit juice

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Abstract   Recently, xylanase has become an essential option for environmental friendly industrial biotechnological applications and the rising demand for its large scale production urge to take the advantage of statistical approach of optimization to investigate the interactive effects of prominent process factors involved to enhance xylanase production. In the present study, xylanase production from *Streptomyces* sp. strain ER1 isolated from Cochin estuarine soil; was optimised using statistical designs- Plackett-Burman and Central composite design. Plackett-Burman design was used to identify important fermentation condition factors affecting the xylanase production using beechwood xylan as the substrate. The optimum levels of these significant factors were determined employing the Central Composite Design. Out of the thirteen factors screened, concentration of beechwood xylan and olive oil, agitation speed, and inoculum age were recognized as the most significant factors. By analyzing the response surface plots and using numerical optimization method, the optimal levels for concentration of xylan and olive oil, agitation speed and inoculum age were determined as 0.37%, 33.10 mg/L, 42.87 RPM and 21.05 h, respectively. The optimised medium resulted in a 1.56-fold increased level of the xylanase (10,220 U/mL) production compared to the initial level (3986.444 U/mL) after 120 h of fermentation. The purified enzyme could successfully clarify orange, mousambi and pineapple juice to 20.87%, 23.64% and 27.89% respectively. Thus the present study has proved that *Streptomyces* sp. strain ER1 (KY449279) is a potential and useful organism for xylanase production and its purified enzyme could clarify the selected fruit juices.

1. Introduction

Enzymes found their way in several new industrial processes after being discovered in the latter half of the 19th century as an effective biocatalyst [8]. The biotechnological applica-

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tions of xylanases started in 1980s. Xylan, the polymer of xylose is the main component of hemicellulose found plentifully in plant biomass. The structure of xylan varies and this heteropolysaccharide is randomly hydrolysed by endoxylanase to form xylooligosaccharides, which are then degraded by xylanolytic enzymes such as xylosidase and arabinofuranosidases. Accessory enzymes are able to cleave side chain groups of heteroxylan [4]. The possible biotechnological applications of microbial xylanases in various industries include the food, feed, fuel, textile, detergents, paper and pulp industries and in waste treatment [21,6].

The high demand and cost of available commercial enzymes urge for bioprospecting and manipulation of microbes for higher productivity and enhancement of existing commercial enzymes [10,11]. Enzyme productivity can be increased by optimizing the production processes [9]. The conventional 'one-variable-at-a-time' approach is time restrictive and also ignores the combined interaction(s) among various physical and nutritional parameters [28]. Consequently, statistical factorial designs like Plackett-Burman design (PBD) screens medium components for their significant result on product formation [9]. The significant media components can then be optimised using response surface methodology (RSM) [30], to get better enzyme yield by designing least number of experiments for a large number of factors [3]. RSM explains the combined effects of all the independent variables in a fermentation process and explores an estimated interaction between a response variable and a set of design-independent variables [10].

Fruit juices prepared by simple extraction are cloudy, viscous and turbid due to the presence of polysaccharides such as starch, pectin, cellulose, hemicelluloses and bound lignin [13]. The cloudy juices contain fewer yields, less acceptability and are difficult to pasteurise and concentrate [24]. Therefore enzymes have been exploited for degradation of all polymeric carbohydrates, thus enabling better processing of pulp to improve the yield of substances contained in the fruit, to liquefy entire pulp for maximum yield, optimal juice clarity and a quality product that ensures consumer appeal [7].

Numerous investigations on varying cultivation conditions for optimal xylanase production by different fungal and bacterial strains have been done; for instance, fungal genera including *Trichoderma*, *Penicillium*, and *Aspergillus* [13,14]; and the bacterial genera such as *Bacillus*, *Cellulomonas*, *Clostridium*, *Thermonospora* and *Arthrobacter* [15]. However, reports on marine bacterial isolates are a promising area of interest [16]. The marine environment contains complex polysaccharides (CP) derived from terrestrial and aquatic sources; hence, it is a rich resource for microorganisms equipped with the enzymes series for CP degradation [17,18]. Furthermore, actinomycetes are a microbial group not fully exploited for hydrolytic enzyme production despite their record of expansive metabolite production [19,20]. *Streptomyces* species are notable producers of several highly active secondary metabolites and extracellular enzymes [22,21].

Keeping the above in view, the present study is an attempt to enhance xylanase production from a hyper xylanolytic *Streptomyces* sp. strain ER1 through optimisation of nutritional/physical parameters by statistical approaches by Plackett-Burman design and RSM at shake flask level. A regression model was established and the experimental verification of the model was validated. This study also investigated

applications of xylanase in juice clarification of selected fruit juices. To our knowledge, this is the first report on xylanase production by *Streptomyces* sp. strain ER1 isolated from Cochin estuary in submerged fermentation.

2. Materials and methods

2.1. Microorganism and inoculum preparation

Actinomycete cultures were isolated from sediment samples of Cochin estuary [23]. One promising strain ER1 with good xylanase activity was selected. To confirm the identification of strain ER1, the isolate was subjected to 16S rRNA gene amplification using the universal primer set (27F: 5′-AGA GTT TGATCCTGG CTC AG-3, 149R: 5′-GGT TAC CTT GTTACG ACTT-3′). The reaction conditions included an initial denaturation at 94° for 5 min, followed by 30 cycles of denaturation at 94° for 40 s, annealing at 55 °C for 40 s, and extension at 72 °C for 1 min 30 s. A final extension at 72 °C for 10 min was also included. The PCR product was sent for sequencing at Scigenom, Kochi. The sequence obtained was submitted to the basic local alignment search tool (BLAST; http://www.ncbi.nlm.nih.gov/BLAST) at NCBI (National Center for Biotechnology Information) to determine the percentage similarity with already identified 16S rRNA sequences in the GenBank database. The sequence was deposited in the Genbank with an accession number KY449279. *Streptomyces* sp. strain ER1 was used for xylanase production in this study. The actinomycete was subcultured in nutrient agar slants containing 1% beechwood xylan (pH 7.0) and incubated at 35 °C for five days. Spores of the culture was harvested and collected.

2.2. Extracellular xylanase production and extraction

The unoptimized fermentation medium is composed of (g/l): Beechwood xylan 0.4%, potassium dihydrogen phosphate 0.03 g, calcium chloride 0.04 g, potassium nitrate 0.09 g, casein 0.0015 g, peptone 0.0015 g, olive oil: 0.0015 g, trace element solution 0.054 ml that comprised of zinc sulphate : 2.8 mg, manganous chloride : 32 mg, ferrous sulphate : 10 mg, cobaltous chloride : 4 mg. The pH was maintained at 7. The fermentation medium was inoculated with 5% (v/v) inoculum containing 452×10^1 colony forming units (CFU) ml^{-1} and incubated at 28 °C for 120 h on an orbital shaker. Each sample was then centrifuged at 24,000g, at 4 °C for 15 min and the clear supernatant was assayed for xylanase activity.

For optimisation studies, the composition of the culture medium and other fermentation conditions was varied according to the experimental data.

2.3. Xylanase assay

Xylanase activity was determined using beechwood xylan (Sigma, Germany) [1]. A 0.2 ml culture supernatant was added to 1 ml xylan solution (1%; pH 7.0; 100 mM sodium phosphate buffer) and incubated at 55 °C. After 30 min; 3 ml 3,5-dinitrosalicylic acid reagent was added and the amount of reducing sugars released in the reaction was estimated by measuring the absorbance at 540 nm [15]. A control was run simultaneously which contained all the reagents but the reaction was

terminated prior to the addition of enzyme extract. One unit of xylanase activity is defined as the amount of enzyme catalysing the release of 1 μmol of reducing sugar equivalent to xylose per minute under the specified assay conditions.

2.4. Screening for essential medium components using Plackett-Burman design (PBD)

PBD was used to screen the medium and physical components for their influence on xylanase production by *Streptomyces* sp. strain ER 1. Using the PBD, 12 independent variables (pH, incubation temperature, agitation speed, inoculum age and size, salinity, substrate concentration, concentration of nitrogen sources (peptone and potassium nitrate), additive (olive oil), metal ion salts (calcium chloride and manganese chloride) were screened by representing them at two levels, low (−) and high (+) in 20 trials (Table 1). The experiments were carried out in triplicate and the average xylanase activity was recorded as the response. F value and P values and the proportion of variance R^2 determined the model were significant at $P = 5\%$ level.

2.5. Statistical optimisation of factors affecting xylanase production by RSM

Response surface methodology was used to optimise the screened significant components at five levels (−1, 0, +1, −alpha, +alpha) and to find out interaction effects between variables for maximisation of xylanase production (Table 3). Quantitative data generated from these experiments were subjected to analysis of regression through response surface methodology to solve multivariate equations. This methodology allows the modelling of a second-order equation that describes the process. $Y = \beta0 + \beta1A + \beta2B + \beta3C + \beta4D + \beta11A2 + \beta22B2 + \beta33C2 + \beta44D2 + \beta12AB + \beta13AC + \beta14AD + \beta23BC + \beta24BD + \beta34CD$, where Y = response variable; $\beta0$ = intercept; $\beta1$, $\beta2$, $\beta3$ and $\beta4$ = linear coefficients; $\beta11$, $\beta22$, $\beta33$ and $\beta44$ = squared coefficients; $\beta12$, $\beta13$, $\beta14$, $\beta23$, $\beta24$, and $\beta34$ = interaction coefficients; A, B, C, D, $A2$, $B2$, $C2$, $D2$, AB, AC, AD, BC, BD and CD = levels of independent variables.

2.6. Software used

The independent variables of the experimental design were optimised and interpreted using Design Expert Version 7.0 (Stat-Ease Inc., Minneapolis, Minnesota, USA) statistical software. ANOVA was used to validate statistical parameters.

2.7. Purification of xylanase

The cell-free media collected was subjected to different steps of purification including cold acetone precipitation, gel filtration and DEAE-cellulose ion-exchange chromatography by using gradient elution buffer (sodium chloride ranging from 0.05 to 1 M at a flow rate of 1 ml/min) [19].

2.8. Enzyme characterization

SDS-PAGE was performed according to Laemmli [12] with the 4% acrylamide stacking gel and 12% acrylamide separating gel to determine the molecular mass and purity of protein. Staining was carried out with coomassie brilliant blue. For xylanase activity staining (zymography), after native-PAGE resolution; the gel (10% resolving gel) was directly immersed in 1% (w/v) beechwood xylan/0.1 M sodium phosphate buffer (pH 7.0) at 60 °C temperature for 3 h. The gel was stained with Congo red solution (0.1%-10 min) followed by repeated washing with NaCl (1 M) solution. After 20 min, the gel was destained in a 5% (v/v) acetic acid solution [22].

2.9. Clarification of fruit juices

Orange (*Citrus sinensis*), Pineapple (*Ananas comosus*) and Mosambi (*Citrus limetta*) were purchased from local markets, washed, peeled and macerated using a blender to form a smooth textured pulp. The enzyme and pulp were incubated at 55 °C. After incubation, the suspension was heated in a boiling water bath for 5 min; cooled, filtered and centrifuged at 24,000g for 15 min. The supernatant (juice) was used for determining juice clarity by recording transmittance at 650 nm taking distilled water as the blank. % Clarification was calculated as follows: % clarification = Tt − Tc/Tc × 100; Tt = Transmittance of

Table 1 Variables and their levels employed in Plackett-Burman design for screening of culture conditions affecting xylanase production by *Streptomyces* sp. strain ER1.

Factor	Name	Units	Minimum (−)	Maximum (+)
A	Day of incubation	Days	5	8
B	Xylan concentration	%	0.2	0.4
C	pH		6	9
D	Age of inoculum	Hours	20	30
E	Size of inoculum	%	3	9
F	RPM		60	150
G	Salinity	ppt	7	18
H	Temperature	°C	30	40
I	Olive oil (additive)	mg	100	200
J	Ammonium chloride (inorganic nitrogen source)	gm	1	10
K	Peptone (organic nitrogen source)	gm	0.1	1
L	Manganese chloride (micro ion salt)	mg	100	200
M	Calcium chloride (macro ion salt)	gm	1	10

Table 2   Statistical analysis of components by Plackett-Burman design for xylanase production by *Streptomyces* sp. strain ER1.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	61594193	4	15398548	52.68084	0.0000000118	Significant
B-xylan concentration	1736685	1	1736685	5.941471	0.027712	
D-age of inoculum	4518820	1	4518820	15.45959	0.001332	
F-RPM	45624139	1	45624139	156.0873	0.00000000249	
J-olive oil	9714549	1	9714549	33.23499	0.0000373	
Residual	4384482	15	292298.8			
Cor Total	65978675	19				

Table 3   Selected factors and their levels employed in Central Composite design for optimisation of culture conditions affecting xylanase production by *Streptomyces* sp. strain ER1.

Factor	Name	Units	Minimum (−1)	0	Maximum (+1)	− alpha	+ alpha
A	Olive oil concentration	mg	5	35	65	2.5	95
B	Xylan concentration	%	0.35	0.40	0.45	0.30	0.50
C	Agitation speed	RPM	42.5	57.5	72.5	27.50	87.50
D	Age of inoculum	Hours	16.5	19.5	22.5	13.50	25.50
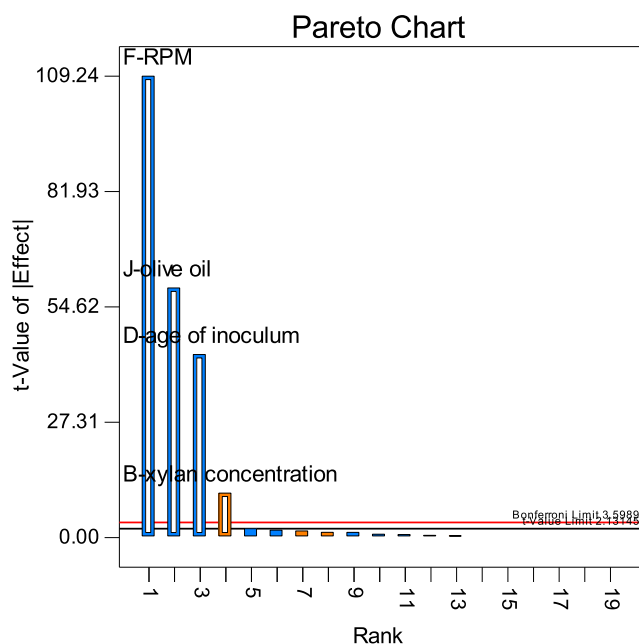
Figure 1   Pareto chart showing the effect of media components on xylanase production.

test; Tc = Transmittance of control. Effect of clarification time for all the juices was studied for 4 h by taking 2 ml of the sample at every 30 min of free enzyme respectively and transmittance was recorded at 650 nm. The flow rate of the juice was determined as described by Sharma and Chand [25]. Total titrable acidity (% citric acid) of the juice was determined as described by Nagar et al. [17]. The amount of reducing sugar was

determined using DNS method. All the experiments were performed in triplicates and their mean values are given. The untreated pulp for each of the fruit was kept as control.

3. Results and discussion

The statistical design approach was used in order to optimise medium components and physical parameters for enhancing xylanase production from *Streptomyces* sp. strain ER1. Previous reports had shown xylanase production from microbial sources using the statistical method [27,29].

3.1. Statistical optimisation of media

In statistical optimisation, initially, Plackett-Burman was designed and used for screening the significant variables affecting the enzyme production. The second step, central composite design optimised significant variables along with their interaction on enzyme production.

3.1.1. Plackett-Burman design

Plackett-Burman is a complete factorial design, to identify the significant factors affecting a response with two-factor interactions. It was carried out by using 20 runs. Twelve factors were analysed for their effects on xylanase production (Supplementary Table 1). Further the polynomial equation which was used to explain the xylanase production as:

$$R1 = 10089.19 + 2946.765 * \text{xylan concentration} - 95.0665$$
$$* \text{age of inoculum} - 33.5637 * \text{agitation speed}$$
$$- 13.9388$$
$$* \text{olive oil, where R1 is the xylanase activity (U/mL)}.$$

Table 4 Statistical analysis of components by Central composite design for xylanase production by *Streptomyces sp.* strain ER1.

Source	Sum of		Mean	F	p-value	
	Squares	df	Square	Value	Prob > F	
Block	6.591E + 007	1	6.591E + 007			
Model	1.387E + 008	14	9.908E + 006	131.26	<0.0001	Significant
A-olive oil	3.657E + 005	1	3.657E + 005	4.84	0.0450	
B-xylan concentration	1.251E + 006	1	1.251E + 006	16.57	0.0011	
C-rpm	4575.25	1	4575.25	0.061	0.8091	
D-age of inoculum	8.402E + 005	1	8.402E + 005	11.13	0.0049	
AB	7.205E + 006	1	7.205E + 006	95.45	<0.0001	
AC	5.940E + 006	1	5.940E + 006	78.68	<0.0001	
AD	8.416E + 005	1	8.416E + 005	11.15	0.0049	
BC	5.396E + 005	1	5.396E + 005	7.15	0.0182	
BD	7.249E + 007	1	7.249E + 007	960.34	<0.0001	
CD	7.094E + 006	1	7.094E + 006	93.97	<0.0001	
A^2	1.437E + 007	1	1.437E + 007	190.36	<0.0001	
B^2	8.285E + 005	1	8.285E + 005	10.98	0.0051	
C^2	3.258E + 006	1	3.258E + 006	43.16	<0.0001	
D^2	2.369E + 007	1	2.369E + 007	313.84	<0.0001	
Residual	1.057E + 006	14	75488.51			
Lack of Fit	3.212E + 005	10	32118.72	0.17	0.9884	Not Significant
Pure Error	7.357E + 005	4	1.839E + 005			
Cor total	2.057E + 008	29				

ANOVA showed that the Model *F*-value of 52.68084 implies the model is significant. Table 2 shows the Student *t*-distribution, the corresponding *P*-value, and the parameter estimate. The coefficient of determination (R^2) for xylanase activity was 0.8613, that is close to 1 and it's explained to 86.13% variability of the response. The predicted R^2 value 0.933547 and the adjusted R^2 value are 0.915826. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of our model is 22.028 which indicated an adequate signal. This model is applicable to the design space.

The Pareto graph is an important tool for analyzing all the parameters and to focus the most significant factors. The less significant factor is identified by having the greatest cumulative effect on the system. In the present analysis, the Student's *t* values for inoculum age, agitation speed, the concentration of olive oil and xylan shows that these were the most significant factors affecting the enzyme production (Fig. 1). Thus the values of Pareto chart & ANOVA table, four most significant factors were selected for second level optimisation by CCD. Other negative factors were eliminated.

3.1.2. Central composite design

CCD was carried in order to verify the optimal concentration of crucial factors and their interaction with each other after the PB designing. In this step inoculum age, agitation speed, the concentration of olive oil and xylan were optimised by response surface methodology (RSM). The results suggested a close agreement between the observed and predicted values of both the responses (Supplementary Table 2), which showed the accuracy of RSM in order to optimise the parameters for enhanced enzyme production. The second order polynomial equation was used to explain the xylanase production and interaction among the factors (in coded form) as:

$$
\begin{aligned}
\text{Xylanase activity} =\ & -1.53810E + 005 + 0.77808 * \text{Olive oil} \\
& + 2.98031E + 005 * \text{Xylan concentration} \\
& + 60.91500 * \text{agitation speed} \\
& + 10581.17684 * \text{age of inoculum} \\
& + 447.37625 * \text{Olive oil} \\
& * \text{Xylan concentration} - 1.35398 \\
& * \text{Olive oil} * \text{agitation speed} - 2.54837 \\
& * \text{Olive oil} * \text{age of inoculum} + 244.84750 \\
& * \text{Xylan concentration} * \text{agitation speed} \\
& - 14190.62917 * \text{Xylan concentration} \\
& * \text{age of inoculum} - 14.79660 \\
& * \text{agitation speed} * \text{age of inoculum} \\
& - 0.80423 * \text{Olive oil}^2 - 69521.04167 \\
& * \text{Xylan concentration}^2 + 1.53173 \\
& * \text{agitation speed}^2 - 103.26404 \\
& * \text{age of inoculum}^2
\end{aligned}
$$

Analysis of variance (*F*-test) showed that the second model is well attuned to the experimental data. The coefficient of variation (CV) indicates the degree of precision with which the treatments are compared. The lower value of CV (3.71) indicates a greater reliability in the experiments performed. The determination coefficient (R^2), which is commonly used to check the goodness of the model, implies that the sample variation of 99.24% for xylanase production is attributed to the independent variables in the current study. The "Pred R-Squared" of 0.9757 is in reasonable agreement with the "Adj
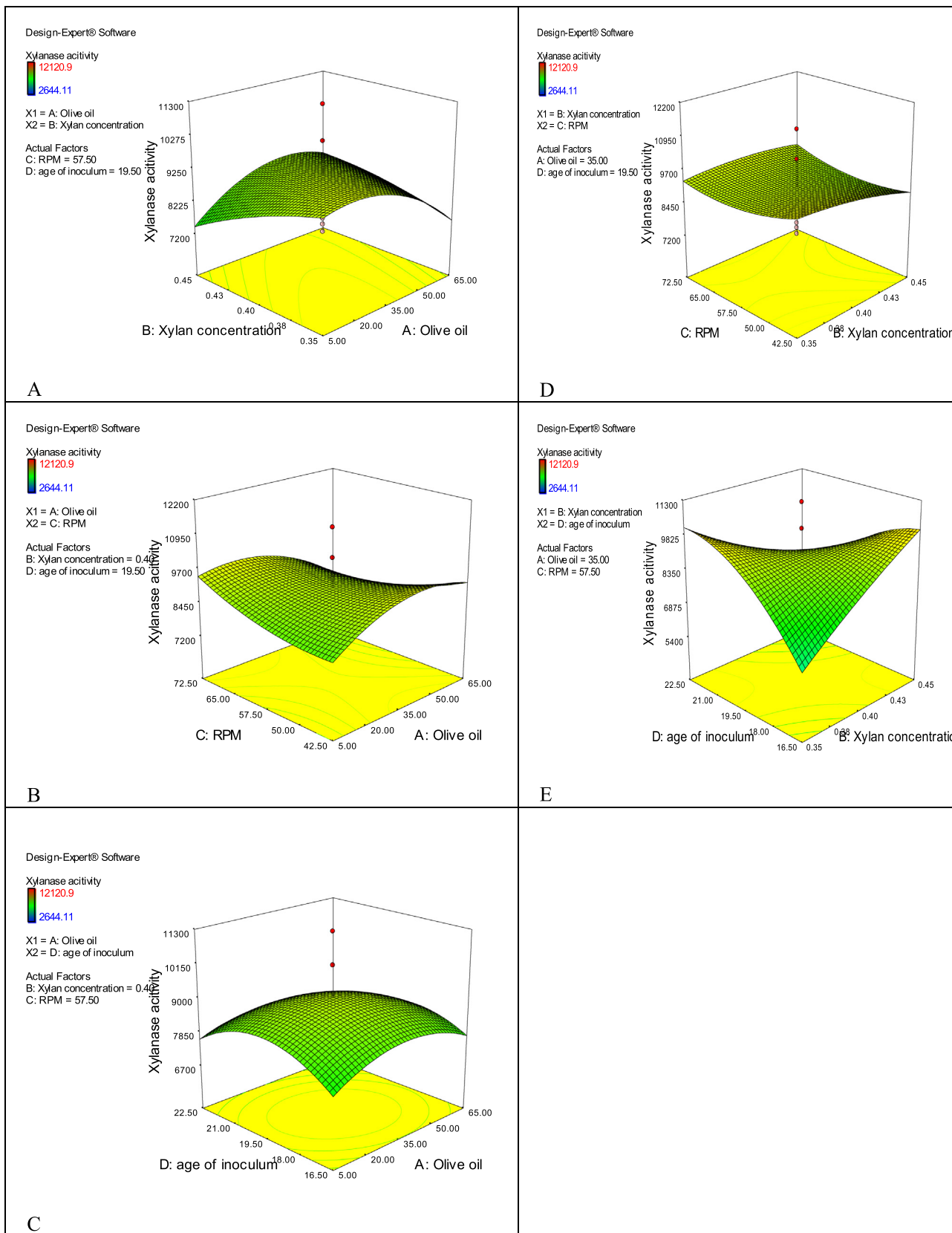
Figure 2   3D graph showing the interaction between significant factors effecting xylanase production. A: concentration of xylan and olive oil; B: RPM and olive oil concentration; C: age of inoculum and olive oil concentration; D: RPM and xylan concentration; E: age of inoculum and xylan concentration.
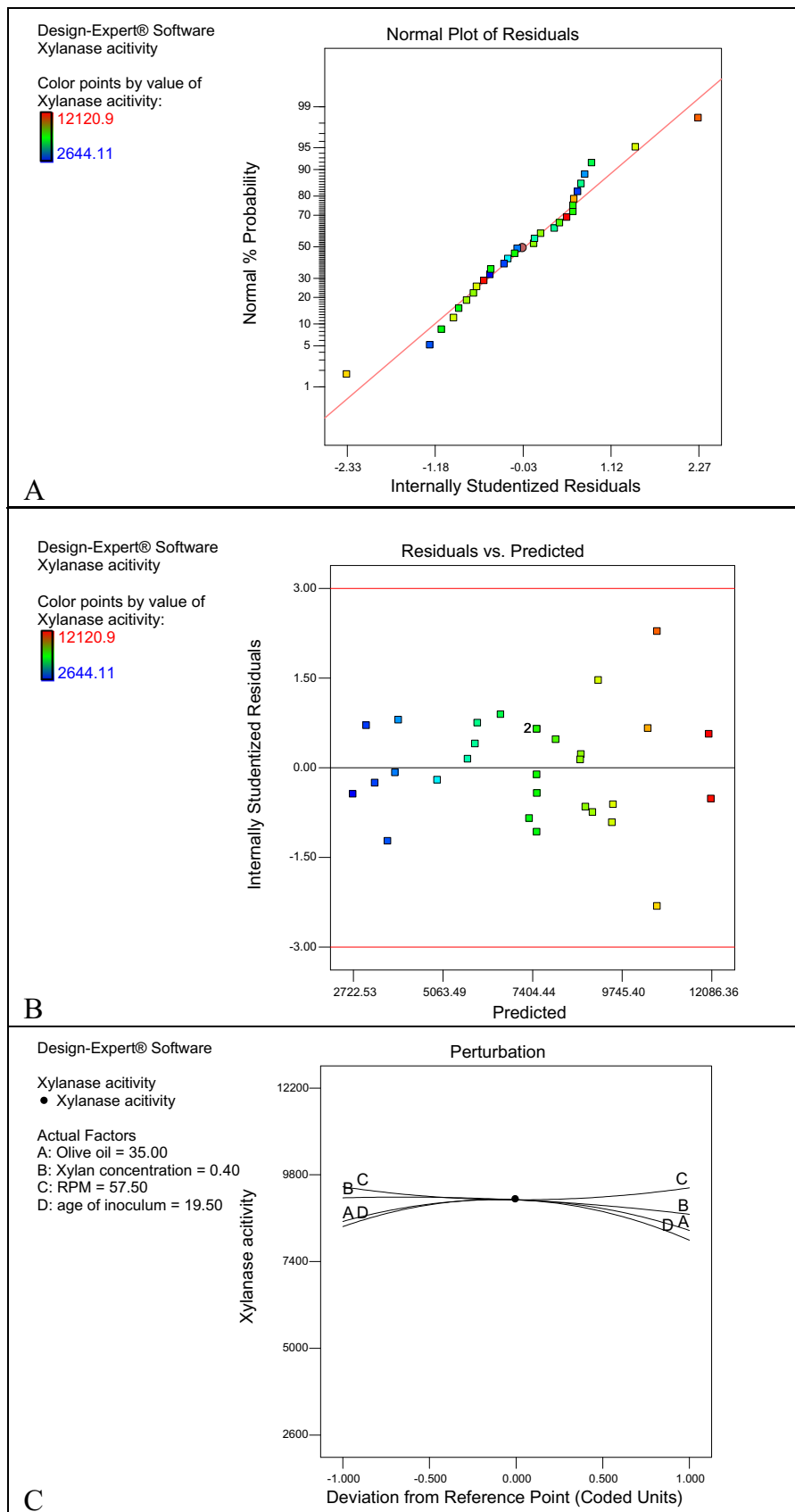
Figure 3 (A) Parity plot showing the distribution of experimental vs predicted values of xylanase production. (B) Graph showing the interaction between the actual and predicted values. (C) Perturbation graph showing the effect of variables on xylanase production by *Streptomyces* sp. strain ER1.

Table 5 Partial purification table for extracellular xylanase produced by *Streptomyces* sp. strain ER1.

Sample	Total protein (mg)	Activity (U)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude extract	200	9776	48.88	100	1
DEAE-Cellulose 52	2.5	734.9333	293.97332	7.52	6.01
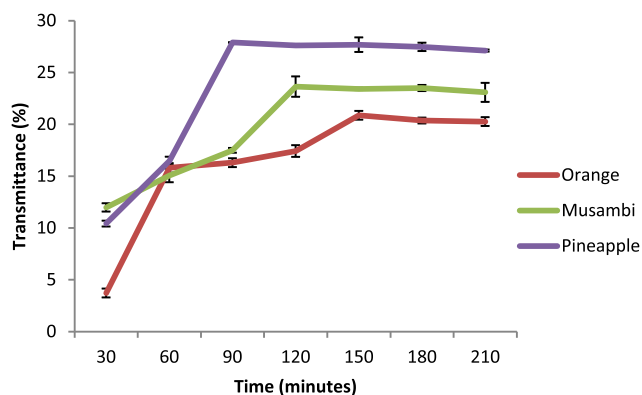
Figure 4 Effect of time on % clarification of fruit juices.

R-Squared" of 0.9849 which depicted adequacy of the model to predict response. Linear, cross product and quadratic terms were significant at the 1% level. Therefore, the quadratic model was selected in this optimisation study. Table 4 shows the Student *t*-distribution, the corresponding *P*-value, and the parameter estimate.

3.2. Interaction of variables

To examine the interaction between two factors on xylanase production, RSM was used and three-dimensional plots were drawn between two factors keeping the third factor at fixed level. The circular shape of the curve indicates no interaction while elliptical shape indicates a good variation of two variables. The interaction between the independent variables such as the concentration of olive oil (additive) and xylan; olive oil concentration and agitation speed; inoculum age and olive oil concentration; xylan concentration and agitation speed; inoculum age and xylan concentration showed a significant effect on xylanase activity by *Streptomyces* sp. strain ER1 (Fig. 2A-E).

3.3. Validation of the experimental model

To obtain the maximum yield of xylanase production, point prediction was used to assess the model validity and to assess optimal level parameters. The model predicted the optimum concentrations of olive oil, xylan, agitation speed and inoculum age were 33.10 mg/l, 0.37%, 42.87 RPM and 21.05 h, respectively, and maximum response 10292 U/ml. The observed xylanase activity versus the predicted xylanase activity (parity plot) under optimum fermentation conditions is shown in Fig. 3A. A repeat fermentation of xylanase by *Streptomyces* sp. strain ER1 under optimal condition was carried out to verify the optimisation. The maximum xylanase level obtained was 10,220 U/ml, which was close to the predicted

value. The residuals versus predicted plot shows (Fig. 3B) that the residuals scattered randomly on the display, signifying that the variance of the original observation was constant for all values of xylanase enzyme activity (Y). Hence, the conclusion can be drawn that the empirical model was adequate to describe the xylanase activity by response surface.

The effect of variations in the level of independent factors on xylanase production can be seen in the perturbation graph. The plot (Fig. 3C) revealed that the produced xylanase activity was most sensitive to inoculum age, followed by olive oil concentration.

3.4. Partial purification of xylanase

Protein precipitated from crude enzyme by acetone precipitation was applied to DEAE-Cellulose anion exchange column (Table 5), and the fractions eluted were subjected to xylanase assay. The active fractions were then pooled and electrophoretic mobility of the fractions was analysed by SDS-PAGE. The monomeric band was found to be approximately 23 KDa which was identical to the enzyme reported from *Streptomyces cyaneus* SN32 [20] and *Streptomyces* sp. SKK1-8 [14]. The zymogram of xylanase exhibited a single significant activity band.

3.5. Clarification of fruit juice

The maximum amount of juice clarification in case of orange, mousambi and pineapple juice was observed at 150, 120 and 90 min respectively. Enzyme treated juice gave 20.87%, 23.64% and 27.89% increase in clarity of orange, mousambi and pineapple juice, respectively. The results are shown in Fig. 4. The clarification is due to disruption of hemicellulosic material and hence polymer xylan is hydrolysed. There is better % clarification in case of the pineapple juice than in mousambi and orange juice. Similar observation was made by Kumar et al. [11]. Prolonged incubation time could result in the formation of haze particles consisting of protein-carbohydrate and protein-protein complexes [26]. Other researchers have documented 30–90 min as the optimum time for xylanase treatment [11].

Titratable acidity determines the quality of fruit juice and an increase in titratable acidity would increase the shelf life of juice [2]. It was found to be higher in pineapple and mousambi juices obtained from xylanase- treated fruit pulps as compared to untreated pulps. However, there was no significant variation observed in the acidic neutrality value of the control and enzyme treated juice of the orange. Similar observations were made by Nagar et al. [17]. Based on our study, an increase in filterability of juice after treatment with xylanase was observed which could be the result of xylan hydrolysis by the enzyme (Supplementary Table 3) Similar results were reported by Dhiman et al. [5].

4. Conclusion

The experimental data obtained after statistical optimisation had resulted in increased enzyme production by *Streptomyces* sp. strain ER1. From the present study, it is clear that PBD and CCD can be used effectively, one after the other, to determine the significant variables and their optimum conditions for enhanced xylanase production. Using PBD, xylan concentration, agitation speed, inoculum age and olive oil concentration were identified as the most influencing parameters affecting xylanase production, and CCD helped to study their interactive effects on enzyme yield. The medium designed using a limited number of experiments, less efforts and time resulted in approx. 1.56-fold increase in xylanase production compared to the conventional method of optimisation which is incapable of reaching the true optimum conditions. The statistical strategy, as a whole, proved to be adequate for the design and optimisation of the bioprocess for enhanced xylanase production.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jgeb.2017.06.001.

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