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Statistical optimization of cellulases by *Talaromyces thermophilus* utilizing *Saccharum spontaneum*, a novel substrate

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ABSTRACT

Background: At present, cellulases are the most important enzymes worldwide, and their demand has been increasing in the industrial sector owing to their notable hydrolysis capability.
Results: In the present study, contrary to conventional techniques, three physical parameters were statistically optimized for the production of cellulase by thermophilic fungi by using response surface methodology (RSM). Among all the tested thermophilic strains, the best cellulase producing fungus was identified as *Talaromyces thermophilus* – both morphologically and molecularly through 5.8S/ITS rDNA sequencing. The central composite design (CCD) was used to evaluate the interactive effect of the significant factors. The CCD was applied by considering incubation period, pH, and temperature as the model factors for the present investigation. A second-order quadratic model and response surface method revealed that the independent variables including pH 6, temperature 50 °C, and incubation period 72 h significantly influenced the production of cellulases. The analysis of variance (ANOVA) indicated that the established model was significant ($P \leq 0.05$) and showed the high adequacy of the model. The actual and predicted values of CMCase and FPase activity showed good agreement with each other and also confirmed the validity of the designed model.
Conclusions: We believe the present findings to be the first report on cellulase production by exploiting Kans grass (*Saccharum spontaneum*) as a substrate through response surface methodology by using thermophilic fungus, *Talaromyces thermophilus*.
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1. Introduction

The enzymatic hydrolysis of the cellulosic biomass by cellulases is a combined action of three principle enzymes i.e., endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) [1]. Cellulose is a complex carbohydrate that is present abundantly on the earth and found as a linear chain of repeating units of glucose, which are linked by β-1,4-glucosidic bonds [2,3]. It is an insoluble and fibrous crystalline molecule with high-molecular-weight [4,5]. Cello oligosaccharides are produced by the combined action of endoglucanase and exoglucanase on cellulose and cellobiose, and then β-glucosidase acts on cellobiose and converts it into glucose [6]. Cellulases have enormous applications in different sectors such as food, beverages, textile, laundry, paper, waste management, and pharmaceuticals [7]. Microbial cellulases are preferred over animal and plant cellulases owing to their fast growth, easier manipulation of genetic material, and the ease of handling with no seasonal effects [8]. A large variety of fungi are capable of producing cellulases, but only few of them can produce a considerable amount of enzyme and possesses the ability to hydrolyze crystalline cellulose [9]. Temperature influences microbial growth, and it is considered as one of the most important factors for optimum enzyme production. Among the various categories, thermophilic organisms have caught the attention of microbiologists and biochemists because of their more stable protein structure and resistance against many chemical reagents. Moreover, the saturated fatty acids are abundant in lipids of thermophilic microorganisms, which is the leading feature that allow thermophiles to maintain stability and function of the membrane

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at high temperature [10]. Submerged fermentation is more in demand in contrast to solid state fermentation caused by efficient heat transfer and ease of handling [11]. Proper agitation and proper aeration are other reasons because of which submerged fermentation (SmF) is mainly preferred. Because the production of cellulase enzyme is a major factor in the hydrolysis of cellulosic materials, it is important to make the process economically viable [12,13]. Many valuable products, such as cellulases, can be produced by using Kans grass as a substrate by the microorganisms because it is cheap lignocellulosic biomass and a common source of sugar [14,15]. *Saccharum spontaneum* (Kans grass) is also known as wild sugarcane, a wasteland weed, a grass with height up to 4 m [16]. Being drought- and flood-tolerant, it is found in Pakistan on a large scale. It possess an extensive root system that helps it to establish firmly in the soil with low lignin content and in the easy dispersal of seeds.

A suitable strategy needs to be designed for process optimization that influences the final yield or optimum productivity of enzymes. The optimization of different physiochemical parameters has been conventionally applied by changing one factor at a time (OFAT) and it is effective as long as the production process is influenced by a limited number of variables. However, this technique does not portray the combined effects of all the involved factors. It takes time and requires to conduct many experiments [17,18]. Therefore, the traditional method of one-factor-at-a-time (OFAT) approach for optimization process is found to be more time-consuming. Nonetheless, it also serves the purpose of coarse estimation of the optimal levels. However, Response Surface Methodology (RSM) is used to overcome this problem because it is a combination of mathematical and statistical techniques. RSM is used to design an experiment and enable researchers to evaluate the interactions among all the factors and responses throughout the experiment [19,20]. In the present study, *Saccharum spontaneum* was used for the production of cellulase by thermophilic fungus *Talaromyces thermophile*. The optimization was performed through RSM by using the central composite design.

2. Materials and methods

2.1. Isolation and identification of thermophilic fungi

To isolate thermophilic cellulolytic fungi, samples were collected in the months of June–July, 2017 (Pakistan) from underground deep soil (20–30 cm), different sites of industrial area (Faisalabad), compost area (Lahore), and piles of litter (Islamabad, Lahore, and Rawalpindi). Fungal strains were isolated by the serial dilution method [21]. The selected thermophilic fungal strain exhibiting the highest cellulase production was identified morphologically by using molecular approaches. The molecular identification was carried out by sequencing the 5.8S/ITS rDNA region [22].

2.2. Inoculum preparation

Inoculum was prepared by adding 10 ml of saline water into a four days old slant containing plentiful fungal growth, which was grown at 40 °C. By using an inoculating loop, the spores were carefully scratched and mixed well to obtain a homogenous suspension [23].

2.3. Submerged fermentation

Sterilized Vogel's medium of 100 ml along with 2 g of *Saccharum spontaneum* was inoculated with 1 ml of inoculum. The fermentation process was carried out in a shaking incubator for 72 h at 40 °C (160 rpm). After 72 h, the medium was filtered with a muslin cloth, and filtrate was centrifuged at 6000 rpm for 15 min to obtain a clear supernatant. After centrifugation, the supernatant was used for the estimation of cellulases.

2.4. Enzyme assay

CMCase and Filter-paperase activity (FPase) was determined in accordance with the method of Gao et al. [12].

One unit of the enzyme activity was defined as the amount of enzyme that liberated 1 μmol of glucose from the appropriate substrate under standard assay conditions [21].

2.5. Molecular characterization

Molecular characterization was carried out in accordance with the CTAB method [24,25].

2.6. Pretreatment of Saccharum spontaneum

Saccharum spontaneum was subjected to an alkaline treatment by following the method of Irfan et al. [26].

2.7. Scanning electron microscopy (SEM) of Saccharum spontaneum

Morphological study of untreated and treated *Saccharum spontaneum* was performed at different magnification powers by using a scanning electron microscope.

2.8. Response surface methodology

In the present study, optimization of the vital physiochemical parameters was carried out through RSM modeling. The statistical model was obtained using the Central Composite Design (CCD) with three independent variables such as initial pH (A), temperature (B), and time course (C). Each variable was considered at two levels, i.e., a low −1 and high +1 value (Table 1). The range values of the three independent variables were checked against a dependent variable Y (CMCase and FPase activity). A total of 20 experiments were performed to determine the effect of three physical factors using Design Expert Version 11. An expression of quadratic polynomial regression model (Equation (1)) was derived to explain the relationship between dependent and independent variables:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \quad (1)$$

In the above equation; Y represents predicted response (activity of cellulases), β_0 is the constant coefficient, β_1, β_2, and β_3 are the linear coefficients, β_{11}, β_{22}, and β_{33} are the quadratic coefficients, β_{12}, β_{13}, and β_{23} are the cross products coefficients, and A, B, and C were point variables.

The enzyme activity was analyzed using the analysis of variance (ANOVA) combined with Fisher's test to evaluate whether a given term has a significant effect ($p \leq 0.05$). The optimum levels of the variables were obtained by graphical and numerical analyses using a Design Expert program.

Table 1
Levels of factors chosen for the experimental design for CMCase and FPase activity.

Factors	Units	Actual levels of coded factors	
pH	–	4	9
Temperature	°C	40	70
Time course	Days	1	5

3. Results and discussion

3.1. Isolation of thermophilic fungi

The choice of an appropriate fungal strain is a fundamental step for the hyper production of cellulases. In the present research, cellulases producing thermophilic fungal strains were isolated from both soil and compost. Among 12 thermophilic strains, the strain TS-9 gave the best activity of CMCase (0.10 ± 0.1 U/ml) and FPase (0.91 ± 0.03 U/ml) and had been identified as *Talaromyces thermophilus* on the basis of morphological characteristics (Table 2). The colony morphology showed a grayish color in the center and possessed brush-like penicillus [27,28] (Fig. 1). Sometimes, morphological identification of fungal cultures becomes challenging because of the limited number of morphological characters. Unlike morphological identification, the molecular identification technique is more specific, fast, and sufficient to distinguish between the species of different fungi [29,30]. The morphologically identified fungal strain was further verified by analyzing at the molecular level by sequencing the specific ITS regions (5.8S rDNA). The ITS rDNA sequences were compared with those in the databases by using NCBI-BLAST. The sequence similarity was checked relative to the corresponding sequences in the GenBank (Fig. 2). The obtained sequence was deposited in NCBI GenBank and obtained accession no. was MW391556. It was also considered that ITS (Internal Transcribed Spacer region) 5.8S rDNA region sequences from the database confirmed that ITS 5.8S rDNA region can be used to differentiate fungi at the species level. Some studies demonstrated that fungal strains can be distinguished on the basis of the size of the ITS/5.8S fragment [31,32,33]. These regions have the potential for phylogenetic analysis owing to their universal distribution, persistent function, and enough conservation that provides a deep view of evolutionary relationship [34]. Phylogenetic analysis was conducted to ascertain the phylogenetic position and the taxonomy of TS-9. A phylogenetic tree was constructed through MEGA 7 by using the Maximum Likelihood method. Moreover, the likelihood method estimates the unknown parameters of a probability model and gives information on probabilities related to the evolution of that sequence [35]. The isolated fungus is closely related to *Talaromyces* sp. with 1000 bootstraps and identified as *Talaromyces thermophilus*.

3.2. Fermentation process

Low cost agro waste, *S. spontaneum*, was used as a carbon source in fermentation media to reduce the cost of production process because its cell wall consists of high amount of carbohydrates i.e., cellulose and hemicellulose which together makes 67.85% of the total carbohydrate content (TCC) of *S. spontaneum* [14]. However, in the present study, different concentrations of *S. spontaneum* ranging from 1%–7% were estimated for CMCase and FPase activity. Among all the concentrations, the maximum cellulase activity was achieved at 2% and decreased thereafter as shown in Fig. 3. The topography of untreated and pretreated *S. spontaneum* was determined through SEM. The images at 500X magnification revealed that the alkaline pretreatment of the substrate had fewer lignin contents in contrast to the untreated substrate (Fig. 4a & b). The alkaline pretreated substrate exhibited a more intact surface which provides better surface area for the enzyme to react toward the degradation of hemicellulose and cellulose. The present results were in agreement with the study of Komolwanich et al. [36].

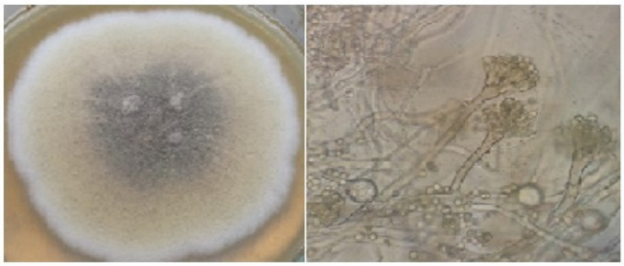


Fig. 1. *Talaromyces thermophilus* (a) Colony (b) Microscopic image.

3.3. Optimization of significant physical factors for the production of cellulases by using the response surface methodology (RSM)

RSM integrates the interactive effect of different variables and help us to optimize various process parameters simultaneously within a minimum number of experimental runs. In the present investigation, a three-factor CCD was used to optimize the independent variables, i.e., time course, pH, and temperature by *T. thermophilus* by using *Saccharum spontaneum*. A series of 20 experiments were conducted with the possible combinations of these three independent variables with two coded levels (−1 and +1). For both CMCase and FPase activities, all the screened parameters were found to have a positive effect on cellulases production (Table 3).

RSM simulation proposed that the quadratic model was the most suited approach to explain the relationship between responses and factors. Second-order polynomial equation showed the empirical relationship between the independent factors and responses (CMCase and FPase) which are given in Equation (2) and Equation (3).

CMCase activity

Table 2
Screening of fungal strains for CMCase and FPase activity.

Sr No.	Fungal Isolates	CMCase activity U/ml	FPase	Macro and microscopic characteristics
1	*Penicillium* sp.	0.02 ± 0.01	0.34 ± 0.05	Initially the colonies appear white-cream to yellow and then turns to green when matures,
2	*Penicillium* sp.	0.06 ± 0.03	0.56 ± 0.09	penicilli comprised phialides and may contain both branches and metulae, Conidiophores are hyaline
3	*Penicillium* sp.	0.07 ± 0.03	0.73 ± 0.07	
4	*Penicillium* sp.	0.03 ± 0.01	0.43 ± 0.12	
5	*Penicillium* sp.	0.08 ± 0.03	0.76 ± 0.05	
6	*Penicillium* sp.	0.06 ± 0.03	0.51 ± 0.04	
7	*Penicillium* sp.	0.05 ± 0.02	0.49 ± 0.09	
8	*Humicola* sp.	0.06 ± 0.02	0.54 ± 0.10	Initially, colonies are colorless and then turns to light brown to black with globose shape.
9	***Talaromyces* sp.**	**0.10 ± 0.10**	**0.91 ± 0.03**	Greyish color in the center and possesses brush like penicillus
10	*Trichoderma* sp.	0.08 ± 0.04	0.71 ± 0.11	Initially, colonies are white and later become yellowish-green to deep green. Flask-shaped conidiophores.
11	*Thermomyces* sp.	0.04 ± 0.02	0.45 ± 0.08	Initially the colonies appear white very thin but soon become grey or greenish and septate.
12	*Aspergillus* sp.	0.07 ± 0.04	0.64 ± 0.05	Colonies are typically blue-green and finely roughened with smooth walled conidiophores.

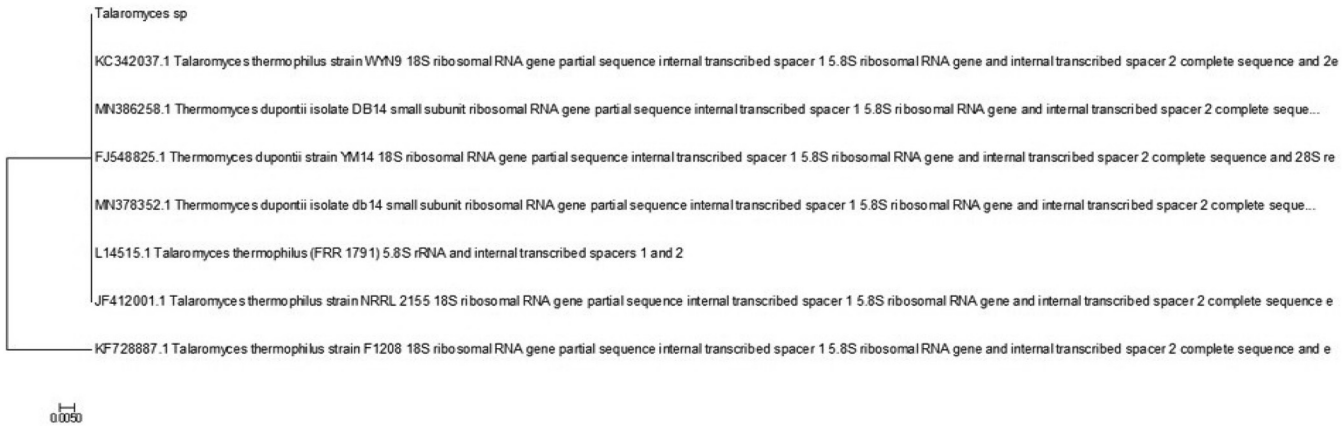


Fig. 2. Phylogenetic tree of *T. thermophiles.*

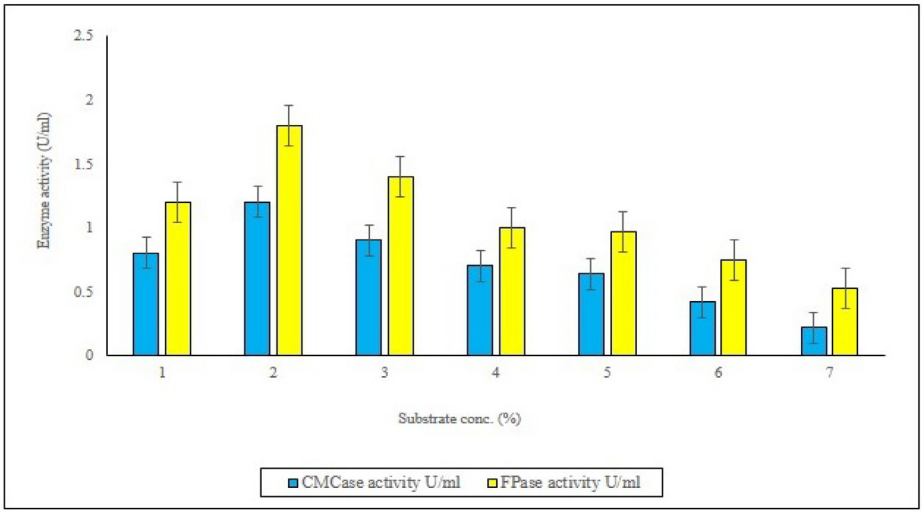
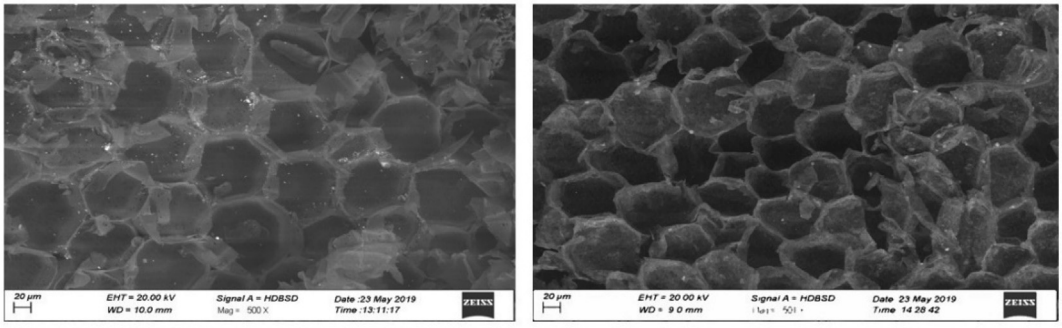


Fig. 3. Effect of different substrate concentration on the cellulase production.



(a) (b)

Fig. 4. SEM images of *Saccharum spontaneum* (a) untreated *Saccharum spontaneum* (b) treated *Saccharum spontaneum.*

$$Y = +0.9042 - 0.0041A + 0.0918B - 0.0136C + 0.0016AB - 0.0463AC - 0.0489BC - 0.0445A^2 - 0.2519B^2 - 0.2238C \quad (2)$$

FPase activity

$$Y = +1.80 - 0.0566A + 0.2776B + 0.1119C + 0.0515AB - 0.1815AC - 0.0952BC - 0.0745A^2 - 0.5355B^2 - 0.4823C$$

where Y is the predicted responses (CMCase and FPase), A is pH, B is temperature and C is time course.

Summary of ANOVA in response to the surface quadratic polynomial models for the production of CMCase and FPase by *Talaromyces thermophilus* are presented in Table 4 and Table 5. The values of "Prob>F" less than 0.05 indicate the significance of the model. The significant model and nonsignificant lack of fitness indicate

Table 3
Experimental runs and response in CCD for CMCase and FPase activity.

Run	A (pH)	B (Temperature) °C	C (Time Course) Days	CMCase activity U/ml	FPase activity
1	4	30	5	0.38	0.72
2	6	40	1	0.5	1
3	9.5	30	1	0.29	0.48
4	9	70	5	0.38	0.63
5	7	40	4	0.65	1.12
6	4	60	2	0.78	1.62
7	3	55	2	0.79	1.58
8	8	40	3	0.89	1.56
9	6	60	3	0.9	1.9
10	6	40	1	0.58	0.9
11	4.5	50	5	0.73	1.46
12	8	55	3	0.9	1.8
13	7	80	4	0.38	0.76
14	7	55	1	0.7	1.41
15	9	40	3	0.75	1.53
16	6	40	1	0.55	1.58
17	4	70	1	0.5	1.05
18	2	60	4	0.7	1.55
19	5	55	3	0.85	1.76
20	6	40	1	0.55	0.9

good model fitness. The *F*-values of 24.4 and 7.82 for CMCase and FPase activity revealed that the present model is significant. This illustrates that there was a 0.01% chance that a larger *F*-value could have occurred because of the noise. The quadratic and interaction terms such as B, B^2, AC, C^2 and C, C^2, and B^2 for CMCase and FPase activity, respectively, were significant. Values greater than 0.1000 indicate that the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model. The *F*-values of lack of fit – 3.50 and 0.1597 for CMCase and FPase activity implies that lack of fit is not significant relative to the pure error, which showed the accuracy of the model and helped in fitting the current model.

The accuracy and reliability of the model were determined by the evaluation of coefficient R^2, predicted R^2, and an adequate precision. Adequate Precision is an index that measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The computed ratios of 8.8 and 15.5 for CMCase and FPase activity, respectively, indicated that the present study had an adequate signal, which means the models can be used to navigate the design space. However, the determination coefficient R^2 = 0.8, and 0.9 for CMCase and FPase activity, specified the goodness-of-fit of the model. Furthermore, the adjusted determination coefficient (adj. R^2 = 0.9, 0.7) for CMCase and FPase activity suggested the high significance of the model. Predicted R^2 determination of coefficient is also in reasonable agreement with adjusted (R^2) which fundamentally specified that the current model fits very well to the experimental data. In addition, it is also confirmed that the predicted CMCase and FPase activity matched well with the observed activity. The present model yielded predicted R-squared values of 0.8 and 0.5 for CMCase and FPase activity, respectively, which showed the reliability of the model. The lower value of the coefficient of variation (CV) confirmed the reliability and precision of the conducted experiments. Here, the CV values of 8.63 and 16.57 for CMCase and FPase, respectively, indicated the greater reliability of the experiments. Fig. 5 illustrates the predicted and experimental values of the model where data points are localized close to the diagonal line suggesting the accuracy of the model (Table 6).

3.4. Graphical interpretation of significant parameters by response surface methodology

Fig. 6, Fig. 7, and Fig. 8 illustrate the profile of the quadratic response surface plots for the optimization of significant variables (temperature, pH, and time course). The shapes of contour plots describe the significance of the interaction between corresponding parameters [37]. Each figure demonstrates the effect of two factors while keeping the other factor fixed. Fig. 6 a & b shows the combined effect of temperature and pH on the CMCase and FPase activity, whereas time course remains at a fixed level.

Table 4
Analysis of Variance (ANOVA) for Quadratic model of CMCase activity.

Source	Sum of Squares	DF	Mean Square	*F*-value	*p*-value	
Model	0.6653	9	0.0739	24.44	< 0.0001	***significant***
A-pH	0.0001	1	0.0001	0.0217	0.8859	
B-temperature	0.0268	1	0.0268	8.86	0.0139	
C-time	0.0005	1	0.0005	0.1689	0.6897	
AB	3.863E-06	1	3.863E-06	0.0013	0.9722	
AC	0.0054	1	0.0054	1.80	0.2098	
BC	0.0082	1	0.0082	2.72	0.1300	
A^2	0.0071	1	0.0071	2.35	0.1563	
B^2	0.2222	1	0.2222	73.46	< 0.0001	
C^2	0.1138	1	0.1138	37.61	0.0001	
Residual	0.0302	10	0.0030			
Lack of Fit	0.0269	7	0.0038	3.50	0.1656	***not significant***
Pure Error	0.0033	3	0.0011			
Corrected Total	0.6956	19				

Table 5
Analysis of Variance (ANOVA) for Quadratic model of FPase activity.

Source	Sum of Squares	DF	Mean Square	*F*-value	*p*-value	
Model	3.09	9	0.3436	7.82	0.0017	***significant***
A-pH	0.0124	1	0.0124	0.2827	0.6065	
B-temperature	0.2453	1	0.2453	5.58	0.0398	
C-time	0.0345	1	0.0345	0.7838	0.3968	
AB	0.0040	1	0.0040	0.0913	0.7687	
AC	0.0835	1	0.0835	1.90	0.1981	
BC	0.0312	1	0.0312	0.7098	0.4192	
A^2	0.0199	1	0.0199	0.4538	0.5158	
B^2	1.00	1	1.00	22.84	0.0007	
C^2	0.5282	1	0.5282	12.01	0.0061	
Residual	0.4396	10	0.0440			
Lack of Fit	0.1193	7	0.0170	0.1597	0.9785	***not significant***
Pure Error	0.3203	3	0.1068			
Corrected Total	3.53	19				

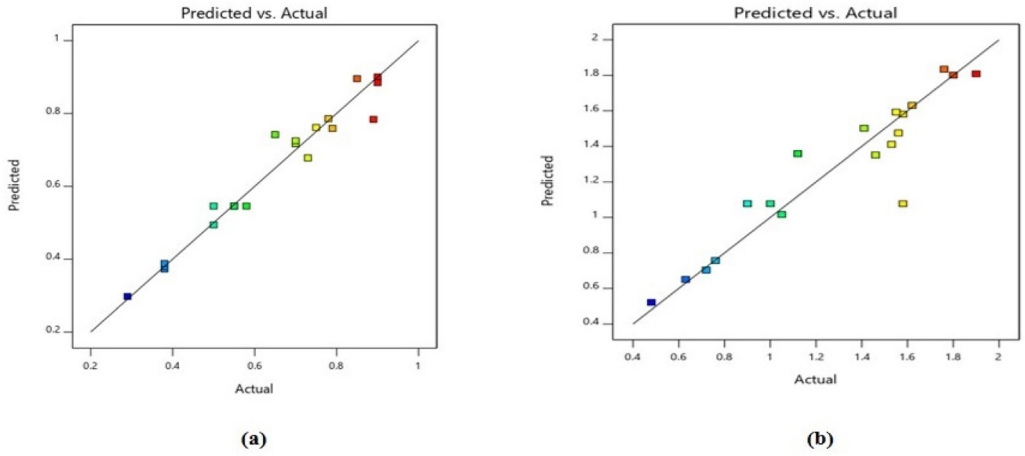


Fig. 5. Fitted diagonal line graphs showing correlation among the actual and predicted experimental values of: (a) CMCase activity (b) FPase activity.

Table 6
Summary of fit statistics for CMCase and FPase activity.

Response	C.V%	R^2	Adjusted R^2	Predicted R^2	Adeq Precision
CMCase	8.63	0.9565	0.9174	0.8184	5.5042
FPase	16.57	0.8755	0.7635	0.5688	8.8586

The surface plot in Fig. 6 a & b are steep in shape which represents that the maximum points are located inside the experimental region. Moreover, the elliptical shape of the contour plot revealed that both the physical factors – pH and temperature, possesses a significant correlation with each other and showed a positive impact on CMCase and FPase yield. In accordance with the corresponding contour plot, it was noted that maximum activity was obtained at pH 6. Hence, above or below the optimum levels there was a gradual decline in both CMCase and FPase activity. The reduction in enzyme activity caused by high or low pH value might be the reason that initial pH affected microbial growth and the protein structure, ultimately influencing the formation of the product. In addition, it affect the solubility of the nutrients, substrate ionization, and its availability to the organisms [38]. The effect of pH on cellulases production was also reported by Gautam et al. [39] who obtained maximal CMCase and FPase activity at pH 6. Our results contradicted with Gupta et al. [21] who reported maximum CMCase and FPase activity at pH 5 and 4.5, respectively. Moreover, temperature effect is considered as one of the important factors that has a profound effect on the enzyme production. The present results showed that the ratio of CMCase and FPase activity was high at 50 °C. In the present study, decline in enzyme productivity caused by low temperature might be because of the fact that it affects the efficiency of the microbes, whereas high temperature might cause thermal denaturation of the enzyme protein tertiary structure [40]. Present findings are close to those of Soeka and Ilyas [41] who recorded maximum cellulase production at 50 °C and pH 6.

Fig. 7 a & b represents the graphical explanation of pH and time course on CMCase and FPase activity. They clearly exhibit that the surface plots for both CMCase and FPase activity were slightly steep having maximum points within the experimental region. In addition, the elliptical contour plot revealed that enzyme yield increased progressively and reached the maximum at pH 6 after 72 h of incubation. Optimization of incubation time is considered as one of the significant factors in the metabolism of a microorganism. The decreasing trend after 72 h of fermentation might be because of the presence of other byproducts that could impede the fungal growth and unavailability or shortage of essential nutrients in the fermentation medium which could cease enzyme production. The present findings were in agreement with the results of Akinyele et al. [42] who reported optimal cellulase

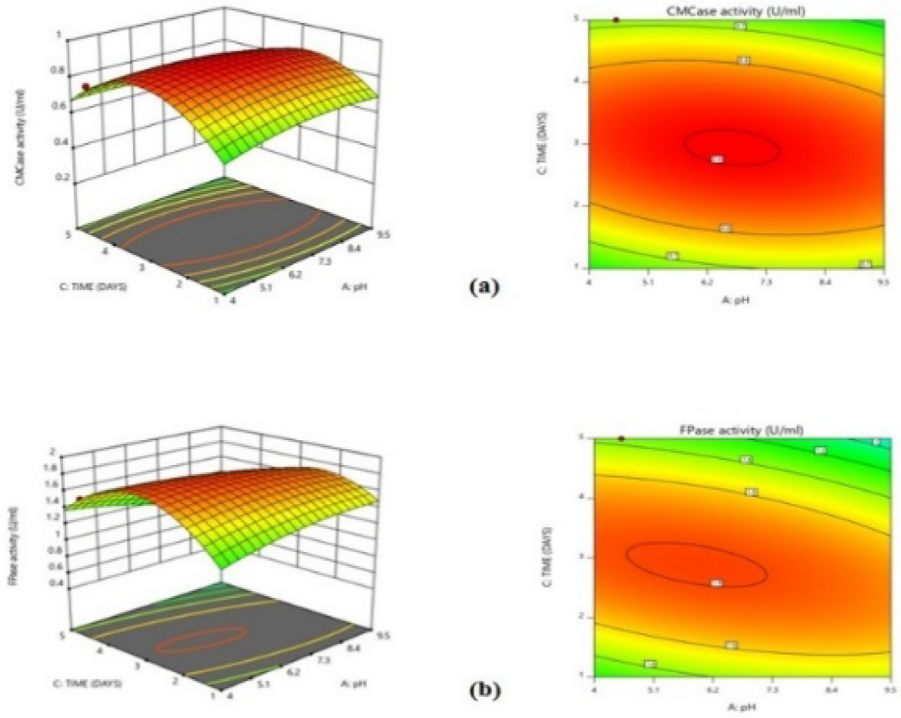


Fig. 6. 3D Response surface plots and contour plots of pH and time course for the optimal enzyme production (a) CMCase activity (b) FPase activity.

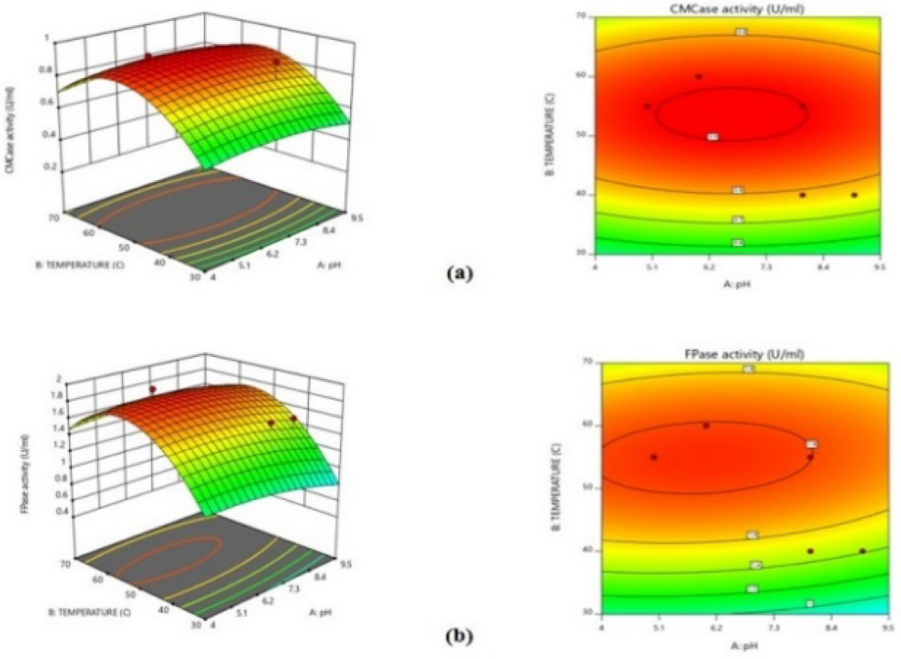


Fig. 7. 3D Response surface plots and contour plots of temperature and pH for the optimal enzyme production (a) CMCase activity (b) FPase activity.

production after 3 d of incubation. Similar results were presented by Gomathi et al. [43] who recorded maximal cellulase production after three days of incubation and maintained pH at 6.

Fig. 8a & b represents the response surface plot and contour plot of two independent variables such as temperature and incubation time, whereas pH remained fixed. The interaction between temperature and time course showed that contour plot was spherical in shape. However, the highest yield of CMCase and FPase activity was noted at 50 °C after 72 h of incubation. These results indicate that the enzymes were secreted at an early growth phase and reached the maximum in the exponential growth phase. The present results were similar to the findings of Gomathi et al. [43] who reported the highest CMCase productivity after 72 h of fermentation. Similar results were reported by Azzaz et al. [44] who noted the maximal cellulase activity after 3 d of incubation at pH 6.

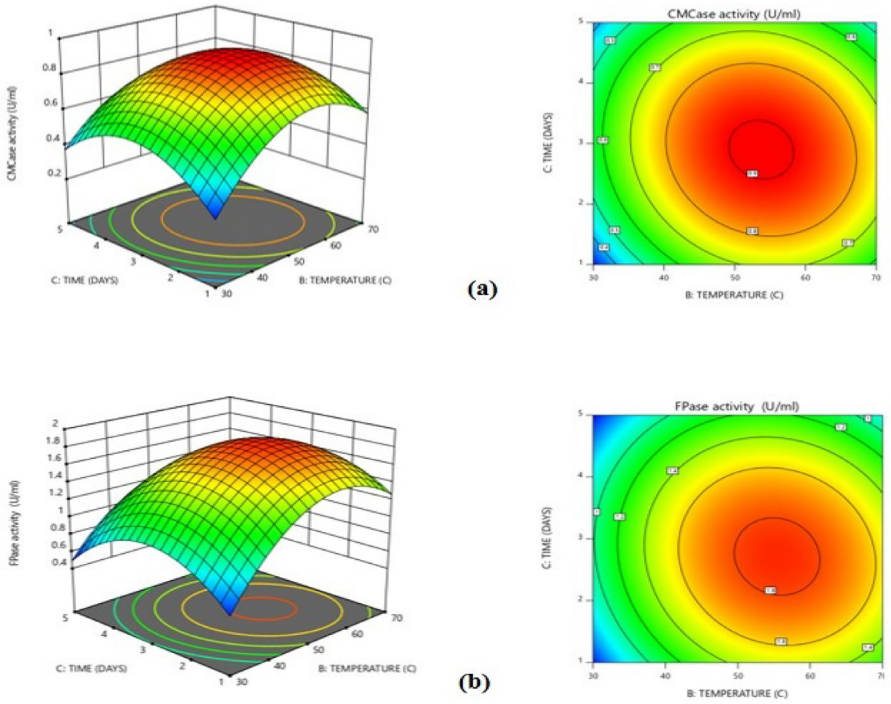


Fig. 8. 3D Response surface plots and contour plots of time course and temperature for the optimal enzyme production (a) CMCase activity (b) FPase activity.

3.5. Model validation

To test the competence of the response surface equations and confirmation of adequacy of response surface model, the experimental and predicted values were compared with the measurement of the activities of CMCase and FPase from *T. thermophilus* under optimal operation conditions by using submerged fermentation. Repeated experiments were performed and the results were compared. As calculated by ANOVA, the experimental value of CMCase and FPase activity was closely related to the predicted value, which demonstrates the adequacy of RSM and linearity of the diagonal graphs exhibits the good fitness of the model. In the present study, good results were obtained without the need for an expensive substrate. We believe that this research work is the first report of cellulase production by exploiting Kans grass (*Saccharum spontaneum*) as a substrate through response surface methodology by using thermophilic fungus, *Talaromyces thermophilus.* However, present study disagrees with Ilyas et al. [14] who reported cellulase production by *Saccharum spontaneum* at 30 °C by *Aspergillus terreus*.

4. Conclusion

The substrate significantly affects the cost of production process and the product yield. The present study shows that *Saccharum spontaneum,* an agricultural waste could be used for cellulase production by using the thermophilic fungus, *Talaromyces thermophiles.* RSM was used for the optimization of significant factors. A quadratic polynomial obtained by the CCD was essential to determine the positive effects of selected parameters on cellulase production. Hence, the present work shows that RSM is a more rapid, less time-consuming, less expensive technique, and an optimized process parameter with high reliability.

Conflict of interest

The authors declared there is no conflict of interest

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