Response Surface Methodology approach for optimization of endoglucanase from alkaliphilic *Fusarium oxysporum* VSTPDK and its potential application in pulp and paper industry

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Abstract

Production of endoglucanase (CMCase) from Fusarium oxysporum VSTPDK has been studied under submerged fermentation technique in a different range of physical and nutritional parameters. Parameters such as incubation time, pH, nitrogen sources and temperature were studied under one factor at a time (OFAT) approach using carboxymethylcellulose (CMC) as sole source of carbon. The use of OFAT produced a maximum CMCase of 3.62U/ml at an optimum ph of 8, temperature 30^{O}C, nitrogen source (1g/L NH_4SO_4) and 8th day incubation time. The use of Response Surface Methodology (RSM) produced 3.91U/ml at pH8.5, temperature 45^{O}C, ammonium sulphate concentration 3% and 8th day incubation. This leads to an increase of 0.39U/ml enzyme production as compared to OFAT.

Unlike convention optimization techniques, Response Surface Methodology (RSM) is a statistical tool which gained attention in the past decade for optimization of cellulase enzyme from various physico chemical and nutritional parameters. The use of RSM in different industries leads to the increase in endoglucane (CMCase) production especially in pulp and paper industries for the removal of ink from waste papers. This alkaliphic Fusarium oxysporum VSTPDK was able to produce considerable amount of CMCase at high pH and temperature. It was also found that rice straw, a cheap and available agro waste in Punjab was used to produce good amount of the enzyme when CMC was replaced with agro cellulolytic wastes.

Keywords: Endoglucanase, RSM, OFAT, *Fusarium oxysporum*, VSTPDK CMCase.

Introduction

Cellulose is among the most abundant and common carbohydrates on earth. The breaking of this complex sugar into simple monosaccharide requires the cleavage of β – 1, 4-glycosidic bonds by action of three cellulase enzymes (endoglucanase, exoglucanase and β-glycosidase). At random, endoglucanase (EG) breaks the inner O-glycosidic bonds leading to the release of glucan chains in different length; this is followed by attack on the ends of the cellulose chains by releasing β-cellobiose as end product by exoglucanase (CBH) while β-glycosidase acts specifically on the breaking down of β-cellobiose disaccharides to glucose[1].

Cellulase enzyme can be produced from different microorganisms like bacteria (*Clostridium spp, Cellulomonas spp*) and fungi (*Trichoderma, Fusarium, Aspergillus spp*) when they grow on cellulosic materials[2]**.**

The enzyme is relatively costly which has a great advantage for its commercial use. Low enzyme yield and substrate cost are some of the problems associated with cellulase production which mainly affect its large scale production. However, these limitations can be overcome by the application of optimizing parameters controlling enzyme yield. This can be done by either optimizing the physical factors like pH, incubation time and temperature[3,4] or nutrient composition of the media such as carbon and nitrogen source[5,6]. The most widely used and considered as most effective deinking method is the conventional chemical deinking method.

However, over the past two decades, different microbial enzymes such as cellulase, hemicellulase, xylanase, laccase and lipase have been studied on their potential application to replace chemicals in removing ink from waste paper[6,7]. *F. oxysporum* is ubiquitous soil inhabitant having the capacity to leave as saprophytes and can degrade lignin as well as carbohydrates associated with soil debris[8]. This research presents production and optimization of endoglucanase from *Fusarium oxysporum* VSTPDK by subsumed fermentation and its subsequent application for deinking of waste paper by pulp and paper industries.

Material and Methods

Chemicals: Reagents and chemicals used in this research are of analytical grades (AR) and were purchased from Hi media (India) and Sigma (USA) unless otherwise stated.

Isolation, Screening and Identification: Alkaline *Fusarium oxysporum* (VSTPDK) was isolated and screened in our laboratory from soil sample of Samana village in Kapurthala District, Punjab, India. Based on the method of

Vega et al[9], the fungus was kept in potato dextrose agar (PDA) and stored in refrigerator at 4^{O}C. It was then screened for the ability to produce endoglucanase using Mandel and Reese media. The broth media was (g/l) of the following composition: Proteose peptone 1.0, Ammonium sulphate $(NH_4)_2SO_4$ 1.4, Potassium dihydrogen phosphate KH_2PO_4 2.0, Urea NH_2CONH_2 0.3, Magnesium sulphate $MgSO_4.7H_2O$ 0.3, Calcium chloride $CaCl_2$ 0.002, Ferrous sulphate $FeSO_4.7H_2O$ 0.005, Mangnese sulphate $MnSO_4.H_2O$ 0.001, Zinc chloride $ZnCl_2$ 0.017 and Carboxy Methyl Cellulose (CMC) 10. The pH of the medium was adjusted to different alkaline level using NaOH and HCl. The fungal organism was identified as *F. oxysporum* by Indian Agricultural Research Institute (IARI) New Delhi.

The fasta format of the partial sequence of *F. oxysporum* strain internal transcribed region is given below. >VSTPDK_NS1_B09.ab1 *F. oxysporum*:
TACCCGCGAAACTGCGAATGGCTCATTATATAAGT
TATCGTTTATTTGATAGTACCTTACTACTTGGATAA
CCGTGGTAATTCTAGAGCTAATACATGCTAAAAAT
CCCGACTTCGGAAGGGATGTATTTATTAGATTAAA
AACCAATGCCCTTCGGGGCTCACTGGTGATTCATG
ATAACTCCTCGAATCGCATGGCCTTGTGCCGGCGA
TGGTTCATTCAAATTTCTTCCCTATCAACTTTCGAT
GTTTGGGTATTGGCCAAACATGGTTGCAACGGGTA
ACGGAGGGTTAGGGCTCGACCCCGGAGAAGGAGC
CTGAGAAACGGCTACTACATCCAAGGAAGGCAGC
AGGCGCGCAAATTACCCAATCCCGACACGGGGAG
GTAGTGACAATAAATACTGATACAGGGCTCTTTTG
GGTCTTGTAATTGGAATGAGTACAATTTAAATCCC
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ATTAAAGTTGTTGTGGTTAAAAAGCTCGTAGTTGA
ACCTTGGGCCTGGCTGGCCGGTCCGCCTCACCGCG
TGTACTGGTCCGGCCGGGCCTTTCCCTCTGTGGAA
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CAGGCCTATGCTCGAATACATTAGCATGGAATAAT
AGAATAGGACGTGTGGTTCTATTTTGTTGGTGTCT
ACGACCGCCCTCATGATTATTAGGGACAGTCAGTG
GCATCAGTATTCACTTGTCAGAGGTGAAATTCTTG
GATGTATTGAAAACTAACTACTGCGACGCCGTTAG
CGAGGATGTTTTCATTATTAAGAACGACCGTACGG
G

Alkaline cellulase productions using different carbon sources: Secondary screening was conducted according to method by Ramanmathan et al[10]. Liquid cellulase enzyme production medium containing 100ml of the modified culture media was replaced with 1% of three different agro cellulolytic waste including Rice straw (RS), wheat straw (WS) and sugarcane bagasse (SB).

This media was autoclaved and prepared with *F. oxysporum* in 250 ml Erlenmeyer flask after alkaline pretreatment of the agro waste. The liquid culture medium was incubated at 150 rpm in a rotary for 12 days with interval of 2 days (2, 4, 6, 8, 10 and 12). In each interval, reducing sugar was measured as per the method of Miller[11].

Enzyme assay: The endoglucanase was measured using dinitrosalicyclic acid method of Miller[11]. In this method, 0.5ml diluted enzyme in 0.05 M citrate buffer (pH 8.5) was mixed with 0.5ml of 1% CMC for endoglucanase. After incubation at 50^{O}C for 30min, the reaction was immediately stopped by the addition of 3ml dinitrosilicyclic acid and heating at 100^{O}C for 10min followed by immediate cooling to stop further reaction. Absorbance was measured by spectrophotometer at 540nm. One unit of CMC and filter paper were defined as the amount of enzyme produced by releasing 1µmole of reducing sugar equivalent to glucose per minute under standard conditions[10].

Optimization of production conditions using one factor at time (OFAT): Conditions of CMCase production parameters by *F. oxysporum* VSTPDK were optimized in 250ml Erlenmeyer flask with Mandel and Reese medium using OFAT approach. The optimum culture conditions pH (6, 7, 8, 9, 10 and 11), temperature (30, 40, 50 and 60^{O}C), incubation period (4th 6th 8th 10th and 12th), nitrogen source (NH_4SO_4, $NaNO_3$), and carbon sources (rice straw, wheat straw, sugarcane baggasse and Carboxymethylcellulose) were determined for maximum *F. oxysporum* production and CMCase activity. In each experiment, triplicate experiments were used and values recorded as a mean standard deviation of the replicate samples.

Statistical optimization: Response Surface Methodology (RSM) was used to statistically designed experiments for optimization of the selected parameters influencing endoglucanase production through central composite design (CCD). Three independent variables which include pH, temperature and ammonium sulphate concentration were selected for enzyme optimization. This leads to the suggestion of 20 different experiments by the model with minimum, medium and maximum (-1, 0 and +1) respectively as shown in table 1.

Design Expert windows vision 6.0.8 portable was the statistical software package used during tabulation and processing process that allows a quick and simple data appraisal. All experiments are in triplicate and mean enzyme production was used as the variable responses *Y*. Equation indicates the second order model used in describing the relationship between independent variable and the response. Experiments are carried out in triplicate while the mean production was used as response variable *Y*. Final RSM predicted response was further validated experimentally.

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

Key: *Y* is the predicted response parameter, β_0, β_1, β_3, β_{11}, β_{22}, β_{33}, β_{12}, β_{13} and β_{23} are constant regression coefficients of the model, β_0 means intercept term, β_1, β_2 and β_3 are linear

coefficients, β_{11}, β_{22} and β_{33} are squared coefficients, β_{12}, β_{13} and β_{23} are interaction coefficients and A, B, C, A^2, B^2, C^2, AB, AC and BC are independent parameters.

Statistical Analysis: Statistical tool used in the research was Analysis of Variance (ANOVA) using a data generated from central composite design (CCD) experiments for CMCase production. All $p < 0.05$ are considered statistically significant.

Results and Discussion

Incubation time: Optimization of incubation time was conducted by inoculating the isolate into the fermentation medium and incubated at different time per day ranging from 4th, 6th, 8th, 10th and 12th days. Maximum endoglucanase production of 3.52U/ml was found on 8th day incubation as indicated in figure 1. The result is in line with work of Ramanathan et al[10] where maximum CMCase (1.92±0.005) was produced from *Fusarium oxysporum* after 8 days incubation. However, a maximum of 0.061U/ml of CMCase was produced from *Aspergillus hortai* after 4 days incubation[12].

Effect of initial pH: Among physical parameters for fungal growth and enzyme production in this research, pH is one of the most vital factors. Generally, enzymes have an optimum value at which highest or lowest activity occurs. This changes in fungal production and enzyme activity from various pH ranges varies from different fungi and enzymes. In this research different pH ranges (6, 7, 8, 9, 10 and 11) were used for the production of endoglucanase. A maximum CMCase activity (3.50U/ml) in a liquid medium at 30^{O}C at 8 days incubation was observed from *F. verticillioides* VSTPDK under shaking condition at pH 6. The work is in accordance with work of Azzaz et al[13] where cellulase has optimum activity of 0.009U/ml from *Aspergillus niger* at pH 6. At a pH 7, Basak and Rangan[14] as well as Saha[15] also reported optimum cellulase production from *Fusarium oxysporum* and *Mucor circinelloides* which is in line with our work. However, significant amount of enzyme was produced across alkaline environment.

An appreciable enzyme activity was also observed at different alkaline condition (3.33, 2.96, 1.69, 0.56 and 0.19U/ml) for pH 7, 8, 9, 10 and 11 respectively. Having such amount of enzyme at alkaline environment indicated this newly isolated *Fusarium oxysporum* as alkaline cellulose producing fungi. Similar results at alkaline environment from different fugal strains were found by other researchers.[9,10,17,18]

Effect of different carbon sources: Three different types of agricultural waste within the University farm which include rice straw (RS), wheat straw (WS) and sugarcane bagasse (SB) were selected and subjected to drying as well as alkaline pre-treatment before. These agro cellulolytic wastes were substituted as sole source of carbon from the fermentation media. Maximum CMCase activity of 2.78U/ml was found from rice straw on 8th day incubation at 30^{O}C and pH 8.5 as shown in fig. 3.

Table 1
Level of independent variables and experimental range from CCD for optimization of CMC produced from *Fusarium oxysporum* VSTPDK

Variable	Factors	Range	Level of experimental variables		
			Low (-1)	Medium (0)	High (+1)
A	pH	6-11	6	8.5	11
B	Temperature (^{O}C)	30-60	30	45	60
C	Ammonium Sulphate (%)	1-5	1	3	5

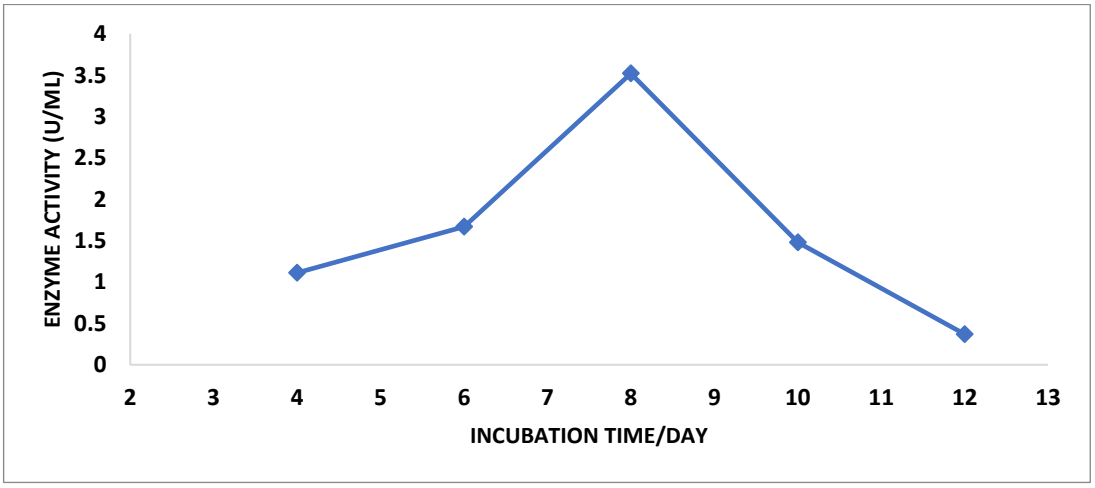

Fig. 1: Effect of incubation time on the production of endoglucanase and exoglucanase from *Fusarium oxysporum* VSTPDK

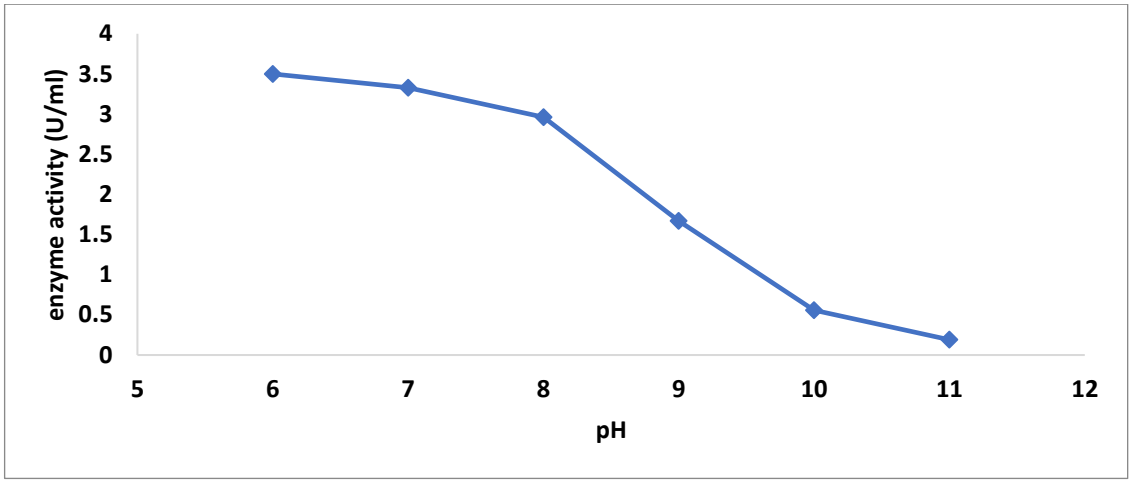

Fig. 2: Effect of temperature on the production of endoglucanase (CMCase) from *Fusarium oxysporum* VSTPDK

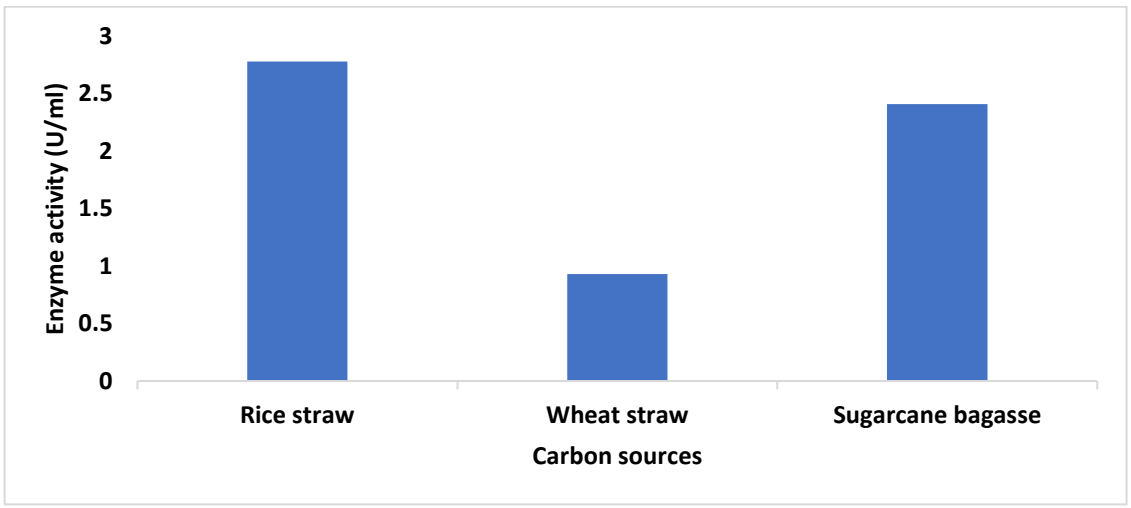

Fig. 3: Effect of different carbon sources on the production of endoglucanase (CMCase) from *Fusarium oxysporum* VSTPDK

This activity in accordance with work of Sasi et al[19] was maximum. CMCase of 0.128g/ml was found in rice bran followed by wheat brand (0.097g/ml) and sugarcane bagasse (0.019) respectively. Bhavsar et al[20] also reported a maximum CMCase production of 7.4U/ml from banana stem followed by rice straw 5.4U/ml using fungal cellulase.

Effect of temperature: Another vital factor for fungal growth and enzyme production is temperature. Different microorganisms and enzymes have different favorable growth temperature as well as different temperature for maximum enzyme production. The research was conducted at different temperature range (30^{O}C, 40^{O}C, 50^{O}C and 60^{O}C) in which maximum CMCase activity (3.50U/ml) was recorded at 30^{O}C as shown in fig. 4.

A similar growth temperature (33^{O}C) from *Fusarium oxysporum* was reported to have a maximum CMCase production[21]. Many researchers also reported maximum CMCase production at high temperature. Remaz et al[22] and Ramanathan et al[10] reported maximum cellulase production from *Aspergillus niger* and *Fusarium oxysporum* at 50^{O}C respectively. Dutta et al[18] also reported a maximum CMCase production at 50^{O}C from *F. solani* SF1404, *F. oxysporum* SF1404 and 1905 as well as *F. chlamydosporum* SF2102.

Effect of different nitrogen sources: One of the most important nutritional factors for influencing microbial growth and enzyme production is the nitrogen source. Different nitrogen sources may have inhibitory or stimulatory effects on fungal growth and cellulase production. Ammonium sulphate $(NH_4)_2SO_4$ was found with maximum CMCase production (3.50U/ml) when four different inorganic nitrogen sources which include ammonium sulphate (NH_4SO_4), ammonium carbonate, ammonium chloride and sodium nitrate were used as shown in fig. 5. The results of the effects of different nitrogen sources obtained here are similar with result reported by Sasi et al[19] who found that ammonium sulphate increases the amount of cellulase enzymes produced from *Aspergillus flavus*.

Optimum cellulase was found when ammonium sulphate was used as nitrogen source. However, it disagrees with work of El-hadi et al[12] and Irfan et al[24] maximum cellulase followed by ammonium sulphate. Urea was also found to be

the best nitrogen source on the production and optimization of cellulase enzyme from *Fusarium oxysporum*[10].

Ammonium sulphate concentration: Effect of various ammonium sulphate concentrations on cellulase production was investigated. The data obtained indicated that 3.0% was the optimum concentration supporting CMCase production of 3.70U/ml as shown in fig. 6. Increase or decrease of ammonium sulphate from the optimum concentration lowered the rate of enzyme production. This result is similar to the result obtained by Vyas et al[23] and Sasi et al[19] where ammonium sulphate was reported to be optimum.

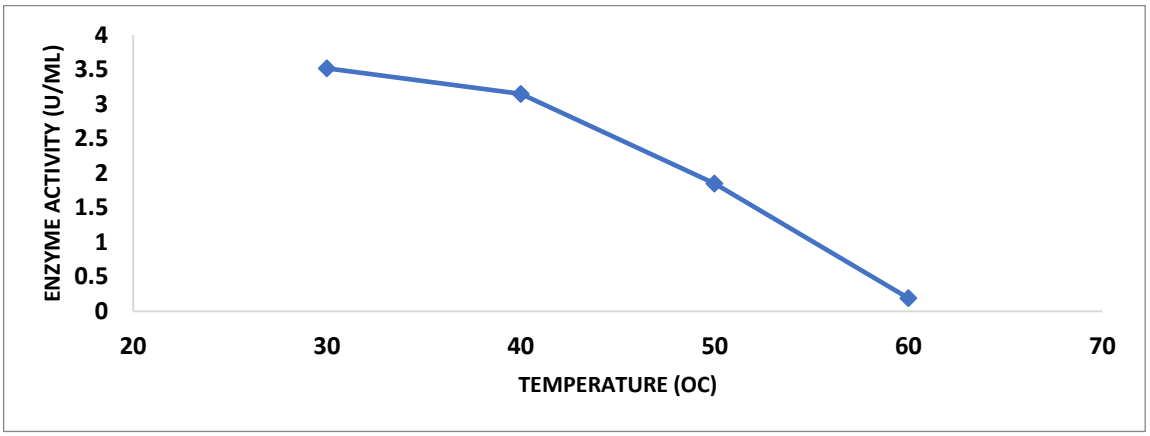

Fig. 4: Effect of temperature on the production of endoglucanase (CMCase) from *Fusarium oxysporum* VSTPDK

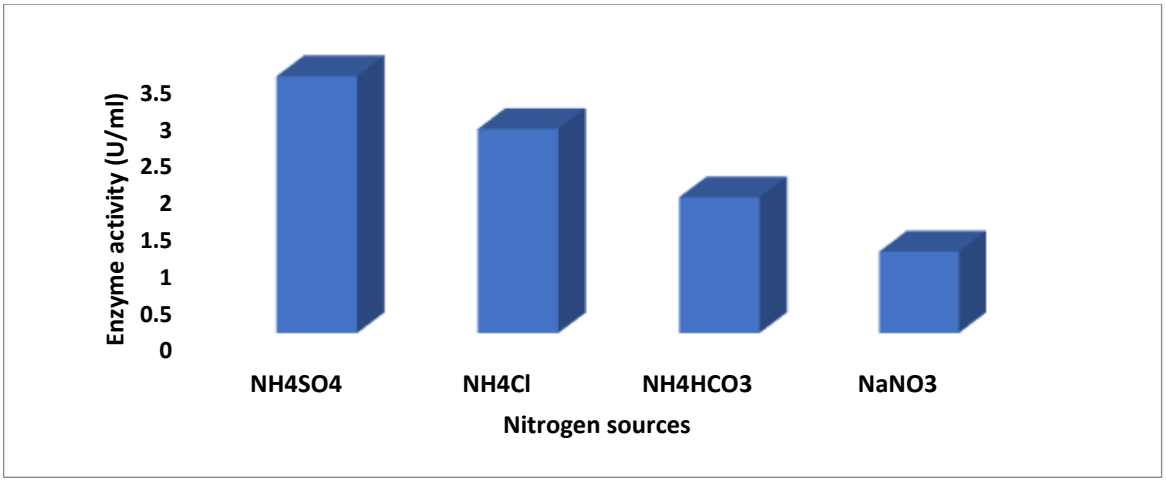

Fig. 5: Effect of different inorganic sources of nitrogen on the production of CMCase from *Fusarium oxysporum* VSTPDK. Error bars represent mean±standard deviation

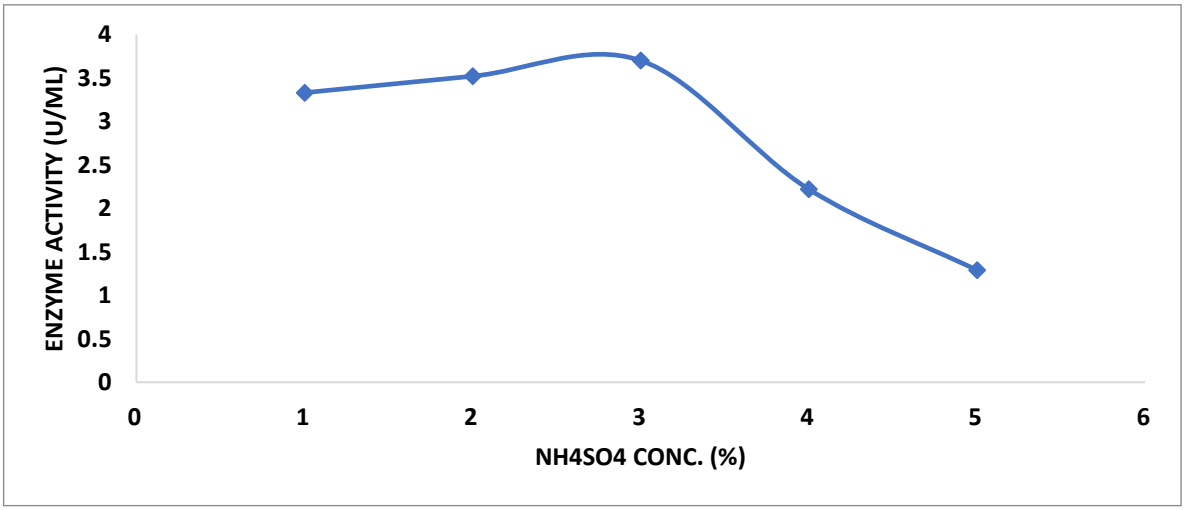

Fig. 6: Effect of ammonium sulphate concentration on the production of endoglucanase and exoglucanase from *Fusarium oxysporum* VSTPDK

Statistical optimization of parameters influencing endoglucanase production; In this research, Response Surface Methodology (RSM) was used to obtain a quadratic model of the experiment through Central Composite Design (CCD). Design expert windows vision 6.0.8 was used to generate the experimental design. A total of 20 experiments were randomly designed by the software and later conducted in our laboratory as predicted. The independent variables pH (A), temperature (B) and concentration of ammonium sulphate (C) were optimized. The predicted and actual responses from central composite experimental plan for CMCase were summarized in table 2.

Regression equation was obtained when Analysis of Variance (ANOVA) gave an estimate of endoglucanase activity as a function of independent variable. The polynomial mathematical model in equation 2 may explain the production of endoglucanase which provides the interaction of different levels of variables where Y is the CMCase production, A is the pH, B is the temperature and C is the ammonium sulphate concentration. Models precisions were normally determined by coefficient (R^2) and its values always range between 0 to 1 where the order of magnitude suggests goodness of the model[25]. As indicated in table 3, R^2 value of CMCase was found to be 0.9933 which is near to 1 and indicated that 99.33% behavior of the model can be explained as model of endoglucanase enzyme production while only 0.67% full variance cannot be explained by the models.

According to Yusuf et al[26], for high accuracy and ability of polynomial model to be good, R^2 value must be close to 1. A similar R^2 value of 0.9873 CMCase was reported by Kumar et al[27]. Adjusted R^2 from this model was found to be 0.9873. This is an indication of good relationship between actual and predicted values. From the result obtained, predicted R^2 values for CMCase (0.9710) agreed with Adjusted R^2 values of 0.9873. Hence, the model provides clarity on how response and independent variables are interrelated. Sufficient precisions of the model measured the signal to noise ratio; for CMCase was it found to be 48.790 indicating an adequate signal while the result showed that the model is significant. This result agreed with that of Sharma, Malik and Satya[28] with adequate precision values of 17.4 while optimizing nutrient supplements on the removal of Cr (VI) by *Aspergillus lentulus* AML05.

Significance of the model is generally measured on the basis of P-value of F-value (prob > F). the higher is the F-value and corresponding lower prob > F value, the better is the importance of the corresponding coefficients (R^2)[29]. For maximum cellulase enzyme production, table 4 summarized the second order response in the form of Analysis of Variance (ANOVA). The result indicated that high model *F*-value for CMCase was 165.31 with respective small prob >F values (P-value) of <0.0001 signifying that the model was significant. This means the probability where F value model could happen due to noise was 0.01%.

In order to know the application of each coefficient, P-values are adopted as the tool. The prob>F<0.05 values showed that the models were significant. This means that A, B, C, A^2, B^2, C^2, AB, AC as well as BC are the significant model terms. The lack of fit *F*-value of the models was 1.83 while 0.2613 lack of fit indicated that lack of fits was not significant and model as very accurate without any noise.

Borugadda and Goud[25] reported that a lack of fit must be estimated in order to examine Analysis of Variance (ANOVA) on each model coefficient and accurate model fit. Manogaran et al[30] and Ibrahim et al[31] reported a non significant lack of fits by describing it as excellent fit. Based on the result obtained, excellent relationship was found between actual and predicted values as depicted in equation 2 and 3 which described actual and coded factors respectively.

CMCase = (0.70) + (-0.63xA) + (1.00xB) + (0.22xC) + (0.21xA^2) + (0.90xB^2) + (0.21xC^2) + (0.65xAB) + (0.094xAC) + (0.14xBC) (2)

CMCase = (19.07785) + (-0.51475*A) + (-0.58746*B) + (-0.16350*C) + (-0.033600xA^2) + (4.00000E-003*B^2) + (-0.052500*C^2) + (0.017300*AB) + (0.018750*AC) + (4.62500E-003*BC) (3)

The interactions effect of parameters on CMCase production was studied on two parameters as well as putting other parameters constant. Response surface plots and contour plots can be used in predicting optimal values of different test series. 3D response surface plots and contours depicted interaction between pH and temperature, pH and $(NH_4)_2SO_4$ concentration as well as temperature and $(NH_4)_2SO_4$ concentration. The interaction between ammonium sulphate concentration and enzyme production showed an important effect. Increases in ammonium sulphate concentration will increases enzyme production from 1 to 3% and subsequent increase showed a decrease of enzyme production.

The optimum enzyme production was indicated at 3% ammonium sulphate and pH 8.5 (Fig. 7). An excellent correlation was also observed between pH and temperature as well as temperature and ammonium sulphate concentration. The interaction between most effective parameters can be changed on the contour plots for cost effective CMCase industrial production.

Validation of experimental Model: The optimum values of the three most important parameters selected were based on the contour plots were 8.5 pH, 45^{O}C temperature and 3% ammonium sulphate concentration. The model was validated experimentally by production of endoglucanase from the optimized values in the same liquid medium using submerged fermentation. The experimental condition leads to an increase of CMCase production by 0.19U.ml as compared to one factor at a time approach (OFAT). It is therefore concluded that CCD based RSM models are more

reliable, accurate and less time consuming for industrial production of CMCase.

Potential industrial applications

Endoglucanase plays an important role in paper and pulp industries for removal of ink from waste paper. Many researchers have reported the application of this enzyme in improvement of paper brightness, reduction of residual ink as compared with conventional method. Remaz et al[22] reported an improved brightness of endoglucanase enzyme treated pulp by 3-4 points as compared to denatured control pulp samples as well as reduction of residual ink speck.

Many researchers have also reported an increase in pulp brightness and decrease in residual ink from CMCase isolated from different bacteria and fungi[23-27]. Therefore, it is expected that production of endoglucanase from alkaliphilic fungi at high temperature can be a good alternative of pulp and paper industries for in ink removal from waste paper

Table 2
Experimental design having coded levels of variables used in Central Composite Design with experimental and predicted value for CMCase activity from *Fusarium oxysporum* VSTPDK

Standard Order	Run	Factor 1 pH	Factor 2 Temperature (^{O}C)	Factor 3 NH_4SO_4conc. (%)	CMCase (U/ml)	
					Actual	Predicted
1	16	0	0	0	3.89	3.91
2	3	1	0	0	1.11	1.16
3	11	1	-1	1	0.37	0.33
4	4	-1	-1	-1	0.19	0.19
5	7	0	0	0	2.96	3.00
6	20	-1	1	-1	0.56	0.63
7	5	0	0	0	0.00	-0.02
8	6	0	0	1	0.19	0.21
9	15	1	-1	-1	1.11	1.11
10	12	1	1	-1	0.00	-0.14
11	17	-1	1	1	2.77	2.59
12	8	0	0	0	0.56	0.60
13	13	-1	-1	1	0.74	0.71
14	9	0	1	0	0.37	0.26
15	10	-1	0	0	0.74	0.70
16	14	0	0	-1	0.74	0.70
17	19	0	-1	0	0.74	0.70
18	1	1	1	1	0.56	0.70
19	18	0	0	0	0.56	0.70
20	2	0	0	0	0.56	0.70

Table 3
Summary of ANOVA result from Central composite design (CCD) for CMCase from *Fusarium oxysporum* VSTPDK

Parameters	Result	Remark
F- value	165.31	
Prob > F	<0.0001	Significant
R^2 value	0.9933	
Adjusted R^2	0.9873	
Predicted R^2	0.9710	
Adequate precision	48.790	Adequate signal to noise ratio
Lack of fit F value	1.83	
Lack of fit prob > F	0.2613	Not significant

Table 4
Analysis of variance (ANOVA) for endoglucanase (CMCase) from *Fusarium oxysporum* VSTPDK

Source	Sum of square	DF	Mean square	*F* value	Prob>F value	
Model	20.48	9	2.28	165.31	< 0.0001	Significant
A = pH	3.94	1	3.94	286.57	< 0.0001	
B = temperature	9.96	1	9.96	723.72	< 0.0001	
C = NH_4SO_4	0.49	1	0.49	35.81	0.0001	
A^2	0.12	1	0.12	8.81	0.0141	
B^2	2.23	1	2.23	161.86	< 0.0001	
C^2	0.12	1	0.12	8.81	0.0141	
AB	3.37	1	3.37	244.66	< 0.0001	
AC	0.070	1	0.070	5.11	0.0473	
BC	0.15	1	0.15	11.19	0.0074	
Residual	0.14	10	0.014			
Lack of fit	0.089	5	0.018	1.83	0.2613	Not significant
Pure error	0.049	5	9.720E-003			
Cor Total	20.61	19				

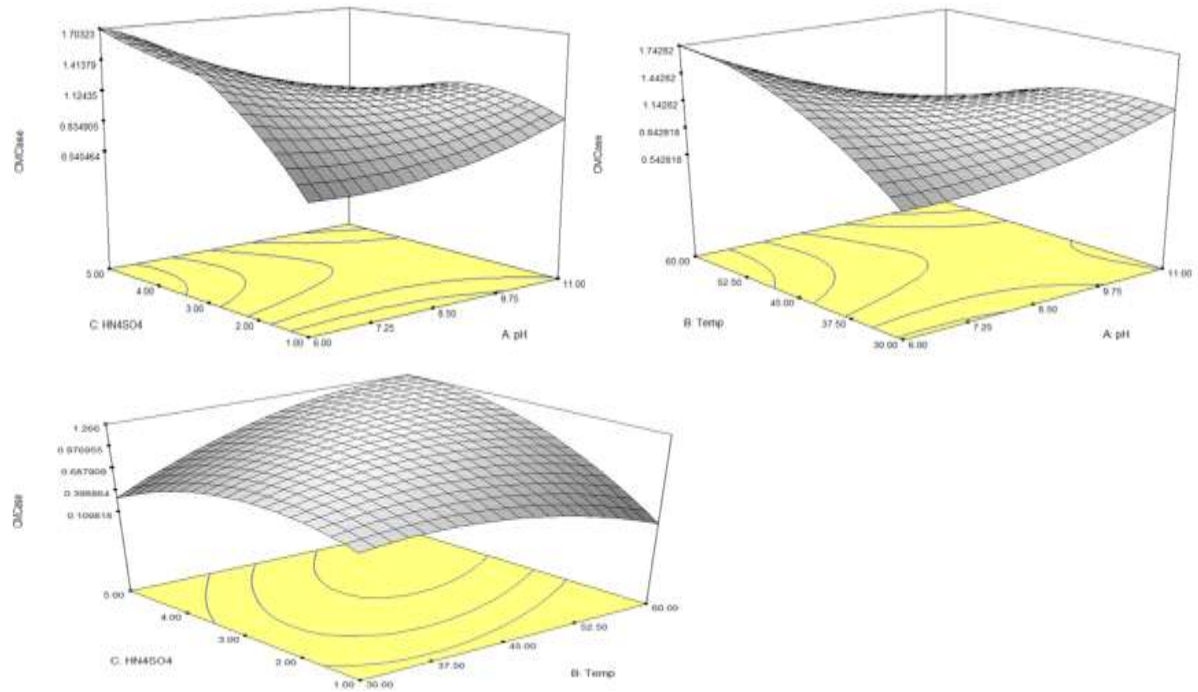

Fig. 7: A three 3D response surface plots for the optimization of endoglucanase from *Fusarium oxysporum* VSTPDK having interactive effects of the three different parameters

Conclusion

Currently, conventional method of enzyme production and optimization such as one factor at a time (OFAT) is now replaced by more accurate and easy statistical approach called response surface methodology (RSM). This statistical model has the ability to generate large number of information from less number of experiments.

In this research, both OFAT and RSM were applied for the production and optimization of endoglucanase using different physico chemical and nutritional factors using alkaliphilic *F. oxysporum* VSTPDK isolated and screened from soil in our laboratory. It was found that using central composite design (CCD) in RSM, there was an increase of 0.39U/ml endoglucanase as compared to OFAT.

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