JEBAT, 2024, Vol 7, No 2, 75-81
https://doi.org/10.54987/jebat.v7i2.1047

JOURNAL OF ENVIRONMENTAL BIOREMEDIATION AND TOXICOLOGY
Website: http://journal.hibiscuspublisher.com/index.php/JEBAT/index

Comparison Between OFAT and RSM Approaches for the Optimization of Endoglucanase Production by Alkaliphilic *Aspergillus oryzae* and Its Application in the Pulp and Paper Industry

Abdulhadi Yakubu[1]*, Aliyu Abdulhamid Omar[2] and Garba Uba[3]

[1]Department of Public Health, College of Health Sciences, Jigawa State Polytechnic, Dutse, P.M.B. 7040, Jigawa State, Nigeria.
[2]Department of Science Laboratory Technology, Faculty of Science, Sa'adu Zungur University, Bauchi State, P.M.B. 065, Nigeria.
[3]Department of Science Laboratory Technology, College of Science and Technology, Jigawa State Polytechnic, Dutse, P.M.B. 7040, Jigawa State, Nigeria.

*Corresponding author:
Abdulhadi Yakubu,
Department of Public Health,
College of Health Sciences,
Jigawa State Polytechnic,
Dutse, P.M.B. 7040,
Jigawa State,
Nigeria.
Email: ayakubu.slt@jigpoly.edu.ng

HISTORY

Received: 18th Aug 2024
Received in revised form: 15th Nov 2024
Accepted: 24th Dec 2024

KEYWORDS

Endoglucanase
RSM
OFAT
Aspergillus oryzae
CMCase

ABSTRACT

The production of endoglucanase (CMCase) from Aspergillus oryzae has been studied under a submerged fermentation technique using a range of physical and nutritional factors. These include temperature, pH, incubation time, and nitrogen sources, which were studied under a one-factor-at-a-time (OFAT) approach using Cellulose Powder as the sole source of carbon. The use of OFAT produced a maximum CMCase of 3.55 U/mL at an optimum pH of 8, temperature 28 ºC, nitrogen source (1 g/L NH_4SO_4) and 7th day incubation time. The use of Response Surface Methodology (RSM) resulted in 3.96 U/ml at pH 8.5, a temperature of 40 ºC, an ammonium sulfate concentration of 3%, and a 7-day incubation. This results in an increase of 0.45 U/mL in enzyme production compared to OFAT. Alkaline endoglucanase from *A. oryzae* was also evaluated for its deinking potential. An A4 paper was printed with the word 'deinking' for approximately 640 words using a Xerox printer. After the enzymatic, chemical, and control pulping processes, as well as flotation, the resultant pulp was prepared according to TAPPI standards for testing pulp brightness, tensile strength, bursting strength, and tearing strength. The percentage brightness result following *A. Oryzae enzymatic treatment indicated an improvement in ISO brightness of 2.36% and 0.48% for the control and chemical, respectively, compared to* the prepared hand sheet. The tensile strength of the printed waste paper improved by 180 N/m and 253 N/m, respectively, when compared to the control and chemical deinking process. In terms of bursting strength, enzymatic deinking was increased by 11 kPa and 8 kPa, while the tearing strength showed an improvement of 3 mN and 9 mN compared to the control and chemical deinking, respectively.

INTRODUCTION

Cellulose is one of the most abundant and common carbohydrates on Earth. The breaking of this complex sugar into simple monosaccharides requires the cleavage of β – 1, 4-glycosidic bonds by the 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 of 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]. The cellulase enzyme can be produced by various microorganisms, including 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 high 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 applying optimizing parameters that control enzyme yield. This can be achieved by optimizing physical factors, such as pH, incubation time, and temperature [3,4], or by adjusting the nutrient composition of the media, including carbon and nitrogen sources [5,6]. The most widely

used and considered the most effective deinking method is the conventional chemical deinking method. However, over the past two decades, various microbial enzymes, including cellulase, hemicellulase, xylanase, laccase, and lipase, have been studied for their potential application in replacing chemicals for removing ink from waste paper [6,7]. This research presents the production and optimization of endoglucanase from Aspergillus oryzae through submerged fermentation and its industrial application for deinking waste paper in the pulp and paper industry.

MATERIAL AND METHODS

Chemicals

Reagents and chemicals used in this research are of analytical grade (AR) and were purchased from HiMedia (India) and Sigma (USA) unless otherwise stated.

Isolation, Screening, and Identification

Alkaline *Aspergillus oryzae* was isolated and screened in our laboratory from a soil sample collected in Jigawa State, Nigeria. Based on the method of Vega et al. [8], the fungus was kept in potato dextrose agar (PDA) and stored in a refrigerator at 4 ºC. 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 levels using NaOH and HCl. The fungal organism was identified as *Aspergillus oryzae*. The FASTA format of the partial sequence of the *Aspergillus oryzae* strain internal transcribed region is given below.

>NS1_B09.ab1 *Aspergillus oryzae*

TACCCGCGAAACTGCGAATGGCTCATTATATAAGTTATCGTTTATTTGATAGTAC
CTTACTACTTGGATAACCGTGGTAATTCTAGAGCTAATACATGCTAAAAATCCCG
ACTTCGGAAGGGATGTATTTATTAGATTAAAAACCAATGCCCTTCGGGGCTCACT
GGTGATTCATGATAACTCCTCGAATCGCATGGCCTTGTGCCGGCGATGGTTCATT
CAAATTTCTTCCCTATCAACTTTCGATGTTTGGGTATTGGCCAAACATGGTTGCA
ACGGGTAACGGAGGGTTAGGGCTCGACCCCGGAGAAGGAGCCTGAGAAACGGCTA
CTACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGG
TAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATTGGAATGAGT
ACAATTTAAATCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGC
CGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGTGGTTAAAAAGCTC
GTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCTCACCGCGTGTACTGGTCCG
GCCGGGCCTTTCCCTCTGTGGAACCCCATGCCCTTCACTGGGTGTGGCGGGGAAA
CAGGACTTTTACTGTGAAAAAATTAGAGTGCTCCAGGCAGGCCTATGCTCGAATA
CATTAGCATGGAATAATAGAATAGGACGTGTGGTTCTATTTTGTTGGTGTCTACG
ACCGCCCTCATGATTATTAGGGACAGTCAGTGGCATCAGTATTCACTTGTCAGAG
GTGAAATTCTTGGATGTATTGAAAACTAACTACTGCGACGCCGTTAGCGAGGATG
TTTTCATTATTAAGAACGACCGTACGGG

Alkaline cellulase production using different carbon sources

Secondary screening was conducted according to the method by Ramanmathan et al. [9]. A liquid cellulase enzyme production medium containing 100 mL of the modified culture medium was replaced with 1% of three different agro-cellulolytic wastes, including Rice straw (RS), wheat straw (WS), and sugarcane bagasse (SB). This medium was autoclaved and prepared with an *A. oryzae* in 250 mL Erlenmeyer flask after alkaline pretreatment of the agro waste. The liquid culture medium was incubated at 150 rpm in a rotary shaker for 12 days with intervals of 2 days (2, 4, 6, 8, 10, and 12). In each interval, the reduction in sugar was measured according to the method of Miller[10].

Enzyme assay

The endoglucanase activity was measured using the dinitrosalicylic acid method of Miller [10]. In this method, 0.5 mL of diluted enzyme in 0.05 M citrate buffer (pH 8.5) was mixed with 0.5 mL of 1% CMC for endoglucanase. After incubation at 50 ºC for 30 min, the reaction was immediately stopped by the addition of 3 mL of dinitrosalicylic acid and heating at 100 ºC for 10 min, followed by immediate cooling to stop further reaction. A spectrophotometer measured absorbance at 540 nm. One unit of CMC and filter paper was defined as the amount of enzyme that produces 1 µmole of reducing sugar, equivalent to glucose, per minute under standard conditions [9].

Optimization of production conditions using one-factor-at-a-time (OFAT)

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

Statistical optimization

Response Surface Methodology (RSM) was employed to statistically design experiments for optimizing the selected parameters influencing endoglucanase production using a central composite design (CCD). Three independent variables, including pH, temperature, and ammonium sulfate concentration, were selected for enzyme optimization. This leads to the model suggesting 20 different experiments, with minimum, medium, and maximum values (-1, 0, and +1), respectively, as shown in **Table 1**.

Design Expert Windows v 6.0.8 portable was the statistical software package used during the tabulation and processing process, allowing for a quick and simple data appraisal. All experiments were performed in triplicate, and the mean enzyme production was used as the variable response, Y. The equation indicates the second-order model used to describe the relationship between the independent variable and the response. Experiments were carried out in triplicate, with the mean production used as the response variable Y. The final RSM-predicted response was further validated experimentally.

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

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.

Determination of Deinked Paper Properties

Following pulping and flotation of the enzymatic, chemical, and control pulps, the resultant hand sheet was prepared according to TAPPI standards and sent to IWOPIN Pulp and Paper Company (IPPC) Limited, Ogun State, Nigeria, for testing of pulp brightness, tensile strength, bursting strength, and tearing strength.

Statistical Analysis

The statistical tool used in the research was Analysis of Variance (ANOVA), which utilized data generated from central composite design (CCD) experiments for CMCase production. The data replicates exhibited a coefficient of variation (CV) below 20%. 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 incubating it at different times per day, ranging from the 4th to 12th days. Maximum endoglucanase production of 3.52U/ml was found on the eighth day of incubation as indicated in **Fig. 1**. The result is in line with the work of Ramanathan et al. [9], where maximum CMCase (1.92±0.005) was produced from *A. oryzae* after 7 days of incubation. However, a maximum of 0.061 U/mL of CMCase was produced from *Aspergillus hortai* after 4 days of incubation [11].

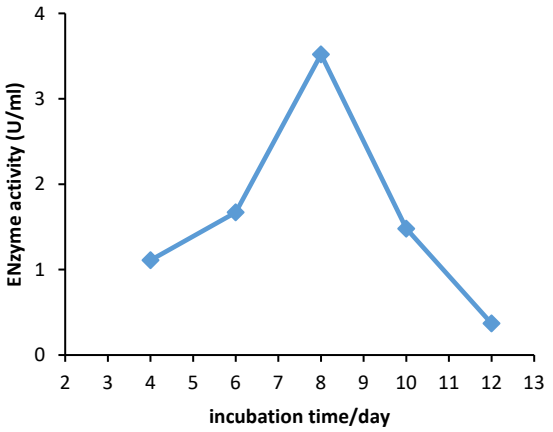

Fig. 1. Effect of incubation time on the production of endoglucanase and exoglucanase from *Aspergillus oryzae.*

Effect of initial pH

Among the 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 their activity is either highest or lowest. The changes in fungal production and enzyme activity across various pH ranges vary among 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 (3.50 U/mL) in a liquid medium at 30 ºC at 7 days incubation was observed from *A. oryzae* under shaking conditions at pH 6.

The work is in accordance with the work of Azzaz et al. [12], where cellulase exhibits an optimum activity of 0.009 U/ml from *Aspergillus niger* at pH 6. At a pH of 7, Basak and Rangan [13] and Saha [14] also reported optimum cellulase production from Fusarium oxysporum and Mucor circinelloides, which is in line with our work. However, a significant amount of enzyme was produced in an alkaline environment. Similar results were found in an alkaline environment by different fungal strains, as reported by other researchers [8,15,16].

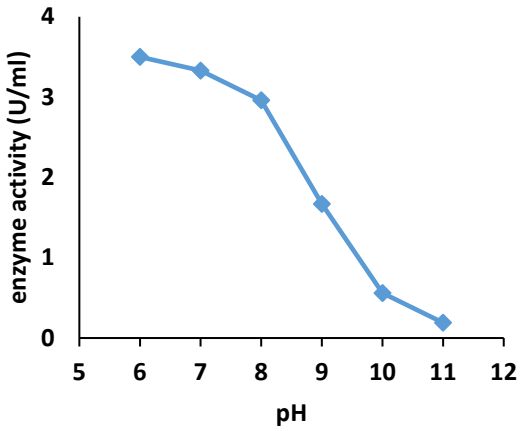

Fig. 2. Effect of temperature on the production of endoglucanase (CMCase) from *Aspergillus oryzae.*

Effect of different carbon sources

Three different types of agricultural waste from the University farm were selected and subjected to drying and alkaline pretreatment: rice straw (RS), wheat straw (WS), and sugarcane bagasse (SB). These agro-cellulolytic wastes were substituted as sole source of carbon from the fermentation media. The maximum CMCase activity of 2.78 U/mL was observed in rice straw on the 8th day of incubation at 30 °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 *Aspergillus oryzae.*

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

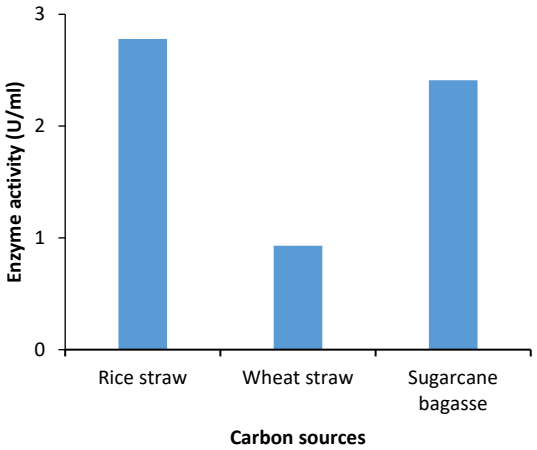

Fig. 3. Effect of different carbon sources on the production of endoglucanase (CMCase) from *Aspergillus oryzae.*

This activity in accordance with the work of Sasi et al. [17] was maximum. A case of 0.128 g/mL was found in rice bran, followed by wheat bran (0.097 g/mL) and sugarcane bagasse (0.019 g/mL), respectively. Bhavsar et al. [18] also reported a maximum CMCase production of 7.4 U/mL from banana stem, followed by rice straw at 5.4 U/mL, using fungal cellulase.

Effect of temperature

Different microorganisms and enzymes have different favorable growth temperatures, as well as different optimal temperatures for maximum enzyme production. The research was conducted at different temperature ranges (30 to 60 ºC), where maximum CMCase activity (3.50 U/ml) was recorded at 30 ºC, as shown in Fig. 4. A similar growth temperature (33 ºC) for *Fusarium oxysporum* was reported to have a maximum CMCase production [19].

Many researchers also reported maximum CMCase production at high temperatures. Remaz et al. [20] and Ramanathan et al. [9] reported maximum cellulase production from *Aspergillus niger* and *Fusarium oxysporum* at 50 ºC respectively. Dutta et al. [16] also reported a maximum CMCase production at 50 ºC 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 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 (3.50 U/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. [17] who found that ammonium sulphate increases the amount of cellulase enzymes produced from *Aspergillus flavus*. Optimum cellulase activity was found when ammonium sulfate was used as the nitrogen source. However, it disagrees with the work of El-Hadi et al. [11] and Irfan et al. [21], which found that maximum cellulase activity followed the addition of ammonium sulphate. Urea was also found to be the best nitrogen source on the production and optimization of cellulase enzyme from *Fusarium oxysporum* [9].

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 3.70 U/mL, as shown in Fig. 6. An increase or decrease in ammonium sulfate from the optimum concentration lowered the rate of enzyme production. This result is similar to that obtained by Vyas et al. [22] and Sasi et al. [17], where ammonium sulfate was reported to be optimal.

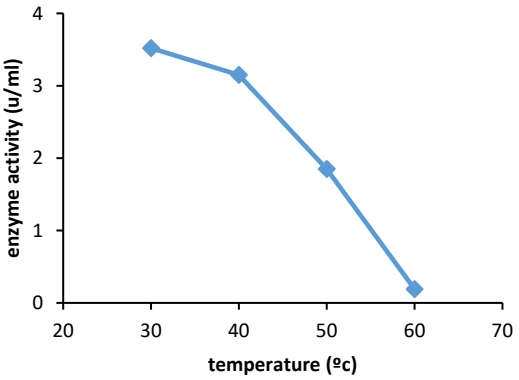

Fig. 4. Effect of temperature on the production of endoglucanase (CMCase) from *Aspergillus oryzae.*

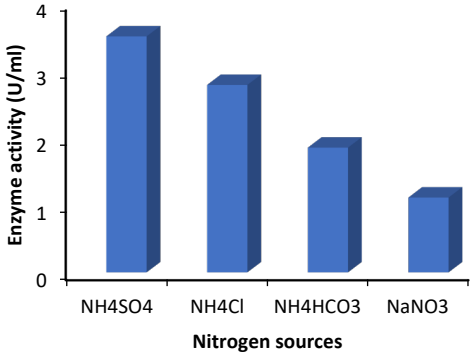

Fig. 5. Effect of different inorganic sources of nitrogen on the production of CMCase from *Aspergillus oryzae*. Error bars represent mean±standard deviation.

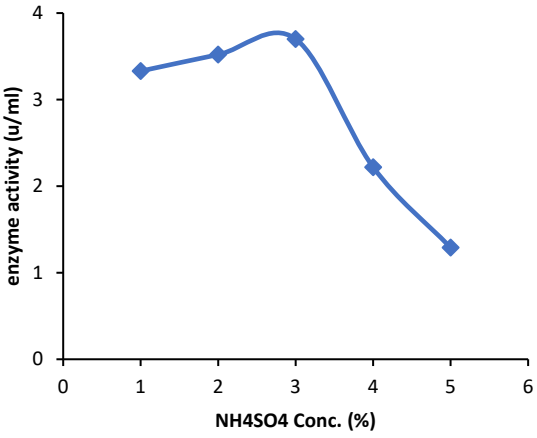

Fig. 6. Effect of ammonium sulphate concentration on the production of endoglucanase and exoglucanase from *Aspergillus oryzae.*

Statistical optimization of parameters influencing endoglucanase production

In this research, Response Surface Methodology (RSM) was employed to develop a quadratic model of the experiment using Central Composite Design (CCD). Design Expert v 6.0.8 was used to generate the experimental design. The software randomly designed a total of 20 experiments, which were 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 the central composite experimental plan for CMCase were summarized in **Table 2**.

A regression equation was obtained when Analysis of Variance (ANOVA) provided an estimate of endoglucanase activity as a function of the independent variable. The polynomial mathematical model in Equation 2 may explain the production of endoglucanase, which involves the interaction of different variables at various levels, where Y represents CMCase production, A represents pH, B represents temperature, and C represents ammonium sulfate concentration. Model precision was normally determined by the coefficient of determination (R^2), and its values always range between 0 and 1, where the order of magnitude suggests the goodness of the model [23]. As indicated in **Table 3**, the R^2 value of CMCase was found to be 0.9933, which is close to 1 and indicates that 99.33% of the model's behavior can be explained by the a model of endoglucanase enzyme production, while only 0.67of the % full variance cannot be explained by the models.

According to Yusuf et al. [24], for a polynomial model to achieve high accuracy and a good fit, the R^2 value must be close to 1. A similar R^2 value of 0.9873 CMCase was reported by Kumar et al. [25]. Adjusted R^2 from this model was found to be 0.9873. This indicates a good relationship between the actual and predicted values. The predicted R^2 values for CMCase (0.9710) agreed with the adjusted R^2 values of 0.9873. Hence, the model provides clarity on how response and independent variables are interrelated.

The model's sufficient precision measured the signal-to-noise ratio; for CMCase, it was 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 [26], with adequate precision values of 17.4, while optimizing nutrient supplements for the removal of Cr(VI) by *Aspergillus lentulus* AML05.

The significance of the model is generally measured based on the P-value of the F-value (prob > F). The higher the F-value and the corresponding lower prob > F value, the greater is the importance of the corresponding coefficients (R^2) [27]. For maximum cellulase enzyme production, **Table 4** summarizes the second-order response in the form of an Analysis of Variance (ANOVA). The result indicated that the high model F-value for CMCase was 165.31, with a respective small P-value of <0.0001, signifying that the model was significant. This means the probability that the F-value model could occur due to noise was 0.01%.

To understand the application of each coefficient, P-values are used as the tool. The p-value < 0.05 showed that the models were significant. This means that A, B, C, A^2, B^2, C^2, AB, AC, and BC are the significant model terms. The lack of fit *F*-value of the models was 1.83, while a lack of fit of 0.2613 indicated that the lack of fit was not significant and the model was very accurate without any noise.

Borugadda and Goud [23] reported that a lack of fit must be estimated to examine Analysis of Variance (ANOVA) on each model coefficient and to ensure an accurate model fit. According to Manogaran et al. [28] and Ibrahim et al. [29], a non-significant lack of fit value indicates an excellent fit of the data to the model. Based on the results obtained, an excellent relationship was found between the actual and predicted values, as depicted in Equations 2 and 3, which describe the actual and coded factors, respectively.

$$CMCase = (0.70) + (-0.63xA) + (1.00xB) + (0.22xC) + (0.21xA^2) + (0.90xB^2) + (0.21xC^2) + (0.65xAB) + (0.094xAC) + (0.14xBC) \quad \text{(Eqn. 2)}$$

$$CMCase = (19.07785) + (-0.51475*A) + (-0.58746*B) + (-0.16350*C) + (-0.033600xA^2) + (4.00000E\text{-}003*B^2) + (-0.052500*C^2) + (0.017300*AB) + (0.018750*AC) + (4.62500E\text{-}003*BC) \quad \text{(Eqn. 3)}$$

Validation of Experimental Model

The optimum values of the three most important parameters, as determined from the contour plots, were 8.5 pH, 45 ºC temperature, and 3% ammonium sulfate concentration. The model was experimentally validated by producing endoglucanase from the optimized values in the same liquid medium using submerged fermentation. The experimental condition leads to an increase in CMCase production by 0.19 U/mL compared to the 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 the industrial production of CMCase, as shown in **Table 5**.

Potential applications of the enzyme in the Pulp and Paper industries

Endoglucanase plays a crucial role in the paper and pulp industries, specifically in the removal of ink from waste paper. Many researchers have reported the application of this enzyme in improving paper brightness and reducing residual ink compared to the conventional method. Remaz et al. [30] reported an improved brightness of endoglucanase enzyme-treated pulp by 3-4 points as compared to denatured control pulp samples, as well as a reduction of residual ink speck. This finding aligns with our results, which show that the enzyme can increase the percentage brightness (0.12%), Tensile strength (297 N/m), bursting strength (9 kPa), and tearing strength (73 mN) of waste paper compared to the chemical deinking process, as presented in **Table 6**.

Many researchers around the world have also reported a significant increase in pulp brightness and a clear decrease in the amount of residual ink when using CMCase enzymes, which are isolated from different kinds of bacteria and fungi. This information shows that such enzymes, especially CMCase, can play a very important role in the pulp and paper industry, particularly in removing ink from used or waste paper materials. Due to these positive results, it is becoming increasingly important to seek better sources of these enzymes that can function more effectively under industrial conditions.

One of the very interesting possibilities is to produce an endoglucanase enzyme from alkaliphilic fungi, which are special types of fungi that thrive and grow well in high-pH environments. Additionally, if these fungi can produce enzymes even at high temperatures, they will be more suitable for use in large-scale processing, where heat is utilized. Therefore, it is expected that producing this enzyme from such fungi at higher temperatures can be a good alternative option for pulp and paper companies seeking a more eco-friendly and efficient method for removing ink from recycled papers [31,32].

Table 2. Experimental design with coded levels of variables used in Central Composite Design, including experimental and predicted values for CMCase activity from *Aspergillus oryzae.*

Standard Order	Run	Factor 1 pH	Factor 2 Temperature (ºC)	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 *Aspergillus oryzae.*

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 *Aspergillus oryzae.*

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				

Table 5. Validation of optimum conditions and results obtained between OFAT and RSM for the optimization of CMCase and FPase from *A. oryzae.*

Factors	CMCase (IU/ml)		FPase (FPU/ml)	
	OFAT	RSM	OFAT	RSM
pH	6	8.5	6	8.5
Temperature (ºC)	30	45	30	45
NH_4SO_4 (%)	3	3	3	3
Enzyme activity	3.52	4.26	3.70	4.26
Enzyme increase	-	0.74	-	0.56

Table 6. Optical and mechanical properties of enzymatic, chemical and control hand sheet.

Test parameters	Unit	Control	Chemical	*Enzymatic Bio deinking*
Brightness	%	79.63	81.51	81.63
Tensile strength	N/m	1070	953	1250
Bursting strength	KPa	56	58	67
Tearing strength	mN	285	279	352

CONCLUSION

Currently, the conventional method of enzyme production and optimization, such as one-factor-at-a-time (OFAT), is now replaced by a more accurate and easier statistical approach called Response Surface Methodology (RSM). This statistical model has the ability to generate a large number of pieces of information from a smaller number of experiments. In this research, both OFAT and RSM were applied to produce and optimize endoglucanase using different physicochemical and nutritional factors with alkaliphilic Aspergillus oryzae isolated and screened in our laboratory. It was found that using central composite design (CCD) in RSM, there was an increase of 0.39 U/mL endoglucanase as compared to OFAT. The endoglucanase enzyme produced by *A. oryzae* was also able to increase the percentage brightness (0.12%), Tensile strength (297 N/m), bursting strength (9 kPa), and tearing strength (73 mN) of waste paper compared to the chemical deinking process.

ACKNOWLEDGEMENT

The authors acknowledge the Tertiary Education Trust Fund (TETFund), Abuja, Nigeria, and Jigawa State Polytechnic, Dutse, Nigeria, for their sponsorship and provision of facilities, respectively.

CONFLICT OF INTEREST

The authors have nothing to declare

REFERENCE

1. Singh R, Kumar M, Mittal A, Kumar P. Microbial enzymes : industrial progress in 21st century. 3 Biotech. 2016;6(2):1–15.
2. Yakubu Abdulhadi. Saikia Upasana & Vyas Ashish. Microbial enzymes and their application in pulp and paper industry. In: Ajar Nath Yadav, Sangram Singh SM and AG, editor. Recent Advancement in White Biotechnology Through Fungi. Springer; 2019. p. 297–317.
3. Yang W, Meng F, Peng J, Han P, Fang F, Ma L, et al. Isolation and identification of a cellulolytic bacterium from the tibetan pig's intestine and investigation of its cellulase production. Electronic Journal of Biotechnology. 2014;17(6):262–7.
4. Zambare VP. Optimization of amylase production from Bacillus sp. Using statistics based experimental design. Emirates Journal of Food and Agriculture. 2011;23(1):37–47.
5. Aanchal, Akhtar N, Kanika, Goyal D, Goyal A. Response surface methodology for optimization of microbial cellulase production. Romanian Biotechnological Letters. 2016;21(5):11832–41.
6. Lee CK, Ibrahim D. Enzymztic and Chemical Deinking of mixed office wastepaper and old newspaper: Paper quality and effluent characteristics. BioResources. 2011;6(4):3859–75.
7. Zhang X, Renaud S, Paice M. Cellulase deinking of fresh and aged recycled newsprint / magazines (ONP / OMG). Enzyme and Microbial Technology. 2008;43:103–8.
8. Vega K, Villena GK, Sarmiento VH, Ludeña Y, Vera N, Gutiérrez-Correa M. Production of Alkaline Cellulase by Fungi Isolated from an Undisturbed Rain Forest of Peru. Biotechnology Research International. 2012;2012:1–7.
9. Ramanathan G, Banupriya S, Abirami D. Production and optimization of cellulase from Fusarium oxysporum by submerged

fermentation. Journal of Scientific and Industrial Research. 2010;69(6):454–9.
10. Miller GL. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry. 1959;31(3):426–8.
11. El-hadi AA, El-nour SA, Hammad A, Kamel Z. Optimization of cultural and nutritional conditions for carboxymethylcellulase production by Aspergillus hortai. Journal of Radiation Research and Applied Sciences. 2014;7:23–8.
12. Azzaz, H.H., Murad, H.A., Kholif, A. M., Hanfy, M.A. and Abdel Gawad MH. Ortimization of culture conditions affecting fungal cellulase production. Research Journal of Microbiology. 2012;7(1):23–31.
13. Basak S& RL. Production, characterization and inhibition of fungal cellulases utilizing rice bran as substrate in solid state fermentation. Microbiology: Current Research. 2018;02(04):8–13.
14. Saha BC. Production , purification and properties of endoglucanase from a newly isolated strain of Mucor circinelloides. Process Biochemistry. 2004;39:1871–6.
15. Muhammad H, Iqbal N, Ahmed I, Zia MA, Irfan M. Purification and characterization of the kinetic parameters of cellulase produced from wheat straw by Trichoderma viride under SSF and its detergent compatibility. Advances in Bioscience and Biotechnology. 2011;2011(June):149–56.
16. Dutta S, Tarafder M, Datta B. Characterixation of cellulolytic enzymes of Fusarium soil isolates. Biocatalysis and Agricultural Biotechnology. 2018;1–12.
17. Sasi A, Ravikumar M, Manthirikani S. Optimization , production and purification of cellulase enzyme from marine Aspergillus flavus. African Journal of Microbiology Research. 2012;6(20):4214–8.
18. Bhavsar NH, Raol B V, Amin SS, Raol GG. Production , Optimization and Characterization of Fungal Cellulase for Enzymatic Saccharification of Lignocellosic Agro-waste. inj j Curr App Sci. 2015;4(3):30–46.
19. Panagiotou G, Kekos D, Macris BJ, Christakopoulos P. Production of cellulolytic and xylanolytic enzymes by Fusarium oxysporum grown on corn sto v er in solid state fermentation. industrial Crops and Products. 2003;18:37–45.
20. Remaz A& B. Optimization of Factors Influencing Cellulase Production by Some Indigenous Isolated Fungal Species. Jordan Journal of Biological Sciences. 2018;11(1):31–6.
21. Irfan M, Nadeem M, Syed Q. One Factor at a time (OFAT) optimization of xylanase production from Trichoderma viride IR05 in solid state fermentation. Journal of Radiation Research and Applied Sciences. 2014;7(3):317–26.
22. Vyas A, Vyas D, Vyas KM. Production and optimization of cellulases on pretreated groundnut shell by Aspergillus terreus AV49. Journal of Scientific & Industrial Research. 2005;64(April):281–6.
23. Borugadda VB, Goud V V. Response surface methodology for optimization of bio-lubricant basestock synthesis from high free fatty acids castor oil. Energy Science and Engineering. 2015;3(4):371–83.
24. Yusuf I, Aqlima S, Phang LY, Syed MA, Shamaan NA, Khalil KA, et al. Keratinase production and biodegradation of polluted secondary chicken feather wastes by a newly isolated multi heavy metal tolerant bacterium-Alcaligenes sp. AQ05-001. Journal of Environmental Management. 2016;183:182–95.
25. Kumar B, Bhardwaj N, Alam A, Agrawal K, Prasad H, Verma P. Production, purification and characterization of an acid/alkali and thermo tolerant cellulase from Schizophyllum commune NAIMCC-F-03379 and its application in hydrolysis of lignocellulosic wastes. AMB Express. 2018;8(1).
26. Sharma S, Malik A, Satya S. Application of response surface methodology (RSM) for optimization of nutrient supplementation for Cr (VI) removal by Aspergillus lentulus AML05. Journal of Hazardous Materials. 2009;164(2–3):1198–204.
27. Hassanzadeh-Tabrizi SA, Taheri-Nassaj E. Modeling and optimization of densification of nanocrystalline Al2O3powder prepared by a sol-gel method using response surface methodology. Journal of Sol-Gel Science and Technology. 2011;57(2):212–20.
28. Manogaran M, Shukor MY, Yasid NA, Khalil KA, Ahmad SA. Optimisation of culture composition for glyphosate degradation by Burkholderia vietnamiensis strain AQ5-12. 3 Biotech. 2018;8(2):1–13.
29. Ibrahim S, Shukor MY, Khalil KA, Helmi MIE, Syed MA, Ahmad SA. Application of Response Surface Methodology for optimising caffeine-degrading parameters by Leifsonia sp. strain SIU. Journal of Environmental Biology. 2015;36(5):1215–21.
30. Vyas S, Lachke A. Biodeinking of mixed office waste paper by alkaline active cellulases from alkalotolerant Fusarium sp. Enzyme and Microbial Technology. 2003;32(2):236–45.
31. Saini S, Chutani P, Kumar P, Sharma KK. Development of an eco-friendly deinking process for the production of bioethanol using diverse hazardous paper wastes. Renewable Energy. 2020;146:2362–73.
32. Tsatsis DE, Papachristos DK, Valta KA, Vlyssides AG, Economides DG. Enzymatic deinking for recycling of office waste paper. Journal of Environmental Chemical Engineering. 2017;5(2):1744–53.