iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 5 **Issue:** XII **Month of publication:** December 2017

DOI:

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor :6.887
Volume 5 Issue XII December 2017- Available at www.ijraset.com

Optimization of AFEX Pretreated Agrowaste Media for Endoglucanase and Xylanase Production by Stenotrophomonas maltophilia

Rohini T[1], Satheeja Santhi V[2], Saranya S[3], Sam Ebenezer R[4], Shakila H[5], *Harshavardhan Shakila*[6],
[1, 2, 3 4, 5] *Department of Molecular Microbiology, School of Biotechnology, Madurai Kamaraj University, Madurai - 625 021, Tamil Nadu, India.*
[6]*Department of Molecular Microbiology, School of Biotechnology, Madurai Kamaraj University, Madurai - 625 021, Tamil Nadu, India,*

Abstract*: Translation of cellulosic biomaterials into simpler commercially beneficial products has gained attention during the past two decades. Endoglucanase and xylanase are such enzymes, which can convert complex lignocellulose into simple sugars, which can further be utilized for production of economically valuable products like ethanol. In this study, optimization of media components for the enhanced production of aforementioned enzymes was carried out by submerged fermentation (SMF) method, using Ammonia Fiber Explosion (AFEX) treated rice straw, a lignocellulosic waste from an agricultural field, as a substrate. Stenotrophomonas maltophilia, isolated from bio-gas waste slurry was used as production organism. The enzyme activities of end oglucanase and xylanase with unoptimized medium were 169.68 U/ml/min and 141.66 U/ml/min respectively. It was further optimized by statistical approaches like Placket-Burman design and Central Composite Design (CCD). Production was found to get significantly enhanced by Cellulose, K_2HPO_4, Yeast extract and peptone. The maximum activities predicted by the model value of 245.31 & 206.14 U/ml/min which was in good agreement with experimental values of 256.4 U/ml/min, 212 U/ml/min. However, lignin peroxidase activity decreased from 53.21 to 37.6 U/ml/min. The optimized production medium was finally determined to contain Cellulose (18.75g/L), Di-Potassium Phosphate (3.75g/L), yeast extract (2.5g/L) and peptone (7.5g/L).

Keywords- Biomass Conversion; Media Optimization; Cellulase; Substrate treatment; Batch Fermentation

I. INTRODUCTION

Bioconversion of cellulosic materials has been receiving attention in recent years since large quantities of cellulosic wastes are generated every day through forestry and agricultural processes [1] which remain un-utilized and gets accumulated in the environment and becomes a cause of pollution [2]. This biomass mainly consist of plant dry matter which is collectively called as Lignocelluloses. It is mainly comprised of cellulose, hemicelluloses, and lignin [3,4]. Cellulose and hemicellulose are carbohydrate polymers while lignin is a polymer with aromatic rings. Cellulose consists of linear chains of hundreds or thousands of glucose molecules, while the most abundant hemicelluloses are comprised of xylans. They also consist of heterogeneous mixtures of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose) and sugar acids [5]. Lignocellulosic materials could be naturally degraded to monomeric sugars by enzymatic hydrolysis done by cellulase and xylanase, which are widely secreted by various bacterial and fungal species [6]. The enzymatic hydrolysates of lignocellulosic materials containing reducing sugars can further be used for the production of biofuels such as hydrogen, ethanol and other high-value chemicals [7].

Cellulases are the enzymes that hydrolyze the cellulose β (1,4) bonds, act synergistically in its degradation, and yield glucose as the main final product, which can be readily fermented into ethanol and other molecules. Three main groups of these enzymes are described: Endoglucanase, exoglucanase, and β-glucosidase. The endoglucanase act on the amorphous region of the fiber and generate non-reducing and reducing terminals for the subsequent action of exoglucanases (CBH İ and CBH Π). CBH İ and CBH Π act simultaneously on the reducing and non-reducing ends to release cellobiose. The β-glucosidase hydrolyze cellobiose to produce glucose. This synergistic action of cellulases promotes a higher yield of the cellulolytic complex when compared to the sum of individual yields [8].

Cellulase is one of the most important industrial enzymes, which is used in the paper, textile, pharmaceutical, food industries, brewing and chemical industries. Cellulase and hemicellulase were efficiently used for de-inking of xerographic and laser- printed

paper [9]. Market trends reveal that cellulase and xylanase take the major portion of share accounting for 20% of the world enzyme market, together with pectinase [10]. In order to attain high yield, the choice of an appropriate substrate in the production media is of great importance. Apart from providing carbon and energy source, a good substrate should provide the necessary inducing compounds for the organism, preferentially for an extended period of time, to increase the overall productivity of the fermentation process [11,12]. Such optimized substrate and production conditions determine the economics of an enzymatic hydrolysis with low production cost [8]. Medium optimization for enhanced production of cellulase production by fungi had been carried out successfully by using different statistical approaches [13-16]. Statistical methods such as Plackett- Burman design (PB), the Box-Behnken design and Central Composite Design (CCD) are the most widely used methods [17,18]. The process of statistical media optimization is generally carried out in important three steps, (i) screening the most significant medium components which enhance enzyme production by using Plackett- Burman design, (ii) Optimization of the most significant medium components by application of central composite design (CCD) of response surface methodology (RSM), and (iii) verification or validation of the model.

PB design is used for screening and selection of significant variables and it will be followed by CCD of response surface methodology (RSM) which provides an important information regarding the optimum level of each variable, their interaction with other variables and effect on the product yield. It involves a minimum number of experiments for a large number of factors, by which improvement in enzyme production had been achieved and reported elsewhere [19-21]. In the present study, attempts were made through the approaches mentioned above to get the maximum production of endoglucanase and xylanase by Stenotrophomonas maltophilia in the submerged batch fermentation process, using rice straw as the substrate.

II. MATERIALS AND METHODS

A. *Microorganism*

Stenotrophomonas matlophilia isolated from slurry samples from the biogas plant was used in this study and it was maintained on CMC (Carboxy Methyl Cellulose) agar slants and stored at 4^{o}C till further process [22].

B. *Agro-Industrial By-Products*

Paddy straw (Rice straw) and Sorghum stubbles were obtained from a nearby agricultural field, Madurai District, Tamilnadu. To enhance the accessibility of microorganism on the substrate for enzyme production, a pretreatment was carried out by the alkaline physiochemical pretreatment process, namely Ammonia Fiber Explosion method (AFEX) [23]. Here the 10g of chopped biomasses were exposed to liquid ammonia at 100 °C for a period of one hour. These treated bio-waste substrates were then sun-dried individually to reduce the moisture content and powdered [24]. The pre-treated biomass substrates obtained by this process were denoted as RP for paddy straw and SP for sorghum straw. Non-pretreated ground biomass from paddy straw and sorghum stubbles were denoted as RN and SN respectively. The AFEX pretreated paddy straw substrate (RPM) provided by the Institute of Chemical Technology (ICT), Mumbai, India was also used for comparative analysis with our pre-treated biomass.

C. *Chemical analysis of Agro-Industrial By-Products*

Estimation of cellulose, hemicellulose, and lignin from treated and non-treated substrates were carried out. Cellulose content was estimated by anthrone method [25]. The amount of acid-insoluble lignin was estimated by the Klason method [26]. Hemicellulose was estimated by the method of Goering and Van Soest [27]. The ash content determination was done by burning the samples at 550^{o}C in a muffle furnace for three hours, according to the Van Soest and Wine [28]. Total reducing sugar and total proteins were also estimated simultaneously [29,30]. All the quantifications were carried out in experimental triplicates.

D. *Enzyme Assay*

Cellulase activity was determined according to the method of the International Union of Pure and Applied Chemistry (IUPAC) and expressed as International Units (IU) [31].

Endoglucanase activity was assayed by adding 0.5ml of diluted enzyme to 0.5ml of 1% (w/v) of carboxymethyl cellulose (CMC) in 50mM Sodium Citrate buffer (pH-4.8) and incubating at 50^{o}C for 30min. The amount of reducing sugars released during the reaction was measured using the DNS method [29] and D-glucose was used as a standard. One international unit of endoglucanase activity is the amount of enzyme that forms 1µmol glucose (reducing sugars as glucose) per minute during the hydrolysis reaction [32].

Xylanase activity was measured by incubating 0.5ml of 1% (w/v) Birchwood Xylan in Sodium Citrate buffer (pH 4.8) and 0.5ml of diluted enzyme extract at 50^{o}C for 30min.The release of reducing sugar was measured by the DNS method [29]. Xylose was used as

a standard. One unit of Xylanase activity is the amount of enzyme that forms 1µmol xylose per minute under standard assay conditions [33].

Lignin peroxidase activity was estimated by adding 0.25ml of enzyme solution with 0.25ml of 1mM veratrol alcohol, 0.2mM H_2O_2, and 0.5ml of 0.1M citrate buffer. This assay is based on the oxidation of the veratryl alcohol (3,4-Dimethoxy benzyl alcohol) to Veratraldehyde in the presence of H_2O_2; the increase in absorbance at 310nm is monitored for one minute at 30^{o}C [34].

E. Selection of Suitable Substrate for Enzyme Production

AFEX treated and non-treated paddy and sorghum straw samples were analyzed comparatively for higher yield of endoglucanase and xylanase enzymes. The minimal medium was supplemented with 1% of treated and non-treated substrates separately. It was autoclaved at 121^{o}C for 20 minutes under 15psi pressure. The medium was cooled down to normal room temperature and checked for its sterility by overnight incubation. Each flask was inoculated with a log phase culture of *S. matlophilia* and incubated at 37^{o}C for 96 hours in the batch fermentation process. After incubation, the culture filtrates were collected by centrifugation at 10,000 rpm for ten minutes. The supernatant was subjected to the enzymatic assay for the production of endoglucanase and xylanase as mentioned previously. The substrate, which yielded increased enzyme activity was taken for further study.

F. Submerged Batch Fermentation

One liter of fermentation media (pH 7.6) containing 10g of pre-treated paddy straw supplemented with 0.7g K_2HPO_4; 0.7g KH_2PO_4; 0.7g $MgSO_4$; 1.0g NH_4Cl; 1g $NaNO_3$; 0.5g NaCl and 0.001g $FeSO_4$, were used for cellulase production. Each flask was plugged with hydrophobic cotton plug and autoclaved. After cooling, inoculation was carried out as mentioned above. Fermentation was carried out in Erlenmeyer flasks and incubated at 37^{o}C on an orbital shaker (150rpm) for 72hrs and culture filtrate was harvested by centrifugation as mentioned previously and used for determination of enzyme activity.

G. Statistical Optimization of Production Media

1) Plackett- Burman Design (PBD): Significant nutrient components were screened and identified by the Plackett-Burman design using statistical software package Design Expert® 10.0.4.0 software version 10 (Stat-Ease Inc., USA) to yield the maximum concentration of cellulase. Based on this design, each factor was evaluated on two levels, -1 for low level and +1 for high level [35]. The factors with their levels are shown in Table 1, whereas Table 3 represents the design matrix. 19 variables were screened in 20 experimental designs. All experiments were carried out in triplicate and the average of the enzyme activity was taken as responses (Table 3).

Table 1: Nutrient screening using a Plackett-Burman design

Factor	Components	Unit	High +	Low–
A	CMC	g/L	30	7.5
B	Starch	g/L	20	5
C	Filter paper	g/L	8	2
D	Glucose	g/L	40	10
E	Cellulose	g/L	30	7.5
F	$Cacl_2$	g/L	1	0.25
G	KH_2PO_4	g/L	20	5
H	$FeSO_4$	g/L	5	1.75
J	$MgSO_4$	g/L	15	4
K	$(NH_4)_2SO_4$	g/L	10	2.5
L	Yeast extract	g/L	20	5
M	K_2HPO_4	g/L	6	1.5
N	Peptone	g/L	4.5	1.5
O	NaCl	g/L	20	5
P	Galactose	g/L	15	5
Q	Malt extract	g/L	7.5	2.5
R	NH_4NO_3	g/L	2	0.5
S	$NaNO_3$	g/L	2	0.5
T	$CoCl_2$	g/L	3	0.75

From the regression analysis the variables, which were significant at or above the 95% level ($p<0.05$), were considered to have the greatest impact on enzyme activity and were further optimized by central composite design.

2) Central Composite Design (CCD): Response surface methodology (RSM) was used to optimize the selected four variables, namely, Cellulose, K_2HPO_4, Yeast extract, Peptone which enhances desired enzyme production. The four independent variables were studied at five different levels (Table 4), and sets of 30 experiments were carried out (Table 5). The statistical software package' Design Expert® 10.0.4.0' was used to analyze the experimental data. All variables were taken as a centrally coded value of zero. The minimum and maximum ranges of the variables investigated are listed in Table-4. Upon the completion of the experiments, the average maximum enzyme activity was taken as the response (Y).

This method is suitable for fitting a quadratic surface and it helps to optimize the effective parameters with a minimum number of experiments as well as to analyze the interaction between the parameters. In order to determine the existence of a relationship between the factors and response variables, the collected data were analyzed in a statistical manner, using regression. A multiple regression analysis of the data was carried out for obtaining an empirical model that relates the response measured by the independent variables as given in Table 4.

A 2^4 factorial CCD was developed by design expert package version 10.0.4.0 with 8 axial points and 6 replicates at the center points leading to 30 runs. The variables were coded according to the following equation.

$$x_i = \frac{X_i - X_0}{\Delta X}, \text{ i=1,2,3,...,k} \quad (1)$$

Where x_i is the dimensionless value of an independent variable, X_i is the real value of an independent variable, X_0 is the value of X_i at the center point and ΔX is the step change. A second-order polynomial model was used to fit the quadratic resulting in the equation,

$$Y = \beta_o + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_5 A^2 + \beta_6 B^2 + \beta_7 C^2 + \beta_8 D^2 + \beta_9 AB + \beta_{10} AC + \beta_{11} AD + \beta_{12} BC + \beta_{13} BD + \beta_{14} CD. \quad (2)$$

Where Y is the measured response (cellulase activity (U/ml)), A, B, C and D are the coded independent input variables, β_0 is the intercept term, β_1, β_2, β_3, and β_4 are the coefficients showing the linear effects, β_5, β_6, β_7, and β_8 are the quadratic coefficients showing the squared effects and β_9, β_{10}, β_{11}, β_{12}, β_{13}, and β_{14} are the cross product coefficients showing the interaction effects.

To determine the significance of each term in the equations fitted and to estimate the goodness of fit in each case, the analysis of variance (ANOVA) was used. The fitted polynomial equation was then expressed in the form of three-dimensional response surface plots to illustrate the main and interactive effects of the independent variables on the dependent ones. The combination of different optimized variables, which yielded the maximum response, was determined to verify the validity of the model. In order to verify the accuracy of the predicted model, an experimental study was conducted with initial and optimized media. The optimal concentrations of the critical variables were obtained by analyzing 3D plots. The statistical analysis of the model was represented in the form of analysis of variance (ANOVA).

III. RESULTS AND DISCUSSION

A. Selection of Substrate

AFEX pretreated and non-pretreated substrates of rice straw and sorghum stubbles were selected for endoglucanase and xylanase production by submerged batch fermentation. The maximum endoglucanase and xylanase production (34.2 & 23.1 U/ml/min) was observed in pretreated paddy straw (RP) followed by the control substrate RPM (32 & 21 U/ml/min, respectively) the other substrates as RN, SP, SN yielded low enzyme production in the similar conditions (Fig. 1). Thus pre-treated paddy straw can be considered as the best substrate for cellulase production because of its global availability and low cost. Several other works have previously reported the suitability of paddy straw as a source of endoglucanase and xylanase production. One among such is the production of the cellulase and D-xylanases by Aspergillus ustus, and Aspergillus niger KK2 using rice straw as a substrate for solid state fermentation [36, 37] and submerged fermentation by Aspergillus niger [38].

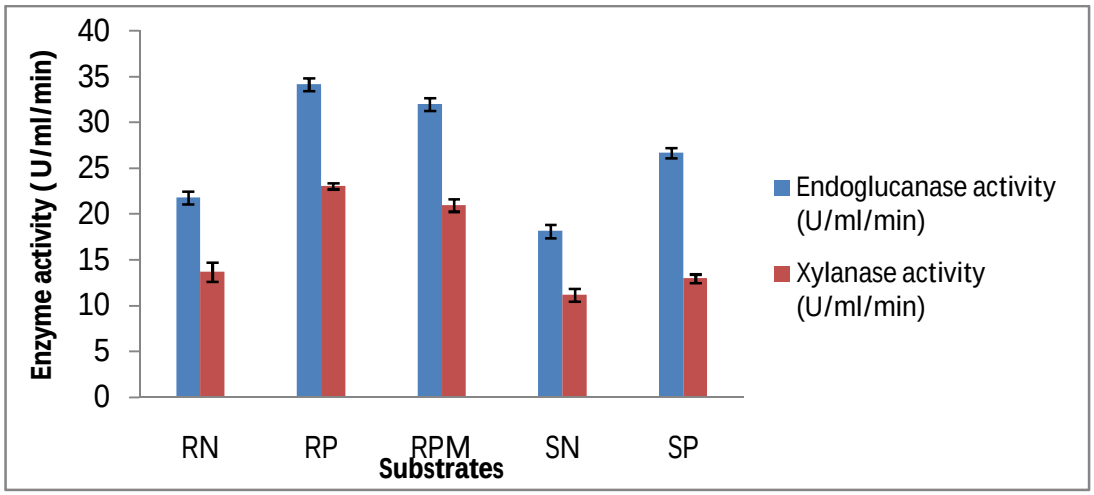


Fig.1: Endoglucanase and Xylanase production with different substrates (RN- Non-Pretreated Rice, RP- Rice Pretreated, RPM- Rice Pretreated Mumbai, SN- Sorghum Non-Pretreated and SP- Sorghum Pretreated).

B. Composition of Agro-Industrial By-Products

In the present study, the AFEX pretreatment was efficient in reducing the hemicelluloses and lignin contents and there was an increase in the cellulose content (Table 2). This pretreatment removed the lignin content to increase the accessibility of the cellulose in the substrate and enables nearly complete saccharification of the polysaccharides. AFEX pretreated paddy straw (RP) showed high cellulose content (45.6%) followed by the AFEX treated control paddy straw (RPM) (40.2%). This, increased conversion of cellulose would increase the available sugar content in the hydrolyzate [39]. Comparison between pretreated and non-pretreated substrates of rice straw and sorghum stumbles along with control (RPM) showed that pretreated substrates RP and SP yield more amount of reducing sugars than untreated substrates RN and SN. The increase in cellulose and hemicelluloses were predominantly leading to the decrease in lignin.

Pretreatment of lignocellulosic biomass is generally used to alter the structure of lignin and hemicellulose components or reducing their level, thereby reducing the crystallinity of cellulose, which further assists the efficient hydrolysis by the cellulase enzyme complex [40]. AFEX pretreatment results in the decrystallization of cellulose [41], partial depolymerization of hemicelluloses, removal of acetyl groups [42] predominantly on hemicelluloses, cleavage of lignin-carbohydrate complex (LCC) linkages, lignin C-O-C bond cleavage, increase in accessible surface area due to structural disruption, and increased wettability of the treated biomass [43]. The ammonia left after the pretreatment process can be recovered, recycled, and reused [44].

Table 2: Chemical Composition of Substrates in Percentage

Substrates	Ash (%)	Protein (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reducing sugar (%)
RPM	6.31±0.26	3.58±0.11	40.2±0.25	21.5±0.36	2.3±0.12	0.692± 0.009
RP	5.96±0.19	4.20±0.25	45.6±0.11	18.4±0.42	1.5±0.08	0.752± 0.016
RN	3.54±0.42	3.64±0.28	32.6±0.32	25.6±0.24	4.2±0.13	0.468 ± 0.012
SP	4.85±0.56	4.86±0.15	40.0±0.56	19.1±0.13	3.1±0.19	0.506 ± 0.018
SN	2.35±0.13	2.80±0.24	35.6±0.68	23.1±0.19	3.4±0.72	0.284 ± 0.024

C. Statistical Optimization of Production Media

Plackett-Burman (PB) experiments (Table-3) showed a wide variation in Endoglucanase, Xylanase & Lignin peroxidase activity. This variation reflected the importance of optimization to attain higher productivity. Based on the results obtained from PB, Pareto chart was created to identify the most significant factor which has the greatest cumulative effect on the system. The significance of each factor is represented as a series of bars in which impact of factors corresponds to its height and colour. From the Pareto chart (Fig. 2&3), the variables, namely cellulose, K_2HPO_4, yeast extract, peptone were selected for further optimization to attain a

maximum production of Cellulase. The concentration of factors (cellulose, K_2HPO_4, yeast extract and peptone) in the culture media and its effect on interactions on cellulase production was determined by CCD using RSM.

Table 3: Plackett-Burman experimental design matrix for screening of important variables for cellulase production

Run Order	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q	R	S	T	Endoglucanase activity (1U/ml/min)	Xylanase activity (1U/ml/min)	Lignin peroxidase activity (1U/ml/min)
1	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	151.19	51.66	72.36
2	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	140.92	59.33	59.54
3	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	136.86	58	63.28
4	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	136.83	61	60.84
5	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	140.97	72.66	63.54
6	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	137.35	93.33	60.54
7	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	169.68	141.66	53.21
8	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	137.40	121	57.35
9	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	137.63	99.66	60.54
10	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	151.48	126.33	59.32
11	+	-	+	-	-	-	-	+	+	-	+	+	-	+	+	+	+	+	-	137.04	112.33	58.64
12	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	40.99	76.66	63.84
13	+	-	+	-	+	-	-	-	-	+	+	-	+	-	-	-	+	+	+	110.75	82.66	56.84
14	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	73.89	74	60.93
15	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	66.38	108	55.64
16	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	55.65	86	55.34
17	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	70.64	79.66	54.34
18	-	-	+	+	+	+	-	+	-	+	-	-	-	+	+	+	-	+	+	26.04	59	61.45
19	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	55.35	34.33	53.45
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	51.94	78.66	56.36

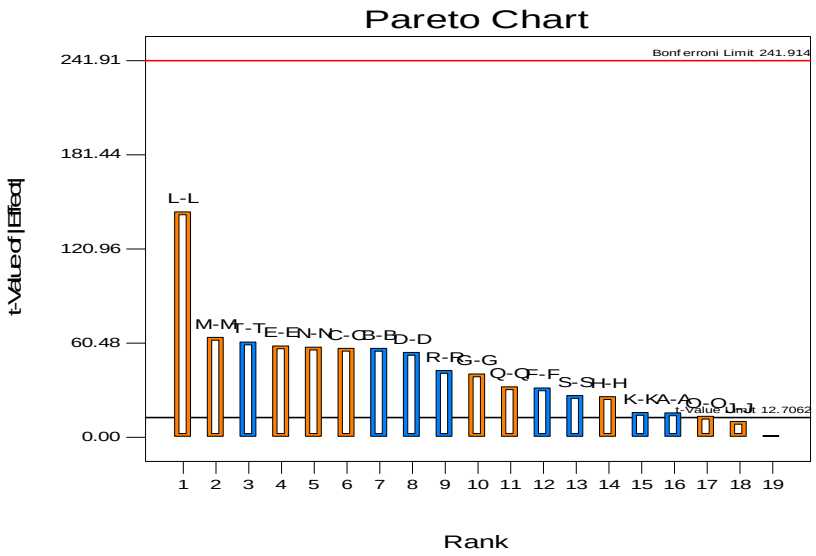


Fig. 2. Pareto chart showing the effect of medium components on Endoglucanase production.

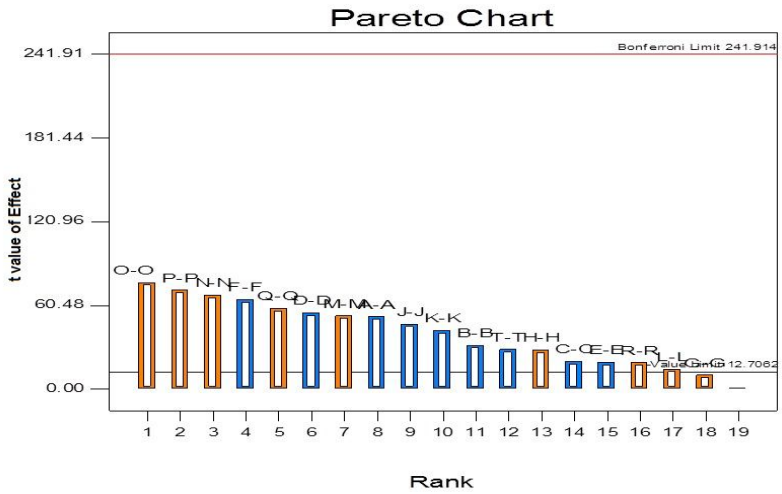


Fig. 3. Pareto chart showing the effect of medium components on Xylanase production.

Table 4: Ranges of independent variables used in RSM

Variables	Code	Levels (g/L)				
		-2	-1	0	+1	+2
Cellulose	A	3.75	7.5	18.75	30	41.25
K_2HPO_4	B	0.75	1.5	3.75	6	8.25
Yeast Extract	C	2.5	5	12.5	20	27.5
Peptone	D	1.5	3	7.5	12	16.5

Table 5: Central composite design (CCD) of factors in coded levels with Enzyme activity as response

Cellulase,Hemi cellulase& Lignin activities					Endoglucanase activity(1U/ml/min)		Xylanase activity (1U/ml/min)		Lignin peroxidase activity (1U/ml/min)	
Run	A	B	C	D	Actual Value	Predicted Value	Actual Value	Predicted Value	Actual Value	Predicted Value
1	-1	-1	-1	-1	250.30	250.67	97.46	106.54	89.23	84.10
2	1	-1	-1	-1	226.50	225.59	84.50	101.83	78.38	76.81
3	-1	1	-1	-1	218.08	225.76	81.17	83.59	77.63	79.89
4	1	1	-1	-1	252.93	235.75	154.28	134.71	77.02	73.35
5	-1	-1	1	-1	78.60	85	26.18	28.99	56.67	57.36
6	1	-1	1	-1	218.74	225.80	142.00	145.67	58.84	60.54
7	-1	1	1	-1	228.36	235.75	153.40	134.71	73.22	73.85
8	1	1	1	-1	212.40	235.75	128.00	134.71	76.10	73.85
9	-1	-1	-1	1	164.40	181.53	112.40	130.17	72.60	68.32
10	1	-1	-1	1	237.70	236.69	138.73	132.08	71.76	69.06
11	-1	1	-1	1	176.13	175.12	142.40	150.24	77.24	72.68
12	1	1	-1	1	227.65	230.69	124.60	116.51	73.15	76.09
13	-1	-1	1	1	237.66	239.44	93.43	103.46	74.24	75.66

14	1	-1	1	1	238.36	224.76	93.40	78.71	72.67	67.84
15	-1	1	1	1	244.11	235.75	112.28	134.71	74.34	73.85
16	1	1	1	1	213.80	209.84	148.85	133.47	61.46	60.28
17	-2	0	0	0	159.78	151.37	88.07	88.68	66.19	67.68
18	2	0	0	0	163.55	168.97	74.65	68.60	74.36	80.69
19	0	-2	0	0	228.90	235.75	142.00	134.71	71.17	73.85
20	0	2	0	0	212.40	203.30	133.00	116.87	73.37	74.18
21	0	0	-2	0	256.40	245.31	212.00	206.14	37.6	44.71
22	0	0	2	0	223.60	217.00	183.60	76.20	72.21	71.51
23	0	0	0	-2	173.20	183.21	136.37	152.62	73.58	77.23
24	0	0	0	2	218.96	221.69	122.00	123.25	54.08	51.73
25	0	0	0	0	247.78	235.75	118.28	134.71	71.23	73.85
26	0	0	0	0	109.03	108.68	38.32	47.01	62.14	58.59
27	0	0	0	0	54.20	47.26	42.60	44.16	68.31	68.92
28	0	0	0	0	169.29	182.21	134.77	147.44	76.88	80.14
29	0	0	0	0	130.87	125.37	119.43	114.46	77.04	78.14
30	0	0	0	0	132.40	128.78	124.68	100.34	71.74	69.24

Thirty experiments were performed in different combinations of the factors as shown in the table 5. The observed responses along with design matrix are presented in the table 6, and the results were analyzed by ANOVA.

Table 6: Analysis Of Variance of the Experimental Results of the CCD

Source	df	F-value			p-value		
		Y_1	Y_2	Y_3	Y_1	Y_2	Y_3
Model	14	33.68	10.03	2.58	<0.0001 #	<0.0001 #	0.0395 *
A	1	0.46	9.00	2.42	0.5102 a	0.0090 *	0.1404 a
B	1	0.31	7.04	3.43	0.5844 a	0.0181 *	0.0840 a
C	1	58.98	15.55	0.076	<0.0001#	0.0013 *	0.0259 *
D	1	9.63	1.14	0.18	0.0073 *	0.3025 a	0.6741 a
AB	1	22.97	21.12	4.15	0.0002 ^	0.0339 *	0.0898 a
AC	1	3.35	13.73	9.99	0.0872 a	0.0021 *	0.0965 a
AD	1	17.13	0.049	7.11	0.0009 ^	0.8271 a	0.0776 a
BC	1	100.83	24.11	0.75	<0.0001 #	<0.0001 #	0.4009 a
BD	1	32.92	0.52	9.063	0.0058*	0.4808 a	0.9254 a
CD	1	1.62	5.45	6.11	0.2231 a	0.0002 ^	0.0659 a
A^2	1	5.06	2.28	1.47	0.0400 *	0.1518 a	0.2444 a

B^2	1	0.65	7.75	0.27	0.4318 [a]	0.0139 [*]	0.6102 [a]
C^2	1	221.33	21.96	0.57	0.1285 [a]	0.0003 [^]	0.4636 [a]
D^2	1	2.59	0.59	0.11	<0.0001 [#]	0.4546 [a]	0.0498 [*]
Residual	15	Y_1 – Endoglucanase, Y_2- Xylanase, Y_3- Lignin peroxidase Prob ≤ 0.0500 indicate model terms are significant. [*]P<0.05 – Significant at the 5% level, [^]P<0.001- Significant at the 1% level, [#]P<0.0001 – Significant at the 0.1% level, [a] not significant.					
Lack of fit	10						
Pure error	5						
Cor total	29						

D. Factors affecting Endoglucanase and Xylanase production:

Three-dimensional response surface plots graphically represented regression equations and were generally used to demonstrate the relationship between the response and experimental levels of each variable.The regression equation for the optimization of endoglucanase (Y_1), xylanase (Y_2) and lignin peroxidase (Y_3) production is provided in the equation 3, 4 and 5 respectively, while the selected variables, namely cellulose, K_2HPO_4, Yeast extract, and Peptone were represented as A, B, C, and D respectively. The ANOVA of the quadratic regression model demonstrated that these equations are highly significant for endoglucanase, xylanase production except that of lignin peroxidase. The model's goodness of fit was checked by determination coefficient (R^2).

On the basis of the quadratic polynomial equation of response surface model, the present model and data analysis not only allowed us to define optimum conditions for enzyme production but also showed the combined effect of independent variables.

$Y_1 = 235.75 + 1.79A - 1.48B - 20.38C + 8.23D - 5.58A^2 - 2.01B^2 - 36.93C^2 - 3.99D^2 - 15.58AB - 5.95AC - 13.45AD - 18.65BC - 32.64BD - 4.13CD.$ (3)

Where Y_1 denotes the predicted response of the endoglucanase production in SSF; A, B, C and D denotes Cellulose, K_2HPO_4, Yeast extract and Peptone respectively.

ANOVA for the response surface of endoglucanase is shown in Table 6. The model F-value of 33.68 implies that the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. $p<0.05$ was considered significant. In this case, the coefficients C, D, AB, AD, BC, BD, A^2, D^2 were found to be highly significant. The fit of the model was checked by the coefficient of determination, R^2 for endoglucanase activity. It was calculated to be 0.9692, which is close to 1, indicating that 96.92% of the variability in the response could be explained by the model. The predicted R^2value of 0.8832 is in reasonable agreement with the "Adj R- Squared" of 0.9404; i.e. the difference is less than 0.2. An adequate precision value greater than 4 is desirable. The adequate precision value of 22.126 indicates an adequate signal and suggests that the model can be navigated to the design space. The above model can be used to predict the Endoglucanase production within the limits of the experimental factors. Among the significant model terms C, BC, and D^2 were significant with a probability of 99.9% and AB and AD were significant at 99%. The interaction between B and C had a significant influence on the increase in Endoglucanase yield.

The equation of the response surface model for xylanase production (U/ml/min) (4) is represented below.

$Y_2 = 134.71 + 10.68A - 9.45B - 14.05C + 3.80D + 5.03A^2 - 9.27B^2 - 15.61C^2 - 2.56D^2 - 24.34AB - 16.16AC + 0.97AD - 10.19BC - 3.15BD - 21.42CD.$ (4)

Where, Y_2 denotes the predicted response of the xylanase production in SSF: A, B, C and D denotes Cellulose, K_2HPO_4, Yeast extract and Peptone respectively.

ANOVA for the response surface of Xylanase is shown in Table 6. The model F-value of 10.03 implies the model is significant. In this case, the coefficients A, B, C, AB, AC, BC, CD, B^2, C^2 was found to be highly significant. The coefficient of determination, R^2 for Xylanase activity was calculated to be 0.9035, which is close to 1. It indicates that 90.35% of the variability in the response could be explained by the model. The predicted R^2 value of 0.5905 is in reasonable agreement with Adj R^2 0.6134. An adequate precision value greater than 4 is desirable. The adequate precision value of 14.357 indicates an adequate signal and suggests that the

model can be navigated into the design space. The optimum concentration of each of these variables in the production media is represented as a three-dimensional response surface plot for the highest yield of Xylanase.

The model terms BC is significant with a probability of 99.9% and CD, C2 were significant with a probability of 99% and A, B, C, AC, and B^2 were significant with a probability of 95%. The Xylanase yield was significantly influenced by the interaction between B and C.

The equation of the response surface model for Lignin peroxidase production (U/ml/min) was fitted with the following quadratic equation (5).

$$Y_3 = 73.01 + 2.45A - 2.92B + 0.44C - 0.68D - 1.79A^2 - 0.77B^2 - 1.11C^2 - 0.48D^2 + 3.93AB - 6.10AC - 5.14AD + 1.67BC + 0.18BD - 4.77CD. \quad (5)$$

Where Y3 denotes the predicted response of the Lignin peroxidase production in SSF: A, B, C and, D denotes Cellulose, K_2HPO_4, Yeast extract and Peptone respectively.

ANOVA for the response surface of lignin peroxidase production is shown in table 6. The model F-value of 2.58 implies the model is significant. There is only a 3.95% chance that a "Model F-value" this large could occur due to noise. In this case, the coefficients C, D^2 was found to be highly significant. The fit of the model was checked by the coefficient of determination, R^2 for Lignin peroxidase activity. It was calculated to be 0.7065, which is close to 1. This indicates that 70.65% of the variability in the response could be explained by the model. The predicted R^2 value 0.4054 is in reasonable agreement with Adj R^2 0.4326. The adequate precision value of 6.766 indicates an adequate signal and suggests that the model can be navigated to the design space. The above model can be used to predict the Lignin peroxidase production within the limits of the experimental factors. The model terms C and D^2 were with a significant probability of 95%. No significant interaction between individual variables to decrease the lignin yield was found.

E. Validation Of The Model

The response surface plots showed the effect of Cellulose, K_2HPO_4, Yeast extract, and Peptone on the production of Endoglucanase and xylanase. The results indicated that the cellulase and hemicellulase response surfaces had a maximum point while it was minimized for lignin peroxidase. Response surface models were useful in determining the concentration of variables in the production media to achieve the maximum production of endoglucanase and xylanase with minimal lignin peroxidase production as reported by Mosier *et al.*, (2005). For endoglucanase activity (Fig 4.) the actual responses of values agree well with the predicted values. The RSM was used and three-dimensional plots were drawn between two factors keeping others at a fixed level. The circular shape of the curve indicates no interaction while elliptical shape indicates the good variation of two variables. The maximum production of endoglucanase would be achieved with the combination of K_2HPO_4 and Yeast extract (Fig. 5).

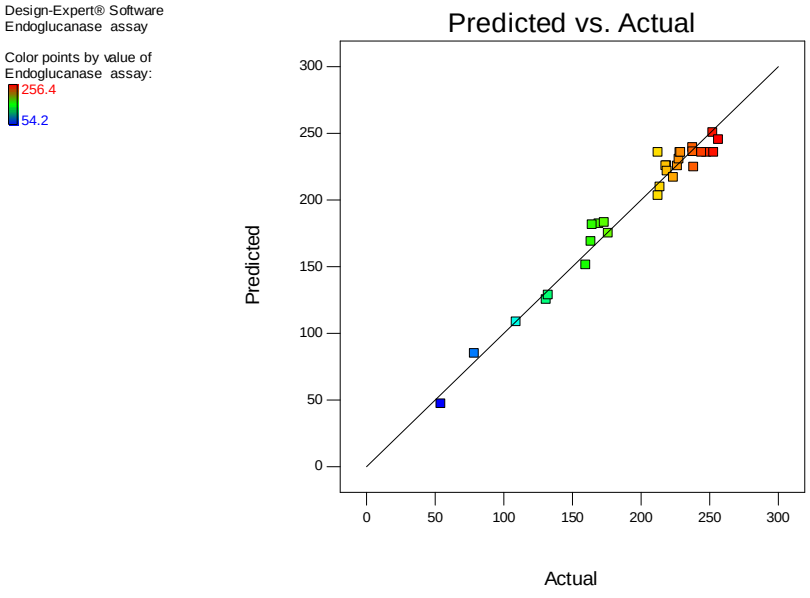


Fig. 4. Predicted vs. actual response of Endoglucanase activity

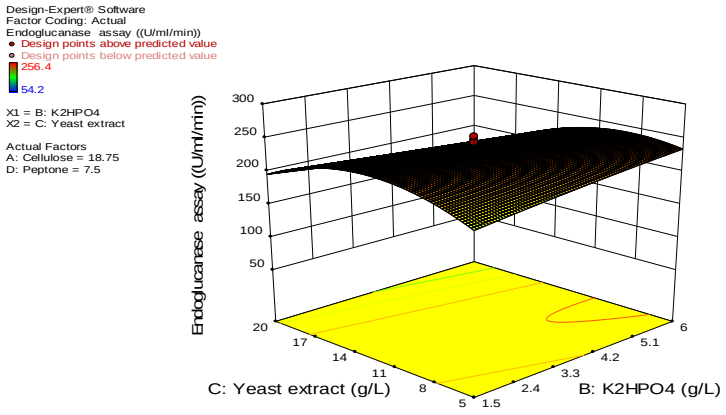


Fig. 5. The response surface plot shows the interactive effect of K_2HPO_4 and Yeast extract on Endoglucanase yield.

For xylanase yield (Fig. 6) comparison of the predicted values with the experimentally obtained actual values indicated that these data are in reasonable agreement. The surface plot of xylanase indicates that the maximum yield using pretreated rice straw could be attained at the combination of K_2HPO_4 and Yeast extract (Fig. 7).

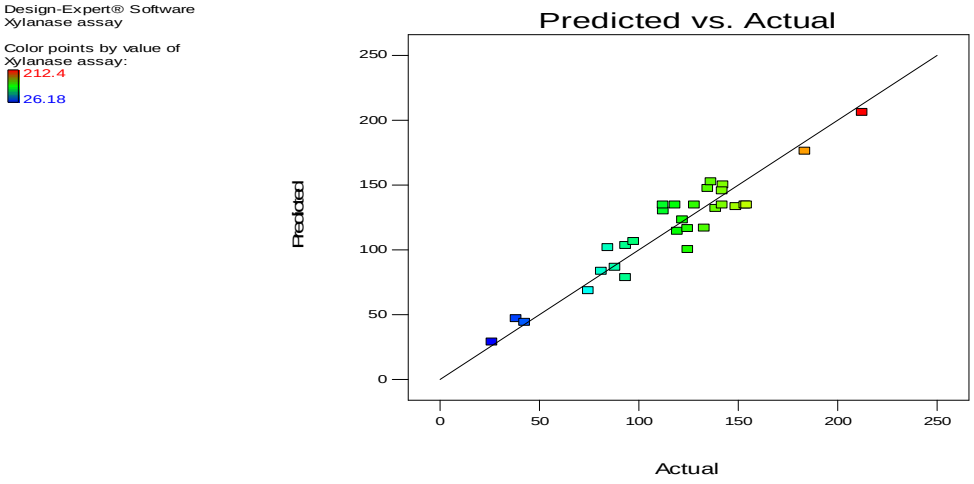


Fig. 6.Predicted vs. actual response of Xylanase activity.

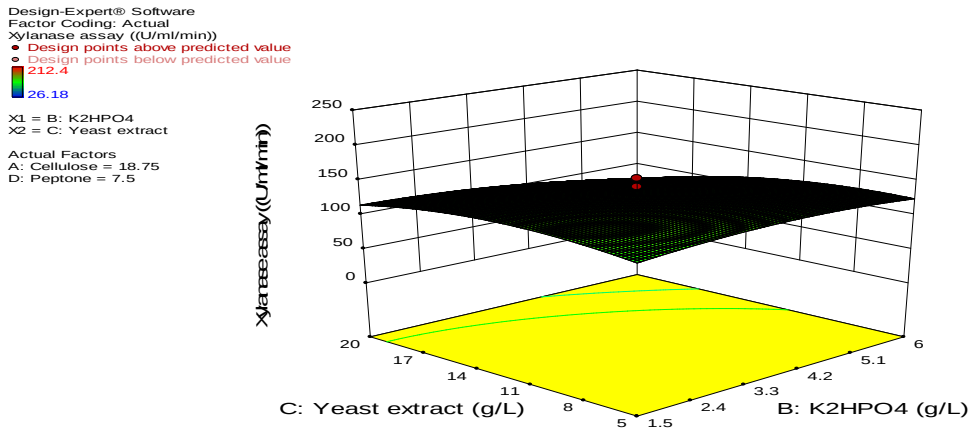


Fig. 7. The response surface plot shows the interactive effect of K_2HPO_4 and Yeast extract on Xylanase yield.

The maximum response predicted from the model was 245.31 and 206.14 U/ml/min (run no. 21 -Table 5) for endoglucanase and xylanase respectively. However, an increase in the activity of both the enzymes was achieved by using the experimentally optimized production media. The maximum yield of endoglucanase and xylanase along with the low lignin peroxidase activities were obtained using the production media containing 18.75g/L of Cellulose, 3.75 g.L of K_2HPO_4, 2.5 g/L of yeast extract and 7.5g/L of peptone (7.5g/L). In these optimized conditions, the maximum Endoglucanase and Xylanase activity were found to be 256.4 U/ml/min and 212 U/ml/min respectively. Validation of the experimental model was tested by carrying out the batch experiment under optimal operation conditions. Three repeated experiments were performed, and the results are compared. The enzyme activity of Endoglucanase, Xylanase, and Lignin peroxidase obtained from experiments was very close to the actual response predicted by the regression model.

The ability of microorganism in saccharification of cheap cellulosic materials such as wheat straw, sugar cane baggage, sugar beet pulp, sawdust and rice straw has been reported by several researchers [45-48]. Type of carbon source is an important factor which affects the inducible production of cellulase by microorganisms [49]. The yield of cellulase can be enhanced by increasing the concentration of cellulose and K_2HPO_4, indicating their importance in the media and reliability of model for enhancing cellulase production using Pseudomonas balearica [50]. The organic nitrogen source was found to be more suitable for optimizing cellulase production by Bacillus subtilis and B. circulars than inorganic sources [51]. Yeast extract and peptone have the significant effect on cellulase production by Cellulomonas fimi NCIM-5015 [52]. A maximum cellulase activity was observed at 2.5g/L of yeast extract. Integration of K_2HPO_4 in culture medium along with other essential components has been shown to increase cellulase production by several times using Clostridium thermocopriae [53] and Trichoderma reesei [54].

The optimized enzyme activity values obtained in this study is much higher than the reported values with other strains. It has been reported that the maximum endoglucanase activity achieved by Penicillium pinophilum NTG (mutant) was 137.61U/ml/min under submerged culture condition [55]. Anuradha et al. reported that maximum Endoglucanase activity (39.56U/ml/min) of A.nidulans was observed when the culture was grown in optimized conditions [56]. In another study, a much lower Endoglucanase production of 2.04U/ml/min was observed under optimized conditions from Bacillus sp., [57]. It has been reported that Aspergillus niger produced maximum xylanase activity 89.51U/ml/min under optimized conditions [58]. Lenartovicz et al., recorded maximum xylanase activity of 125.1 U/ml/min by A. fumigates [59]. In Chaetomium thermophilum it has been reported that maximum xylanase activity was 61 U/ml/min under optimized conditions [60].

IV. CONCLUSION

The results reported in this paper indicate that Stenotrophomonas sp., can be cultivated under submerged fermentation for the production of endoglucanase and xylanase using as carbon source available agricultural waste. This work demonstrates the statistical steps comprising of Plackett-Burman and central composite factorial design to optimize condition that contributes the maximum enzyme production. Initially, 19 media components were screened using PBD for their effect on the production of Endoglucanase and Xylanase. Out of them, cellulose, K_2HPO_4, yeast extract, and peptone were found to be statistically significant, which significantly enhanced cellulase activity. Followed by the PBD, CCD was employed to optimize these selected nutrients. The components of production media were optimized to be, cellulose (18.75g/L), K_2HPO_4 (3.75g/L), yeast extract (2.5g/L) and peptone (7.5g/L). Nearly 45% of an increase in enzyme yield was achieved by using the optimized media. These results show a close concordance between the predicted and obtained activity level. This study showed that the AFEX pretreated rice straw constitutes a good carbon source for the production of endoglucanase & xylanase.

V. ACKNOWLEDGEMENT

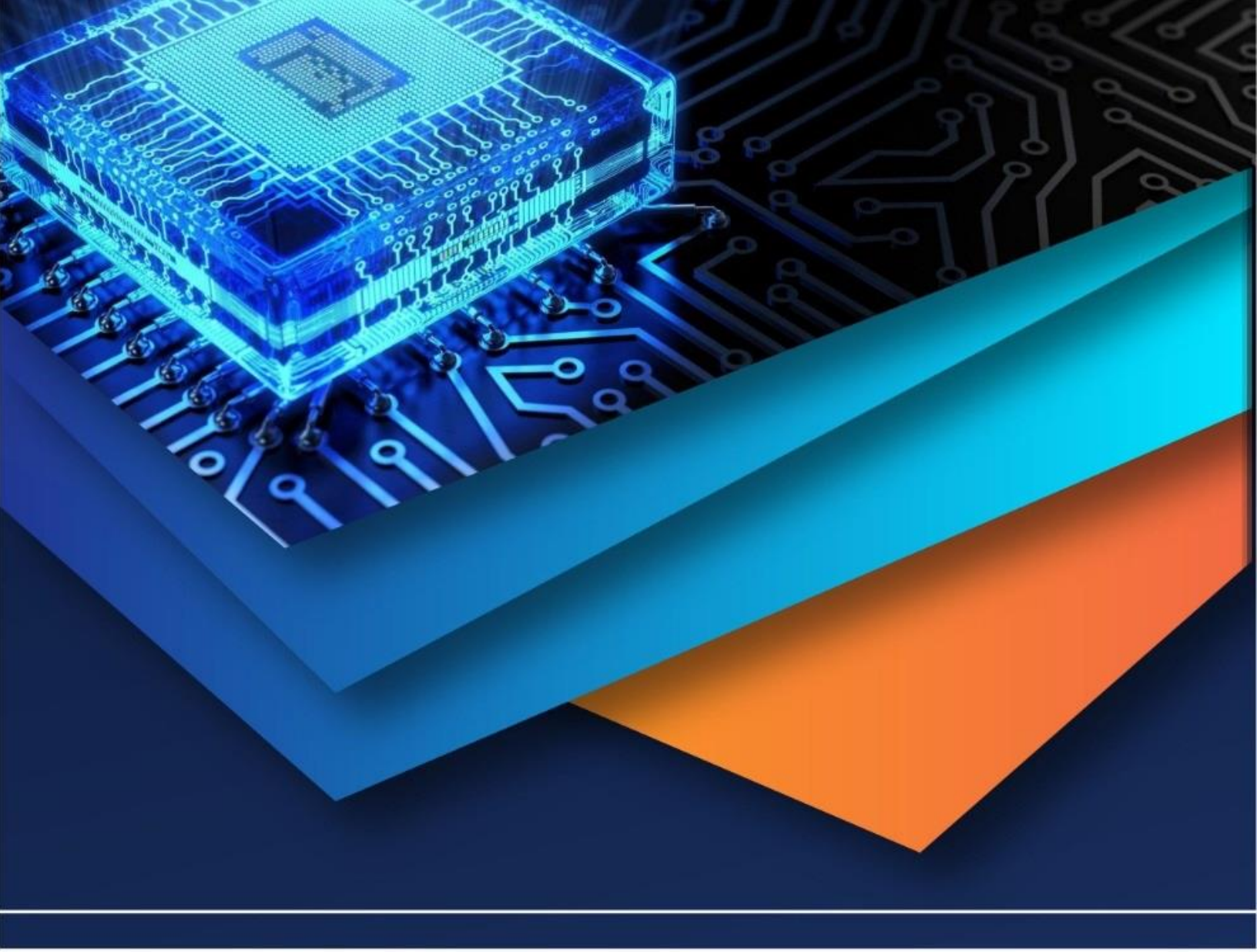
The authors are grateful to late Professor Dr. S.R.D Jebakumar for providing all the facilities and his encouragement**.** T. Rohini gratefully acknowledges UGC for providing Non-NET fellowship.

REFERENCES

[1] P.B. Acharya, D.K. Acharya and H.A. Modi, “Optimization for cellulose production by Aspergillusniger using saw dust as substrate”, Afr J Biotechnol,Vol. 7, pp. 4147-4152, 2008.
[2] M.A. Milala, A. Shugaba and A. Gidado, “Studies on the use of agricultural wastes for cellulase enzyme production by Aspergillus niger”, Res J Agric Bio Sci,Vol.1, pp. 325-328, 2005.
[3] L. Sanchez, “Lignocellulosic residues: Biodegradation and bioconversion by fungi”, Biotechnol. Adv,Vol. 27(2), pp. 185-194, 2009.
[4] R. Muthuvelayudham and T. Viruthagiri, “Application of Central Composite Design Based Response Surface Methodology in parameter Optimization and on Cellulase Production Using Agricultural Waste”, Int.J.chem.Biol.Eng, Vol. 3(2), pp. 97-104, 2010.
[5] B.C. Saha , “Hemicellulose bioconversion”, J Ind Microbiol Biot ,Vol. 30(5), pp. 279-291, 2003.

[6] L.M. Xia and X.L. Sheng, "High-yield cellulase production by Tricoderma reesei ZU-02 on corncob residues", Bioresour Technol, Vol. 91(3), pp. 259-262, 2004.
[7] B. Amouri, A. Gargouri, "Characterization of a novel β-glucosidase from a Stachybotrys strain", Biochem. Eng. J,Vol. 32, pp. 191-197, 2006.
[8] L.R. Lynd, P.J. Weimer, W.H. Van Zyl and I.S. Pretorius, "Microbial cellulose utilization: Fundamentals and biotechnology", Microbiol Mol Biol Rev,Vol. 66(3), pp. 506-577, 2002.
[9] G.M. Gubitz, S.D. Mansfield, D.Bohm and J.N. Saddler, "Effect of endoglucanases and hemicellulases in magnetic and flotation deinking of xerographic and laser-printed papers", J. Biotechnol,Vol. 65, pp. 209-215, 1998.
[10] M.L. Polizeil,A.C. Rizzatti, R. Monti and H.F. Trenzi, "Xylanases from fungi: properties and industrial applications", Appl Microbiol Biotechnol,Vol. 67(5), pp. 577-591, 2005.
[11] S.J.B. Duff and W.D. Murrayh , "Bioconversion of forest products industry waste cellulosic to fuel ethanol", Bioresour Technol,Vol. 55, pp. 31-33, 1996.
[12] D. Haltrich,B. Nidetzky, K.D. Kulbe, W. Steiner and S. Zupancic, "Production of Cellulase and Xylanase by Aspergillus terreus KJ829487 Using Cassava Peels as Sbustrate", Bioresour Technol, Vol. 58, pp. 137-161, 1996.
[13] S.R. Senthilkumar ,B. Ashokkumar ,K. Chandra Raj and P. Gunasekaran, "Optimization of medium composition for alkali-stable xylanase production by Aspergillus fischeri Fxn 1 in solid-state fermentation using central composite rotary design", Bioresour Technol, Vol. 96(12), pp. 1380-1386, 2005.
[14] X.C. Hao ,X. Yu and Z.L. Yan , "Optimization of the medium for the production of cellulase by the Mutant Tricoderme ressei WX-112 Using Response Surface Methodlogy", Food Technol. Biotechnol, Vol. 44(1), pp. 89-94. 2006.
[15] F. Francis ,A. Sabu ,K.M. Nampoothiri , "Use of response surface methodlogy for optimizing process parameters for the production of α-amylase by Aspergillus oryzae". Biochem.Eng.J, Vol. 15(2), pp. 107-115, 2003.
[16] G. Dey ,A. Mitra ,R. Bannered ,B.R. Maiti , "Enhanced production of amylase optimization of nutritional constituents using response surface methodology", Biochem. Eng. J, Vol. 7(3), pp. 227-231, 2001.
[17] M. Rafaat , I.A. Elsanhoty, Al-Turki, M.F. Ramadan , "Screening of medium components by Plackett- Burman design for carotenoid production using date (Phoenix dactylifera) wastes", Ind Crops Prod, Vol. 36(1), pp. 313-320, 2012.
[18] R. Soni, A.Nazir and B.S.Chadh , "Optimization of cellulase production by a versatile Aspergillus fumigates Fresenius strain (AMA) capable of efficient deinking and enzymatic hydrolysis of Solka floc and bagasse". Ind Crops Prod, Vol. 31(2), pp. 277-283, 2010.
[19] R.C.L.B. Rodrigues, M.A. Felipe, J.B.A. Silva and M.Vitolo , "Response surface methodlogy for xylitol production from sugarcane bagasse hemicellulosic hydrolyzate using controlled vacuum evaporation process variables", Process Biochem, Vol. 38(8), pp. 1231- 1237, 2003.
[20] A. Majumder and A. Goyal , "Enhanced production of exocellular glucansucrase from Leuconostoc dextranicum NRRL B-1146 using response surface method", Bioresour Technol, Vol. 99(9), pp. 3685-3691, 2008.
[21] N.B. Ghanem, H.H. Yusef and H.K. Mahrouse, "Production of Aspergillus terreus xylanase in solid-state cultures: application of the Plackett-Burman experimental design to evaluate nutritional requirements", Bioresour Technol, Vol. 73(2), pp. 113-121, 2000.
[22] T. Shankar,V. Mariappan and L. Isaiarasu, "Cellulolytic Bacteria from the Mid-Gut of the Popular Composting Earthworm, Eudriluseugeniae (Kinberg)", World J Zool, Vol. 6(2), pp. 142-148, 2011.
[23] M.T. Holtzapple, J.H.Jun, G.Ashok, S.L.Patibandla and B.E. Dale, "The ammonia freeze explosion (AFEX) process", Appl. Biochem. Biotechnol, Vol. 28/29, pp. 59-74, 1991.
[24] V.S. Santhi, A. Gupta,S. Saranya and S.R.D. Jebakumar, "A novel marine bacterium Isoptericola sp. JS-C42 with the ability to saccharifying the plant biomassesfor the aid in cellulosic ethanol production", Biotechnol Rep. Vol. 1-2, pp. 8-14, 2014.
[25] D.M. Updegroff , "Semimicro Determination of Cellulose in Biological Materials", Anal Biochem, Vol. 32, pp. 420-424, 1969.
[26] KCL Massan, ja puun kokanaisligniinipitoisuus, Total lignin content of wood and pulp-in Finnish, Espoo, Finland, Vol.115, pp. 3, 1982.
[27] H.K. Goering and P.J. Van Soest, "Forage fiber analysis"-Agricultural Research Service, Handbook: Vol. 379, 1975.
[28] P.J. Van Soset and R.H. Wine , " The determination of lignin and cellulose in acid detergent fiber with permanganate", J Assoc of Off Anal Chem , Vol. 51, pp. 780-787, 1968.
[29] G.L. Miller, "Use of dinitrosalicyclic acid reagent for determination of reducing sugar", Anal Chem,Vol. 31(3), pp. 426-428, 1959.
[30] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R. Randall, " Protein measurement with the Folin phenol reagent", J.Biol. Chem, Vol.193, pp. 265-275, 1951
[31] T.K. Ghose ,"The measurement of cellulose activities", Pure Apppl. Chem, Vol. 59(2), pp. 257-268, 1987.
[32] R.M. Teather and P.J. Wood, "Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen", Appl. Environ. Microbiol, Vol. 43, pp. 777-780, 1982.
[33] B. Adney, J. Baker, "Measurement of Cellulase Activities", NREL, Golden, 2008.
[34] M.Tien and T.K. Kirk, "Lignin degrading enzyme from Phanerochaete chrysosporium: purification , characterization and catalytic properties of unique H_2O_2-requiring oxygenase", Proceedings of the Natl. Acad. Sci, Vol. 81(8), pp. 2280-2284, 1983.
[35] R.L. Plackett, J.P. Burman, "The design of optimum multifactorial experiments", Biometrika, Vol. 33, pp. 305-325, 1946.
[36] T.R. Shamala, K.R. Sreekantish, "Production of cellulases and D-xylanases by some selected fungal isolates", Enzyme Microb. Technol, Vol. 8, pp. 178-182, 1986.
[37] S.W. Kang, Y.S. Park, J.S. Lee, S.I. Hong and S.W. Kim, "Production of cellulases and hemicellulases by Aspergillus niger KK2 from lignocellulosic biomass", Bioresour Technol , Vol. 91, pp. 153-156, 2004.
[38] S. Mrudula and R. Anitharaj, "Pectinase production in solid state fermentation by Aspergillus niger using orange peel as substrate", Global Journal of Biotechnology and Biochemistry,Vol. 6, pp. 64-71, 2011.
[39] P.R. Mohan,B. Vijaya kumar and O.V.S. Reddy, "Optimization of pretreatment conditions for increased cellulose conversion of sugarcane bagasse using peracetic acid employing central composite design", Songklanakarin J. Sci. Technol, Vol. 35(2), pp. 177-185, 2013.
[40] N. Mosier, C. Wyman,B. Dale, R. Elander, Y.Y. Lee, M. Holtzapple and M. Ladisch, "Features of promising technologies for pretreatment of lignocellulosic biomass", Bioresour Technol. Vol. 96, pp. 673-686, 2005.
[41] L.E. Gollapalli,B.E. Dale and D.M. Rivers, "Predicting digestibility of ammonia fiber explosion (AFEX) treated rice straw", Appl Biotechnol,Vol .98-100, pp. 23-35, 2002.

[42] J.J. O'Connor, "Ammonia explosion pulping: a new fiber separation process", TAPPI,Vol. 55, pp. 353, 1972.
[43] T. Rohini, V. Satheeja Santhi, R. Sam Ebenezer, H. Shakila, "Purification and characterization of an extracellular cellulase produced using alkali pretreated rice straw by Stenotrophomonas maltophilia", pp. 155-164, 2003.
[44] H. Alizadeh, F. Teymouri, T.I. Gilbert and B.E. Dale, "Pretreatment of switchgrass by ammonia fiber explosion (AFEX)", Appl Biochem Biotechnol, Vol. 121, pp. 1133-1142, 2005.
[45] Y.J. Lee, B.K. Kim, B.H. Lee, K.I. Jo,N.K. Lee and C.H. Chung, " Purification and characterization of cellulase produced by Bacillus amyoliquefaciens DL-3 utilizing rice hull", Bioresour Technol, Vol. 99, pp.378-386, 2008.
[46] P. Pachauri, S.B. Sullia and S. Deshmukh , "Statistical optimization for cellulase production with Aspergillus niger using lignocellulosic biomass by Response Surface Methodology", International Journal of Innnovative Research in Technology, Science & Engineering, 2016.
[47] L. Olsson, T.M.I.E. Christensen, K.P. Hansen and E.A. Palmqvist, "Influence of the carbon source on production of cellulases, hemicellulases and pectinases by Trichoderma reesei Rut C-30", Enzyme andMicrob Technol,Vol. 33, pp. 612-619, 2003.
[48] M. Jeya, N.P.T. Nguyen, H.J. Moon, S.H. Kim and J.K. Lee, "Conversion of woody biomass into fermentable sugars by cellulase from Agaricus arvensis", Bioresour Technol, Vol. 101, pp. 8742-8749, 2010.
[49] W. Yang, F. Meng, J. Peng, P. Han, F. Fang and L. Ma,"Isolation and identification of a cellulolytic bacterium from the Tibetan pig's intestine and
[50] investigation of its cellulase production", Electron. J. Biotechnol, Vol. 17, pp. 262–267, 2014.Dhaliwal, S. More, "Optimization of cellulase production by soil bacteria using statistical design", Int.J. Innov. Biol. Chem. Sci, Vol. 9, pp. 1-7. 2016.
[51] A.K. Ray, K. Sarkar Ghosh and S.K.Sen, " Optimization of fermentation conditions for cellulase production by Bacillus subtilis CY5 and Bacillus circulans TP3 isolated from fish gut," Acta Ichthyol. Pisc, Vol. 37 (1), pp. 47-53, 2007.
[52] S.B.R. Ali,R. Muthuvelayudham and T. Viruthagiri, "Enhanced production of cellulase from tapioca stem using response surface methodology", Innov Rom Biotechnol, Vol. 12, pp. 40-51, 2013.
[53] P. Saravanan, R. Muthuvelayudham and T. Viruthagiri, "Enhanced production of Cellulase from pineapple waste by Response Surface Methodology", J. Eng, pp. 1-8, 2013.
[54] D. Deka, P. Bhargavi, A. Sharma, D. Goyal, M. Jawed and A. Goyal, "Enhancement of cellulase Activity from a New Strain of Bacillus subtilis by Medium Optimization and Analysis with Various Cellulosic Substrates", Enzyme Res, Vol.1-8, 2011.
[55] A.J. Brown, S.A. Collin and T.M. Wood, "Development of a medium for high cellulase, xylanase and beta-glucosidase production by a mutanat strain (NTG III/6) of the cellulolytic fungas Penicillium pinophilum", Enzyme and Microb Technol, Vol. 9, pp. 355-360, 1987.
[56] J.S. Anuradha, N.C. Valli, "A new combitional statistical approach for cellulase optimization in Aspergillus nidulans", Indian J Sci Technol, Vol. 3,pp. 8, 2010.
[57] J. Singh, P. Kaur, "Optimization of process parameters for cellulase production from Bacillus sp. JS14 in solid substrate fermentation using response surface methodlogy", Braz Arch Biol Techn, Vol. 55, pp. 505-512, 2012.
[58] P. Khonzue, T. Laothanachareon, N. Rattanaphan, P. Tinnasulanon, S. Apawasin, A. Paemanee, V. Ruanglek, S. Tanapongpipat,V. Champreda and L. Eurwilaichitr, "Optimization of xylanase production from Aspergillus niger for Biobleaching of Eucalyptus pulp", Biosci. Biotechnol. Biochem, Vol. 75(6), pp. 1129-1134, 2011.
[59] V.Lenartovicz, C. de Souza, F. Moreira and R. Peralta, "Temperature effect in the production of multiple xylanases by Aspergillus fumigates", J. Basic Microbiol, 42, 388-395, 2002.
[60] P. Katapodis, V. Christakopoulou, P. Kekos and P. Christakopoulos, "Optimization of xylanase production by Chaetomium thermophilum in wheatstraw using response surface methodology", Biochem. Eng. J, Vol. 35, pp. 136-141, 2007.



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY