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STATISTICAL OPTIMIZATION OF PHYSICAL AND NUTRITIONAL FACTORS FOR LACCASE PRODUCTION BY ENVIRONMENTAL FUNGAL ISOLATE NS-1

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ABSTRACT

Laccases are multi-copper oxidases. They are lignin degrading enzymes and are found in bacteria, fungi, plant, insects. Fungal laccases possess high redox potential and wide substrate specificity and has been widely used in various environmental and biotechnology allied areas. Different production parameters were tested for enhanced laccase production by isolate NS-1 and the interactive effect of $MnSO_4.7H_2O$, wheat bran, pH and $CuSO_4.5H_2O$ were found to significantly enhance enzyme yield. Consequently, the concentration of these significant factors was further optimized using statistical experimental design. A ten-fold increase in laccase production was achieved under optimized conditions.

KEY WORDS: Laccase, bacterial**,** optimization, enzyme yield, pulp bio-bleaching.

INTRODUCTION

Laccase (benzenediol: oxygen oxide reductase; EC1.10.3.2.) are multicopper blue oxidases. They have broad specificity and catalyze the oxidation of phenolic and non-phenolic compounds and create a free radical[31]. Laccases are widely found in nature; they are produced by plants[27], fungi[8], bacteria[2,14], insects[11] and crustaceans[6], and are alter various phenolic compounds with oxidation, demethylation, polymerization or depolymerization. These enzymes are responsible for lignification in plants[5, 13]. In addition to this, in fungi laccases have been involved in different biological processes such as sporulation[7], pigment formation[16] and pathogenesis[12] in which laccase could play a potent role in necrosis of lignified areas via various pathogen immune evading responses.

Laccase production by fungi is strongly influenced by many growth factors such as time, culture conditions, various inducer molecules and chemical substances[23], aeration[10], and protease[24]. Standardization of media components are necessary to obtain laccases in high yield that may be used in various industrial applications and analytical downstreaming. It has wide substrate specificity due to which it can be used in various applications like drug analysis, juice and wine clarification, pulp biobleaching[29], decolorization of synthetic dyes[4], organic synthesis[25], laundry cleaning[17], bioremediation[21] and biosensors[30]. Thus, for an efficient laccase expression system, an effective optimization program is needed to increase laccase production under submerged fermentation conditions. Since fungal laccase expression system is influenced largely by culture parameters, pH of the medium and the presence of lignocellulosic materials[18]. This approach not only allows fast screening of experimental conditions, but also the interactive role of each component.

The determination of optimum conditions by the one factor at a time method is not always appropriate for enzyme production. Multi variable experiment designs are more precise to conduct and can significantly reduce the count of experimental runs needed to obtain optimized measures. This approach also extends interpretation and relative significance and interaction of several factors.

The study was conducted to increase laccase production by optimizing various physico-chemical parameters used for the growth and laccase production under submerged fermentation conditions using statistical experimental design. Prior to this study, the ability of the selected isolate to grow and produce laccase in the original basal liquid medium was evaluated previously in our lab. The present study is thus aimed to accomplish the optimization of conditions to obtain maximum yield of laccase from the fungal isolate NS-1.

2 MATERIAL AND METHODS

2.1 Fungal Strain

The fungal strain in this work, isolate NS-1 which was isolated in our lab. On the basis of macroscopic and microscopic features the fungal isolate NS-1 was found

to be closely related to Alternaria and Curvularia. The organism was grown and maintained at 30°C and sub-cultured on PDA (Potato Dextrose Agar) media**.**

2.2 Growth Medium

The medium for laccase production was prepared by adding 0.2% yeast extract, 0.2% tryptone, 100µM $CuSO_4.5H_2O$ into M162 (1X) and pH was adjusted to 7.0 with 1 N NaOH. M162 agar was prepared by adding 2.0% agar in it.

2.3 Composition of M162 (per litre) (10X)[9]

0.4g $CaSO_4.2H_2O$, 2.0 g $MgCl_2.6H_2O$, 1.0 g Nitriloacetic acid (NTA), 1.0 ml Micronutrient solution (10x) and 5.0 ml Ferric citrate solution (0.01M).

2.4 Micronutrient solution (per litre) (10X)

5.0 ml H_2SO_4 (conc), 2.28 g $MnSO_4.2H_2O$, 5.0 g $ZnSO_4.7H_2O$, 5.0 g Boric acid, 250 mg $CuSO_4.5H_2O$, 250 mg Sodium molybdate dehydrate and 450 mg Cobalt chloride hexahydrate. Final medium preparation was constituted by addition of 0.2% each of yeast extract and tryptone and 100µM $CuSO_4.5H_2O$ into M162 (1X) and pH was adjusted to 7.0 with 1 N NaOH. M162 agar was prepared by adding 2.0% agar in it.

2.5 Substrate

p-phenylenediamine and guaiacol, were purchased from Hi-media.

2.6 Chemicals

All the chemicals and reagents used in the study were of high purity obtained from Hi media, Merck, Qualigens and Sigma.

2.7 Laccase assay

Laccase activity was determined using 2 mM guaiacol as substrate, at 60°C in 0.1 M acetate buffer (pH 5.0). The change in absorbance due to oxidation of substrate in the reaction mixture was monitored for 5 min of incubation. Enzyme units were expressed in nkat (nmoles of substrate converted/second/ml of enzyme).

2.8 Determination of laccase activity

$$\text{Laccase activity (nkat)} = \frac{O.D * 10^9}{\varepsilon \text{ X } 60 \text{ X } T}$$

$\varepsilon = 12000\ M^{-1}cm^{-1}$

OD= Optical Density

T= Incubation time of reaction in min

2.9 Statistical Optimization of laccase production

2.9.1 Plackett–Burman design (PDB) was employed for screening the significant factors affecting laccase production by isolate NS-1. The factors were chosen from the preliminary studies done on isolate NS1.

About 19 factors were taken for analysis in submerged fermentation using Plackett-Burman. Using statistical methods set of 20 experiments was made using the Plackett-Burman design.

From the pareto chart analysis, the factors showing highly positive effects on laccase production were shown to highly influential and further optimized by Response Surface Methodology (RSM).

2.9.2 Response Surface Methodology

A Central Composite Design (CCD) of RSM was performed to interpret the response of laccase production in the optimum region. The range of variables used are given in the table 2. A five level, four factor CCD requiring 30 experiments was employed in the study.

2.9.2.1 Validation of the model

To check the validity of the chosen quadratic model, the best fit experiments were selected from the numerical optimization using the predicted optimum values of the parameters. The laccase activity was measured and compared with the predicted values.

Table 1: Placket-Burman design showing the lowest and the highest values of the coded variables.

Factor	Name	Units	Type	Low	High
A	Temperature	degrees	Numeric	25.00	30.00
B	pH	pH	Numeric	7.00	9.00
C	Time	Days	Numeric	4.00	7.00
D	Inoculum size	no.of beads	Numeric	1.00	4.00
E	CuSO4	micromoles	Numeric	0.00	100.00
F	Yeast extract	grams	Numeric	0.20	0.80
G	Tryptone	grams	Numeric	0.20	0.80
H	MnSO4	micromoles	Numeric	0.00	100.00
J	FeSO4	micromoles	Numeric	0.00	100.00
K	Catechol	micromoles	Numeric	0.00	100.00
L	Vanillin	micromoles	Numeric	0.00	100.00
M	Pyrogallol	micromoles	Numeric	0.00	100.00
N	Wheat Bran	grams	Numeric	0.50	1.00
O	Isopropanol	percentage	Numeric	0.00	2.50
P	Methanol	percentage	Numeric	0.00	2.50
Q	Glucose	percentage	Numeric	0.00	0.50
R	Fructose	percentage	Numeric	0.00	0.50
S	Ferulic acid	millimoles	Numeric	0.00	100.00
T	Ammonium nitrate	millimoles	Numeric	0.00	0.50

Table 2: Coded values of experimental variables.

Variable	Units	Type	Std. Dev.	Low	High
$MnSO_4$	µmole	Factor	0	150	400
wheat Bran	Gram	Factor	0	1.5	4
pH	pH	Factor	0	7	9
$CuS0_4$	µmole	Factor	0	150	400

3 RESULTS AND DISCUSSION

3.1 Fungal Strain

The fungal strain in this work, *isolate NS-1* was isolated in our lab. On the basis of macroscopic and microscopic features the fungal isolate NS-1 was found to be closely related to Alternaria and Curvularia. The organism was grown and maintained at 30°C and sub- cultured on PDA (Potato Dextrose Agar) media**.**

3.2 Selection of parameters

To find out the significant nutritional and environmental conditions for the production of laccase from fungal *isolate NS-1*, nineteen parameters were selected on the basis of the previous studies in our lab a total of 20 experiments were done. Parameters, experimental runs and responses of Placket-Burman design used for the selection of the influential parameters have been shown in Table 3.

Data obtained from these experimental trial runs was statistically analyzed to evaluate and rank the variables by their degree of impact on the fermentation process for enzyme overproduction. After modifying the order of the design, ignoring two factor (2-F) interactions and excluding the dummies, the effect list was analyzed (in the form of Pareto chart) to check the positive or negative effect of various factors on the production of the enzyme (Fig 1).

The positive factors included are MnSO4, wheat bran, pH, CuSO4, inoculums size, vanillin, temperature, tryptone, ferulic acid. pH was the most influential factor and wheat bran accounts for more than 50% positive influence at interactive level in case of fungus *Coriolopsis caperata* RCK2011[24].

Laccase production was reported maximum when fermentation medium was supplemented with $CuSO_4$ in *P. sajor-cajo* and *P. ostreatus*[1].

3.3 Final equation in terms of coded factors

Laccase Activity =
+206.35+38.04*A+90.93*B76.40*C+61.26*D+70.79*E
+22.43*G+186.88*H-56.85*J-28.65*K+41.15*L-
18.74*M+109.13*N-24.10*O-111.29*P-26.01*Q-
53.57*R+21.65*S-152.21*T.

Table 3: Plackett –Burman design for production media optimization and measured response.

Std	Run	Temp (^{0}C)	pH	Time (days)	Inoculum size (No. of beads)	$CuSO_4$ (µM)	Yeast extract (gm)	Tryptone (gm)	$MnSO_4$ (µM)	FeSO4 (µM)	Catechol (µM)	Vanillin (µM)	Pyrogallol (µM)	Wheat bran (gm)	Isopropanol (%)	Methanol (%)	Glucose (%)	Fructose (%)	NH_4NO_3 (%)	Ferrulic acid (µM)	Activity nanocatal/ml
20	1	25.00	7.00	4.00	1.00	0.00	0.20	0.20	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	111.110
6	2	25.00	7.00	7.00	4.00	0.00	0.80	0.80	0.00	0.00	100.00	100.00	100.00	1.00	0.00	2.50	0.00	0.50	0.00	0.00	3.330
17	3	25.00	9.00	7.00	4.00	100.00	0.20	0.80	0.00	100.00	0.00	0.00	0.00	0.50	2.50	2.50	0.00	0.50	100.00	0.00	0.833
8	4	25.00	7.00	4.00	1.00	100.00	0.80	0.20	100.00	100.00	0.00	0.00	100.00	1.00	2.50	2.50	0.00	0.50	0.00	0.50	12.780
19	5	30.00	7.00	4.00	4.00	100.00	0.80	0.80	0.00	100.00	0.00	100.00	0.00	0.50	0.00	0.00	0.50	0.50	0.00	0.50	2.780
18	6	25.00	7.00	7.00	4.00	100.00	0.80	0.20	100.00	0.00	100.00	0.00	0.00	0.50	0.00	2.50	0.50	0.00	100.00	0.50	4.720
7	7	25.00	7.00	4.00	4.00	100.00	0.20	0.80	100.00	0.00	0.00	100.00	100.00	1.00	2.50	0.00	0.50	0.00	100.00	0.00	1000.00
14	8	30.00	9.00	4.00	4.00	0.00	0.80	0.20	0.00	0.00	0.00	100.00	100.00	0.50	2.50	2.50	0.00	0.00	100.00	0.50	6.110
9	9	30.00	7.00	4.00	1.00	0.00	0.80	0.80	0.00	100.00	100.00	0.00	0.00	1.00	2.50	2.50	0.50	0.00	100.00	0.00	1.390
3	10	30.00	7.00	7.00	4.00	0.00	0.20	0.80	100.00	100.00	100.00	0.00	100.00	0.50	2.50	0.00	0.00	0.00	0.00	0.50	14.450
4	11	30.00	9.00	4.00	4.00	100.00	0.20	0.20	100.00	100.00	100.00	100.00	0.00	1.00	0.00	2.50	0.00	0.00	0.00	0.00	913.890
2	12	25.00	9.00	7.00	1.00	0.00	0.80	0.80	100.00	100.00	0.00	100.00	0.00	1.00	0.00	0.00	0.00	0.00	100.00	0.50	486.110
10	13	25.00	9.00	4.00	1.00	0.00	0.20	0.80	100.00	0.00	100.00	100.00	0.00	0.50	2.50	2.50	0.50	0.50	0.00	0.50	2.220
11	14	30.00	7.00	7.00	1.00	0.00	0.20	0.20	100.00	100.00	0.00	100.00	100.00	0.50	0.00	2.50	0.50	0.50	100.00	0.00	0.833
12	15	25.00	9.00	4.00	4.00	0.00	0.20	0.20	0.00	100.00	100.00	0.00	100.00	1.00	0.00	0.00	0.50	0.50	100.00	0.50	5.000
1	16	30.00	9.00	4.00	1.00	100.00	0.80	0.80	100.00	0.00	100.00	0.00	100.00	0.50	0.00	0.00	0.00	0.50	100.00	0.00	772.220
13	17	30.00	7.00	7.00	1.00	100.00	0.20	0.20	0.00	0.00	100.00	100.00	0.00	1.00	2.50	0.00	0.00	0.50	100.00	0.50	02.780
5	18	25.00	9.00	7.00	1.00	100.00	0.80	0.20	0.00	100.00	100.00	100.00	100.00	0.50	2.50	0.00	0.50	0.00	0.00	0.00	56.940
16	19	30.00	9.00	7.00	4.00	0.00	0.80	0.20	100.00	0.00	0.00	0.00	0.00	1.00	2.50	0.00	0.50	0.50	0.00	0.00	725.000
15	20	30.00	9.00	7.00	1.00	100.00	0.20	0.80	0.00	0.00	0.00	0.00	100.00	1.00	0.00	2.50	0.50	0.00	0.00	0.50	4.440

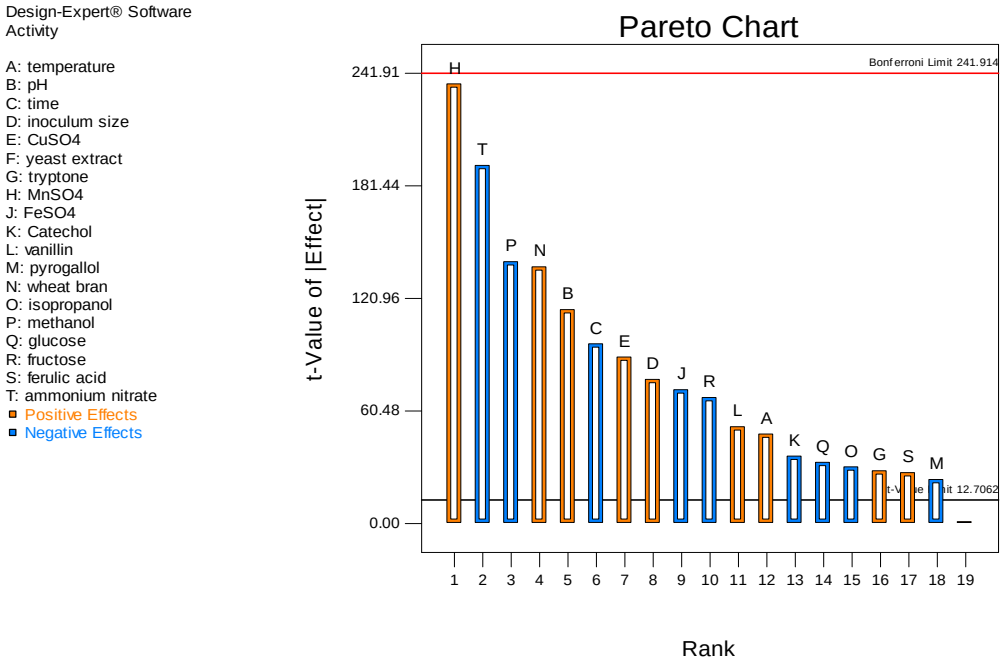


Figure 1. Effect of various parameters on laccase production

Where

* A temperature * B- pH * C-time * D-inoculum size
* E-CuSO4 * G- tryptone * H-MnSO4 * J-FeSO4
* K-catechol * L-vanillin * M-pyrogallol * N-wheat bran
* O-isopropanol * P-methanol * Q-glucose * R-fructose
* S-ferulic acid * T-ammonium nitrate

The diagnostics of Placket-Burman was done by analyzing and comparing the enzyme activity of the results of predicted runs with the results of actual runs. More the values lying on the intercept reveals more proximity of actual experimentation values to the predicted experimentation values. As shown in fig 2, the maximum activity of the actual experimental runs was around 1000 U/ml which is shown by the red blot and the minimum activity was around 4.80 U/ml. Most of the activity values were observed to be lying on the intercept which depicts the close proximity of the actual experimentation with the predicted one.

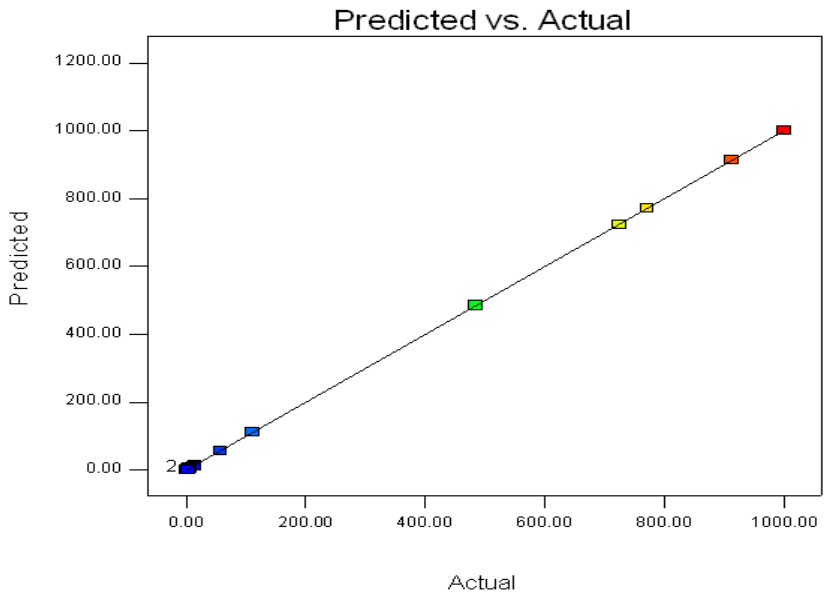


Fig 2. Comparison of actual runs with the predicted runs.

3.4 Response Surface Methodology

Central composite design of response surface was used for the optimization of the variable parameters which were most significantly affecting the production of laccase enzyme. Nine factors were effecting the production of the enzyme positively in Placket-Burman design, and out of these four parameters $MnSO_4$, wheat bran, pH and $CuSO_4$ were selected for further optimization with RSM as these factors were effecting the production most. The factors which were having negative impact on the enzyme production were excluded (Table 4). A total of 30 experimental runs were conducted.

Table 4. Description of variables as used in RSM

Name	Units	Type	Std. Dev.	Low	High
MnSO4	µmole	Factor	0	150	400
wheat Bran	Gram	Factor	0	1.5	4
pH	pH	Factor	0	7	9
CuS04	µmole	Factor	0	150	400

The response of this design is obtained as laccase activity through experimentation as designed by RSM and the data is fed as response.

Table 5 Laccase Activity as measured response.

Std	Run	Block	Type	Factor 1 $MnSO_4$ (µmoles)	Factor 2 wheat bran (grams)	Factor 3 pH	Factor 4 $CuSO_4$ (µmoles)	Activity (nkatal/ml)
18	1	Block 1	Center	400	2.75	8	275	1547.2
7	2	Block 1	Factorial	150	4	9	150	664.87
14	3	Block 1	Factorial	400	1.50	9	400	916.128
2	4	Block 1	Factorial	400	1.50	7	150	967.57
12	5	Block 1	Factorial	400	4	7	400	1095.02
13	6	Block 1	Factorial	150	1.5	9	400	890.03
4	7	Block 1	Factorial	400	4	7	150	1111.16
20	8	Block 1	Center	275	2.75	8	275	1537.86
17	9	Block 1	Center	275	2.75	8	275	1581.26
19	10	Block 1	Center	275	2.75	8	275	1571.01
15	11	Block 1	Factorial	150	4	9	400	694.08
8	12	Block 1	Factorial	400	4	9	150	782.552
10	13	Block 1	Factorial	400	1.50	7	400	863.34
16	14	Block 1	Factorial	400	4	9	400	1143.62
9	15	Block 1	Factorial	150	1.5	7	400	865.7
1	16	Block 1	Factorial	150	1.5	7	150	1453.23
3	17	Block 1	Factorial	150	4	7	150	1118.36
6	18	Block 1	Factorial	400	1.5	9	150	1004
5	19	Block 1	Factorial	150	1.5	9	150	1101
11	20	Block 1	Factorial	150	4	7	400	998.35
22	21	Block 2	Axial	525	2.75	8	275	1029.74
21	22	Block 2	Axial	25	2.75	8	275	1003.45
28	23	Block 2	Axial	275	2.75	8	525	1013.23
24	24	Block 2	Axial	275	5.25	8	275	964.91
29	25	Block 2	Center	275	2.75	8	275	1535.25
26	26	Block 2	Axial	275	2.75	10	275	1238.45
25	27	Block 2	Axial	275	2.75	6	275	1417.92
23	28	Block 2	Axial	275	0.25	8	275	958.09
27	29	Block 2	Axial	275	2.75	8	25	1189.56
30	30	Block 2	Axial	275	2.75	8	275	1550.23

The statistical analysis of the quadratic regression model demonstrated that the model was highly significant. It was evident from the Fisher's F-test F= (120.64) with a very low probability value 0.0001 that the model is significant one. (Table 6). The Model F-value of 35.35 implied the model was significant. There was only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicated model terms were significant.

Table 6. Analysis of Variance (ANOVA)

Source	Sum of squares	Df	Mean square	F value	p-value prob >F
Block	59871.53	1	59871.53	35.35	<0.0001 significant
Model	2.093E+006	14	1.495E+005	0.22	0.6443
A-MnSO4	941.08	1	941.80	1.90	0.1895
B-wheat bran	8042.46	1	8042.46	26.35	0.0002
C-pH	1.114E+005	1	1.114E+005	11.69	0.0042
D-CuSO4	49427.90	1	49427.90	21.84	0.0004
AB	92356.30	1	92356.30	11.83	0.0040
AC	50048.85	1	50048.85	16.05	0.0013
AD	67877.64	1	67877.64	9.44	0.0083
BC	39908.05	1	39908.05	22.90	0.0003
BD	96833.38	1	96833.38	22.49	0.0033
CD	52824.05	1	52824.05	12.49	<0.0001
A^2	6.567E+005	1	6.567E+005	155.28	<0.0001
B^2	7.788E+005	1	7.788E+005	184.15	<0.0001
C^2	1.619E+005	1	1.619E+005	38.29	<0.0001
D^2	4.891E+005	1	4.891E+005	115.64	<0.0001
Residual	59212.02	14	4229.43		
Lack of fit	57874.30	10	5787.43	*17.31*	0.0072 Non-significant
Std. Dev. 65.03 R-Squared 0.9725 Mean 1126.91 Adj R-Squared 0.9450 C.V. % 5.77 Pred R-Squared 0.8573					

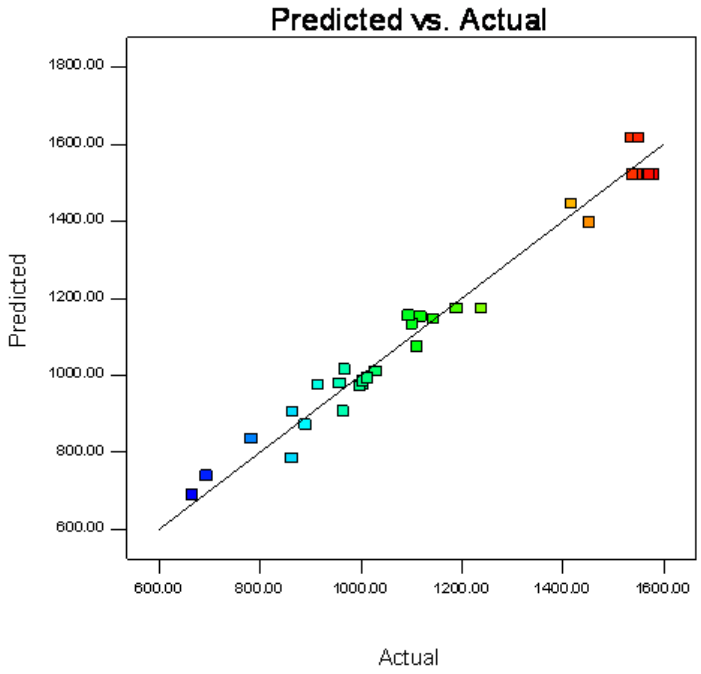


Fig.3 comparison of actual runs with predicted.

3.5 Final Equation in Terms of Coded Factors

laccase activity = +1569.60+6.26 * A-18.31* B-68.14 * C-45.38* D+75.98 * A * B+55.93 * A * C+65.13 * A * D-49.94 * B * C+77.80 * B * D+57.46* C * D-154.74 *A^2-168.51* B^2-76.84* C^2-133.53 * D^2

Where A-$MnSO_4$, B-wheat bran, C-pH and D-$CuSO_4$

The analysis of variance (ANOVA) indicated that the main effects of the independent variables, effect of interaction between varying wheat bran levels and different $MnSO_4$ conc. was significant.

3.6 Interaction effect of two parameters: The 3D response surface are the graphical representation of the regression equation.

3.6.1 Interactive effect of wheat bran and $MnSO_4$

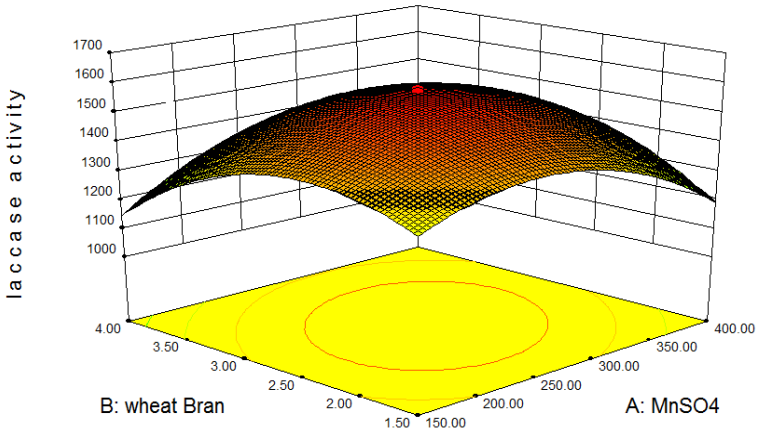
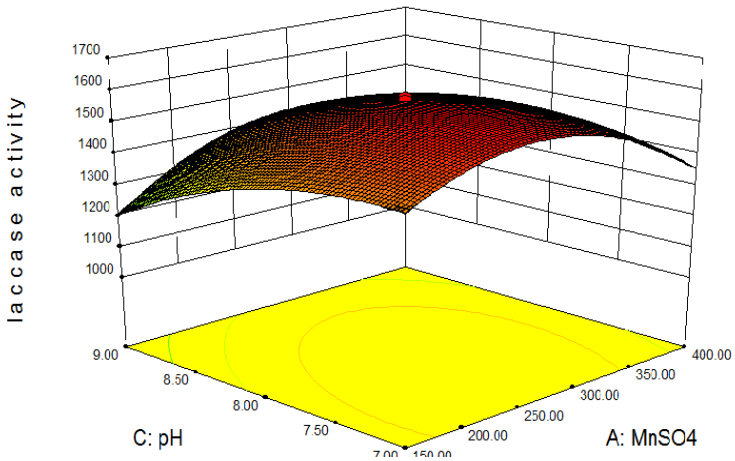
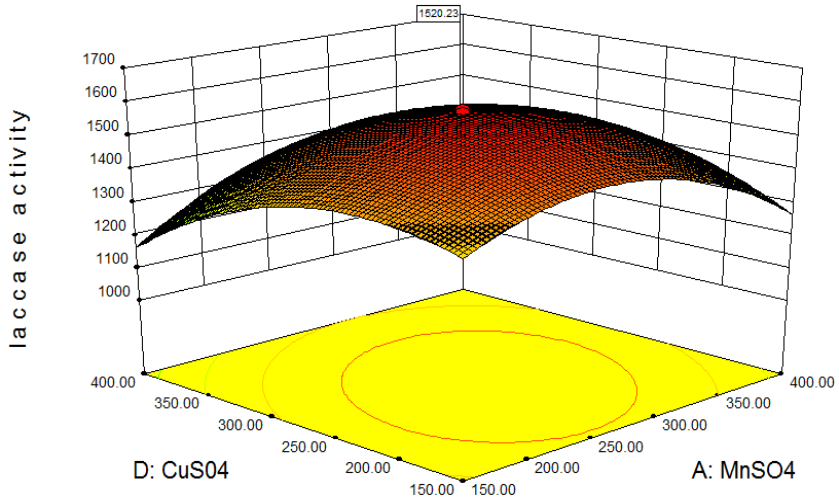


Figure.32 depicts the interaction between varying levels wheat bran and $MnSO_4$ while keeping pH (8) and $CuSO_4$ (275 µmoles)constant. The interaction of above two factors was found to be positive. On increasing the conc. of $MnSO_4$ and wheat bran, laccase activity is enhanced. The graph shows that at 150 µmole $MnSO_4$ and 1.5 g/100ml of wheat bran, maximum enzyme activity was found to be 1501.4 U/ml.

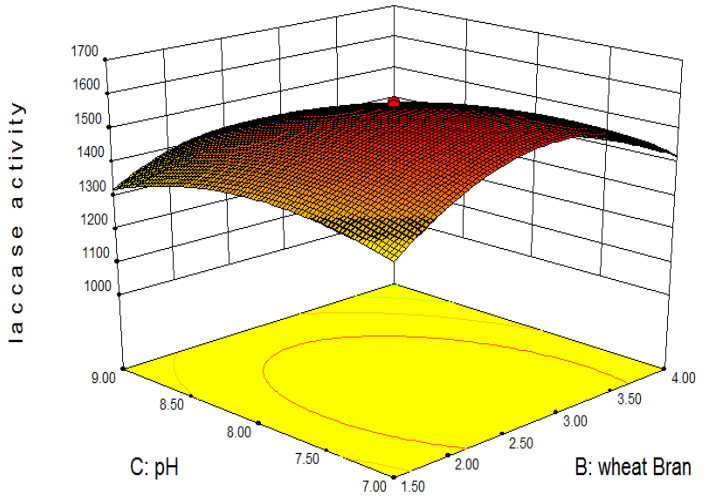
3.6.2 Interactive effect of pH and $MnSO_4$



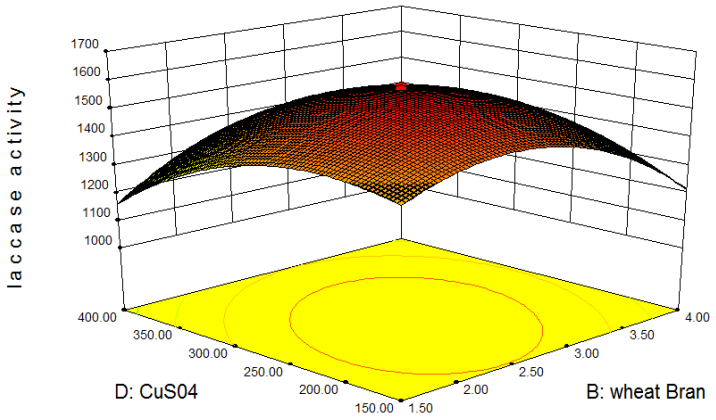
3.6.3 Interactive effect of $CuSO_4$ and $MnSO_4$



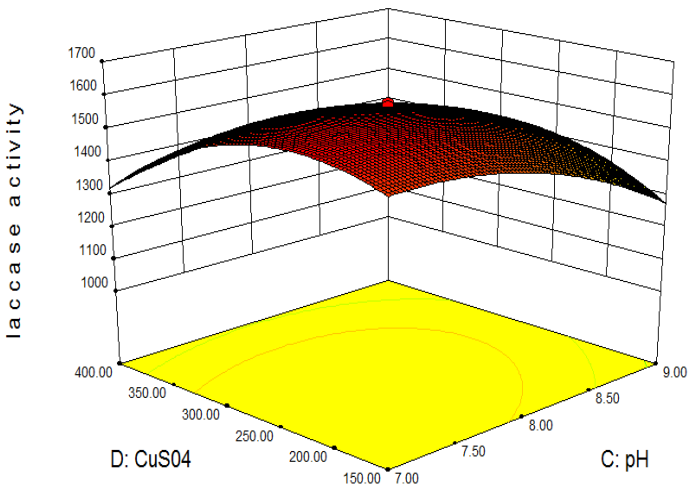
3.6.4 Interactive effect of pH and wheat bran



3.6.5 Interactive effect of $CuSO_4$ and wheat bran



3.6.6 Interactive effect of $CuSO_4$ and pH



The response surface methodology is very efficient for determining the optimal condition of laccase production[28] or the factor interaction effect on enzymatic assay[20]. This study optimize the conditions for maximum laccase production by fungal *isolate NS-1*. The results conclude that laccase activity is increased in the presence of $CuSO_4$ (206.31 µmole), $MnSO_4$ (254.12µmole) at pH 7.65 by supplementing the medium with 2.78 w/v wheat bran.

Through RSM it had been concluded that laccase activity was induced by $CuSO_4$ in S.maltophila.[15] and $MnSO_4$. $CuSO_4$ is widely used as a promising enhancer for laccase production. In some white rot fungi *P. chrysosporium* ME44631, *T. pubescens* 6 and *P.ostreatus* addition of copper increases laccase production. Among the various concentrations tested 1mM copper sulphate produced maximum laccase activity of 17074 U/L. Growth as well as laccase production was suppressed due to addition of concentrations above 1mM. It has been concluded that addition of above1.5 mM concentration of copper sulphate to the cultures of *Lentinula edodes has* inhibited the total growth[3]. Laccase production in *P. sanguineus* was enhanced by copper sulphate supplementation at levels of 0.1 mg/l. Further increase in levels of copper sulphate did not improve the activity in *P.sanguineus*[26].

The activity of laccase was strongly affected by pH (19). In general laccase production was achieved with either limited carbon and nitrogen ratio or higher carbon and nitrogen ratio in the cultivation medium. Of all tested lignocellulosic material, wheat bran showed maximum activity of 9300U/l in *T.hirsuta* (Bakkiyaraj et al.,2013). It was reported that wheat bran was the good substrate for the growth of *Coriolopsis caperata RCK2011* and *Coriolopsis unicolor* and its soluble cellulose and hemicelluloses fractions served as carbon source which led to a sufficient carbon and nitrogen ratio for efficient laccase production[22]. pH contributed maximally (47%) on the overall enzyme production and wheat bran the minimum (7.76 %) in Coriolopsis caperata RCK2011. The optimized conditions in Coriolopsis caperata RCK2011 enhanced laccase production of 59.71% i.e. from 681.66 to 1692.16 $Ugds^{-1}$. (22).

3.7 Numerical optimization and Validation

After numerical optimization, the RSM model predicted that a medium containing wheat bran 2.78 w/v, 206.31 µmole $CuSO_4$ concentration, pH 7.65, and 254.12 µmole $MnSO_4$ can give maximum laccase production of 1582.44 U/ml. On experimentation, 1581.26 U/ml of laccase was obtained. The experimental values were found to be very close (99.99%) to the predicted values and hence the model was successfully validated.

Through statistical optimization, activity was increased by ten-fold.

CONCLUSION

Attempts have been made for optimizing enzyme production parameters to have better suitability in industrial processes. But the search is still on to find organisms giving high yields of enzyme. A significant increase in enzyme yield was achieved by optimizing various physico-chemical parameters of fermentation. Laccase from isolate NS-1 can be commercially explored for various applications such as pulp bio-bleaching, clarification of fruit juices, enzyme based hydrogen peroxide free hair dye etc.

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