Optimization of selective production media for enhanced production of xylanases in submerged fermentation by *Thielaviopsis basicola* MTCC 1467 using L_{16} orthogonal array

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Abstract Enzymes have been the centre of attention for researchers/industrialists worldwide due to their wide range of physiological, analytical, food/feed and industrial based applications. Among the enzymes explored for industrial applications, xylanases play an instrumental role in food/feed, textile/detergent, paper and biorefinery based application sectors. This study deals with the statistical optimization of xylanase production by *Thielaviopsis basicola* MTCC 1467 under submerged fermentation conditions using rice straw, as sole carbon source. Different fermentation parameters such as carbon source, nitrogen source, inorganic salts like KH_2PO_4 , MgSO_4 and pH of the medium were optimized at the individual and interactive level by Taguchi orthogonal array methodology (L_{16}). All selected fermentation parameters influenced the enzyme production. Rice straw, the major carbon source mainly influenced the production of xylanase (~34 %). After

media optimization, the yield of enzyme improved from 38 to ~60 IU/ml (161.5 %) indicating the commercial production of xylanase by *T. basicola* MTCC 1467. This study shows the potential of *T. basicola* MTCC 1467 for the efficient xylanase production under the optimized set of conditions.

Keywords Xylanase · Rice straw · *Thielaviopsis basicola* MTCC 1467 · Optimization · Submerged fermentation · L_{16} orthogonal array

Nomenclature

DNS	Di-nitro salicylic acid
ANOVA	Analysis of variance
OA	Orthogonal array
RS	Rice straw

Introduction

Rising interests in sustainable development and environmentally benign practices, microbial enzyme mediated transformation processes have shown advantages as compared to the conventional chemical conversion processes (Chandel et al. 2011a). In the last two decades, xylanases (EC.3.2.1.8) have shown a great deal of attention due to their vast biotechnological applications in food, feed and paper industries (Chandel et al. 2007). Furthermore, xylanases have shown a pivotal role for the economic production of value-added products such as D-xylitol, cellulosic ethanol, organic acids etc. (Beg et al. 2001). Hemicellulose is one of the main components of lignocellulose, the most abundant and readily available carbohydrate source on earth after cellulose (Chandel et al. 2011b). Xylan is the major backbone in the hemicellulosic fraction of the plant cell wall,

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linking compounds like arabinose, glucose, mannose and other sugars through an acetyl chain (Chandel et al. 2011b). Xylanases are the enzymes produced by prokaryotes, eukaryotes and mechanistically act precisely on xylan to release xylose as monomeric constituent (Beg et al. 2001).

A large number of bacteria and fungi are known to produce xylanases under varying cultivation systems using different carbon and nitrogen sources (Kulkarni et al. 1999; Subramaniyan and Prema 2002). To economize the any metabolite production from microbial systems, selection of appropriate carbon and nitrogen source is pivotal (Sreenivas Rao et al. 2008; Suresh et al. 2011; Húngaro et al. 2011). Fungi are the most potent xylanase producers, as they secrete high level of enzymes than those of yeast and bacteria (Qinnghe et al. 2004). Fungus *Thielaviopsis basicola* which cause black rot in roots of several plants (Baruah et al. 1980) is the selective producer of xylanases and cellulases from an industrial point of view due to the extracellular release of xylanases in fermentation broth with an appreciable yield as compared to yeast and bacteria (Haltrich et al. 1996; Steiner et al. 1987). Production of enzymes like other microbial derived metabolites is generally influenced by several physical and physico-chemical parameters such as incubation temperature, pH of medium, inorganic metals, agitation, aeration and others (Sreenivas Rao et al. 2008). In order to obtain the maximum production of enzymes from microorganism during fermentation reaction, it is essential to optimize these important parameters (Prakasham et al. 2006 and 2007a, b; Subba Rao et al. 2008a, b; Sreenivas Rao et al. 2008). Optimization of fermentation medium and other influencing parameters is also important to produce cost competitive xylanase from microbial systems. There is no defined medium for the optimum production of any metabolite due to the genetic diversity of microbial sources and their varying specific growth conditions (Subba Rao et al. 2008a, b). Therefore, it is essential to optimize all fermentation parameters, but optimizing all factors by one parameter at-a-time is a conventional methodology since it involves numerous experimental trails (Sreenivas Rao et al. 2004 and 2008). In contrast, statistically designed experiments significantly reduce the number of experiments by developing a specific design of experiments (Balakrishnan and Pandey 1996; Sreenivas Rao et al. 2008).

The main aim of this study was to provide the best production conditions for extra cellular xylanase by *T. basicola* MTCC 1467 under submerged fermentation conditions utilizing rice straw as main carbon source.

Materials and methods

Materials D-glucose, Bovine serum albumin (BSA), D-xylose, Di-nitro salicylic acid (DNS), Xylan (Birchwood),

Citric acid, Potato dextrose broth, Agar-Agar type I, Peptone, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, MnSO_4 , $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, KH_2PO_4 , MgSO_4 , Sodium potassium tartrate and Sodium hydroxide were obtained from High media, Mumbai, India. Rice straw was obtained from the local agricultural fields and was further processed in our laboratory. It was used as a sole carbon source for the xylanase production.

Microorganism and maintenance The strain *Thielaviopsis basicola* MTCC 1467 was obtained from IMTECH (Institute of Microbial Technology), Chandigarh, India. It was maintained on potato dextrose agar (PDA) slants at 4 °C and was sub cultured every month.

Submerged fermentation Experiments were performed for xylanase production optimization by *T. basicola* MTCC 1467 considering five factors at selected levels (Table 1). A total of 16 experimental trails were performed (Table 2). All experiments were conducted in 250 ml Erlenmeyer flasks containing 100 ml of medium with the media components such as rice straw (carbon source), peptone (nitrogen source), KH_2PO_4 , MgSO_4 (Table 1) and MnSO_4 0.5 mg, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.15 mg and $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ 0.2 mg. Prior to use, the rice straw was pretreated with NaOH (10 %w/v), autoclaved for 20 min at 121 °C. After pretreatment, the substrate was washed with running tap water to remove the traces of NaOH and dissolved lignin. Thereafter, it was dried in hot air oven at 50 °C for overnight and subsequently sieved by metal mesh (20 mesh size) to obtain uniform particle size of 4 mm. This processed rice straw was used as a carbon source in the fermentation medium. The sterilized flasks were inoculated with 5 % of inoculum and subsequently incubated in an orbital shaker (JSSI-300 C, USA) at 150 rpm at room temperature. The enzyme activities presented in this study are the average values of three individual experiments.

Xylanase optimization by L_{16} -orthogonal array method The orthogonal matrix L_{16} (4^4) (Fukushima and Sukahara 1990) method was used to study the relationship between the variables of medium components and to optimize their

Table 1 Selected fermentation factors and their assigned levels for xylanase production by *Thielaviopsis basicola* MTCC 1467

Components (Factors)	L1 g/100 ml	L2 g/100 ml	L3 g/100 ml	L4 g/100 ml
RiceStraw	1	2	3	4
KH_2PO_4	0.1	0.2	0.3	0.4
Peptone	0.5	1	1.5	2
MgSO_4	0.05	0.1	0.15	0.2
pH	4	5	7	8

Table 2 Experimental set up (L_{16} orthogonal array) and S/N ratios for xylanases by *Thielaviopsis basicola* MTCC 1467

Variables trails	Rice straw	KH ₂ PO ₄	Peptone	MgSO ₄	pH	Observed IU/ml	Predicted IU/ml	S/N ratio
1	1	1	1	1	1	4.4	4.398	12.86905
2	1	2	2	2	2	12	11.998	21.58362
3	1	3	3	3	3	47	46.998	33.44196
4	1	4	4	4	4	55.3	55.298	34.8545
5	2	1	2	3	4	58.08	58.078	35.28053
6	2	2	1	4	3	52.2	52.198	34.35341
7	2	3	4	1	2	50.4	50.398	34.04861
8	2	4	3	2	1	55.92	55.918	34.95134
9	3	1	3	4	2	55.8	55.798	34.93268
10	3	2	4	3	1	43.08	43.078	32.68551
11	3	3	1	2	4	52.68	52.678	34.43292
12	3	4	2	1	3	41.76	41.758	32.41521
13	4	1	4	2	3	59.64	59.638	35.51075
14	4	2	3	1	4	23.04	23.038	27.24965
15	4	3	2	4	1	30.12	30.118	29.5771
16	4	4	1	3	2	34.56	34.558	30.77147

concentration for higher yields of enzyme production. This method uses a special set of arrays called orthogonal arrays. These standard arrays stipulate the way of conducting the minimal number of experiments, which could give the full information of all the factors that influence enzyme production. L_{16} -orthogonal array method is based on modified methods described by Taguchi (Taguchi and Wu 1980 and 1986), which overcomes many problems associated with conventional methodology. The modified version consists of four distinct phases: (a) designing the experiments, (b) running and analyzing, (c) analysis and (d) validation of assumptions (Prakasham et al. 2005). Five typical fermentation factors i.e. carbon source (rice straw), nitrogen source (peptone), KH₂PO₄, MgSO₄ and pH which have a significant influence on enzymes production were considered by performing one at-a-time experiments (data not shown) and the chosen parameters were varied at four different levels in Taguchi design of experiments (Table 1). Conditions such as temperature (30 °C), agitation (150 rpm), and media volume ratio (volume of media/volume of flask) were not changed and fixed at standard prescribed conditions for the micro-organism. The selected variables were arranged into an orthogonal array (L_{16} orthogonal array for the representative experiments) (Table 2). With respect to medium optimization, each column would correspond to individual medium components, and each row would represent trails with different combinations of factors. Each of the above selected components were taken at four defined concentrations, covering the range over which its effect can be determined. The objective of medium optimization was to maximize the enzyme production. For this, following

signal-to-noise ratio function designed by Taguchi design was used.

$$S/N = -10 \log \left[\frac{i}{n} \sum_{n=1}^n \left(\frac{1}{Y_i^2} \right) \right]$$

Where S/N is the signal-to-noise ratio, n is the number of trials with given concentration, and Y_i is the yield of correspondent trials. For each component the optimal conditions are those that give the largest S/N ratio.

AEDPP, Analysis of experimental data and prediction of performance Qualitek-4 software (Nutek Inc., MI) was used for automatic design of experiments in the present study. The data obtained was processed using Qualitek-4 software and MINITAB 15 (Trial version). The processed data was used to identify the influence of individual factors on the enzymes production and to determine the optimum fermentation conditions of *T. basicola* MTCC 1467 for maximum xylanase production.

Validation [V] To validate the determined optimized methodology, fermentation experiments were carried out in triplicates and the samples collected were assayed for xylanase production profile.

Extraction of enzyme The enzyme samples were extracted after every 24 h for about 9 days and centrifuged at 8,000 rpm at 4 °C for 30 min. Supernatant was collected and filtered through Whatman No. 1 filter paper (High media, Mumbai) to remove the suspended spores. The filtrate was used to determine the activity of xylanase.

Enzyme activity Xylanase activity was determined by incubating 1 ml of reaction mixture which contains 0.5 ml of 2 % xylan, 0.4 ml of citrate buffer of pH 5.5 and 0.1 ml of culture filtrate at 50 °C for 10 min. The enzyme blank and substrate blank were simultaneously incubated. The reducing sugar (xylose) released was estimated by DNS method (Miller 1959). One unit of xylanase activity was defined as the number of micromoles of reducing sugar (xylose) released per ml of enzyme solution in 1 min under the assayed conditions.

Estimation of protein concentration Protein concentration was estimated by Bradford's method (Bradford 1976). 1 ml of Bradford's reagent was added to suitably diluted protein samples and to the protein blank. The absorbance was read within 5 min at 595 nm. The amount of protein in the samples was determined from the standard bovine serum albumin plot.

Results and discussion

Carbon source In order to study the influence of carbon source, xylanase fermentation studies were performed using various substrates (% w/v) such as xylose, carboxy methyl cellulose (CMC), xylan, rice straw, vegetable waste, rice brawn, wheat straw, cotton leaves and green chilly leaves (Fig. 1). The microorganism, *T. basicola* MTCC 1467 was able to utilize xylan and rice straw effectively as potential carbon sources for the selective production of xylanase. The carbon source is one of the most significant factors during the growth and metabolic process of any microorganism. The selection of an appropriate carbon source always plays a crucial role on the process economics of xylanase production (Beg et al. 2001; Pandya and Gupte 2012). With the aim of reducing the substrate cost, rice straw a major hemicellulose containing lignocellulosic biomass (Park et al. 2011)

was selected as a principle carbon source in the present study. Several raw materials have been indicated in the literature as suitable carbon sources for the production of lignocellulolytic enzymes by microorganisms such as oat wheat, oat spelt xylan (Chivero et al. 2001; Georis et al. 2000), wheat bran, arabinoxylan (Bataillon et al. 2000), wheat bran (Fujimoto et al. 1995), sugarcane bagasse (Martinez-Trujillo et al. 2003), rice bran (Dhillon et al. 2000) and wild sugarcane (*Saccharum spontaneum*) leaves (Uma et al. 2008). Use of xylan and CMC as the carbon source for large scale production of enzymes is uneconomical because of their high cost. Selection of appropriate carbon source for the production of any metabolite is pivotal in any cultivation system. Production of maximum extracellular chitin deacetylase (460.4 ± 14.7 unit/g initial dry substrate) was obtained by *Colletotrichum lindemuthianum* ATCC 56676 under solid substrate fermentation (Suresh et al. 2011). Hence, use of cost-effective substrates such as lignocellulosic waste material is highly recommended (Wejse et al. 2003; Sá-Pereira et al. 2002).

Enzyme production Xylanase production was studied at 30 °C for 10 days in production medium (100 ml) containing rice straw 2 g, MgSO_4 0.1 g, KH_2PO_4 0.2 g, peptone 1 g, and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 mg, MnSO_4 0.15 mg and $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ 0.2 mg with the medium pH of 5 (Uma et al. 2008). Xylanase production reached maximum on the 9th day (Baby Rani et al. 2008, 2012) of incubation. Xylanases are usually expressed at the end of the exponential phase and harvesting time was correlated to the medium under consideration (Wejse et al. 2003). The phenomenon of sudden increase and subsequent downfall in enzyme activities during the cultivation period has also been observed in xylanase production by *Aspergillus sydowii* MG-49 (Ghosh et al. 1993) and *Streptomyces* sp. CH-M-035 (Flores et al. 1996).

Fig. 1 Xylanase production by *Thielaviopsis basicola* MTCC 1467 using various carbon sources

Evaluation of fermentation conditions for xylanase production Metabolite production by microorganism depends on the physiological, nutritional, and biochemical nature of the microbe employed, improvement of production and the factors which play major role for this may vary from organism to organism (Prakasham et al. 2005; Subba Rao et al. 2008a, b). Preliminary investigations indicated that fermentation factors such as medium pH (7), carbon source (rice straw), nitrogen source (peptone), and major salts are critical for enzymes production by *T. basicola* 1467. Under the above optimized conditions, the enzyme yield enhanced from 38 to ~60 IU/ml indicating the significance of fermentation factors on fungal metabolism and its associated enzyme yields (Fig. 2). This data suggested that optimization for each factor of growth, nutritional requirement, and production yields are essential requirements for understanding the potential of xylanase production at the industrial scale (Sreenivas Rao et al. 2006; Prakasham et al. 2007a, b; Suvarna Lakshmi et al. 2009). Similar results were noticed for biocatalyst production with other microbial strains (Sreenivas Rao et al. 2006). However, the interactive

influence of these factors on enzyme production was not possible with conventional optimization, which is known to positively regulate the microbial metabolism (Prakasham et al. 2007a, b). Therefore, optimization of fermentation factors for enzyme yields with rice straw as carbon source was investigated using five fermentation critical factors and their selected levels (Table 1).

Statistical optimization of xylanase production The Taguchi L_{16} orthogonal array revealed significant variation in the enzyme production (Table 2). The crux of the orthogonal array method lies in choosing the level combinations of the input design variables for each experiment (Fukushima and Sukahara 1990). Taguchi L_{16} orthogonal array method is a statistical method for design of factorial experiments and analysis of the results with accuracy eventually saving a lot of time by reducing the number of experiments (Taguchi 1986). This design has shown many advantages over conventional methodologies of media optimization and predict the accurate results by exploiting the fundamental principles of statistics, randomization, and replication (Subba Rao et

Fig. 2 Impact of selected variables (Rice straw, KH_2PO_4 , MgSO_4 , peptone and pH) on xylanase production by *Thielaviopsis basicola* MTCC 1467 under submerged fermentation

Fig. 3 Performance distribution on xylanase production by *Thielaviopsis basicola* MTCC 1467 under submerged cultivation conditions

al. 2008a, b). The present data suggested a significant improvement in enzyme yields compared to the conventional media optimization (Fig. 3). Similar variation of enzyme yields with optimization experiments were found for xylanase production by *Aspergillus terreus* (Suvarna Lakshmi et al. 2009), alkaline protease production by *Bacillus circulans* (Subba Rao et al. 2008a, b) and xylitol production by *Candida tropicalis* (Sreenivas Rao et al. 2006). Using L_{16} orthogonal array method the parameters were optimized for their concentrations.

The production capacity and signal-to-noise ratio (larger is better) produced by the L_{16} orthogonal array is given in Table 2. The S/N ratio values help to assess which combination of factors have the greatest effect on the response characteristic of interest. A higher S/N ratio value indicates greater effect of those components in combination. The present study based on main effects of individual components (Table 3). The order of components could be ranked as rice straw > $MgSO_4$ > pH > peptone > KH_2PO_4 , suggesting that carbon source had a major effect and KH_2PO_4 showed least effect on enzyme production by *T. basicola* MTCC 1467.

Table 3 ANOVA table for xylanase production with *Thielaviopsis basicola* MTCC1467

Col	Factor	DOF (f)	Sum of Sqrs (S)	Variance (V)	Pure Sum (S)	Percent (P)%
1	RS	3	1463.9	487.966	1463.898	33.933
2	KH_2PO_4	3	511.2	170.406	511.219	11.848
3	Peptone	3	770.2	256.744	770.234	17.852
4	$MgSO_4$	3	837.8	279.272	837.816	19.418
5	pH	3	731.2	243.761	731.283	16.949
Other	Error	0				
Total		15	4314.4			100 %

The main effect plots are shown in Fig. 2. These plots show how each factor affects the response characteristic. A main effect is present when different levels of a factor affect the characteristic differently. MINITAB software creates the main effects plot by plotting the characteristic average to each factor level. A line connects the points for each factor. When the line is horizontal (parallel to the x-axis), then there

Table 4 Interaction influence of selected factors on xylanase production by *Thielaviopsis basicola* MTCC 1467 using rice straw as sole carbon source

Interacting factor pairs (In order of severity)	Column ^a	SI ^b (%)	Col ^c	Opt ^d
$KH_2PO_4 \times$ Peptone	2×3	84.97	1	[1,4]
$MgSO_4 \times$ pH	4×5	81.39	1	[2,3]
$KH_2PO_4 \times$ pH	2×5	74.65	7	[1,3]
Peptone × $MgSO_4$	3×4	70.63	7	[4,2]
$KH_2PO_4 \times$ $MgSO_4$	2×4	59.99	6	[1,2]
Peptone × pH	3×5	43.7	6	[4,3]
RS × KH_2PO_4	1×2	12.22	3	[4,1]
RS × pH	1×5	11.87	4	[4,3]
RS × $MgSO_4$	1×4	1.88	5	[4,2]
RS × Peptone	1×3	1.55	2	[4,4]

^a Represent the column locations to which the interacting factor assigned

^b Interaction severity index (100 % for 90 ° angle between the lines, 0 % for parallel lines)

^c Shows column that should be reserved if this interaction effects were to be studied (2- L factors only)

^d Indicates the factor levels desirable for the optimum condition (based strictly on the first 2 levels). If an interaction included in the study and found significant (in ANOVA) the indicated levels must replace the factor levels identified for the optimum condition without considerations of any interaction effects

Table 5 Xylanase production optimum conditions and performance by *Thielaviopsis basicola* MTCC 1467 using rice straw as carbon source

Factors	Levels description	Levels	Contribution
RS ^a	4	4	16.109
KH ₂ PO ₄	0.1	1	9.4168
Peptone	2	4	10.930
MgSO ₄	0.1	2	12.147
pH	7	3	11.035
Total Contribution From All Factors			59.640
Current Grand Average of Performance			42.248
Expected Result at Optimum Condition			59.638

^a RS stands for Rice Straw: Carbon source

is no or minimal main effect present. Different levels of the factor affect the enzyme characteristics in various manners. The greater the difference in the vertical position of the plotted points (greater the deviation from the parallel x-axis), greater is the magnitude of the main effect. In the present study, it can be seen that for each of the five variables at four levels, one level increases the activity compared to the other level. This difference is a main effect which verifies that rice straw at level 4, MgSO₄ at level 2, pH at level 3, peptone at level 4, and KH₂PO₄ at level 1 showed main effect on xylanase production (Fig. 2). These levels also represent the optimal concentrations of the individual components in the medium. Response tables can also be used to predict the optimal levels of each component used in the study.

Influence of selected factors interaction on xylanase production To better understand the overall xylanase production process and activities by *T. basicola* MTCC 1467, software-created interaction effects were analyzed individually since any independent factor may interact with single or all of the other factors. The severity index (SI) of each selected factor on enzyme production was estimated which

can help to understand the influence of two individual factors at defined levels (Table 4). From Table 4, it can be observed that KH₂PO₄ and peptone interaction (level 2 and 3; column 1) had the highest impact (84.97 %). It was inquisitive to note that, in both of above interaction one of the factor such as KH₂PO₄ which is having a least impact percent factor of 11.848 % (Table 3) interacted with medium factor, peptone (17.85 %). Among all the selected factors, rice straw and peptone interaction showed the lowest severity index, 1.55 % (level 1 and 3; column 2). It was evident from these interaction results, that the influence of selected factors (individually) on xylanase production is different while in grouping the production yield of enzyme is independent of individual factor influence.

ANOVA (Analysis of variance) Understanding the impact of each individual factor on selective metabolite production is the key for a successful fermentation process. ANOVA was used to analyze the results of the orthogonal array experiments and to determine how much variation each factor has contributed towards xylanase production. The F ratio is a test static used for multiple independent variables (factors). This can be used to determine the quality of the experimental results. The main principle used in the test statistics is signal-to-noise ratio in which the systematic variance is divided by unsystematic variance and the larger ratio is better. In Table 2, the S/N ratios of the experimental results can be observed. The test static can be calculated as follows.

Test statistic = system variance/unsystematic variance

If the variance is the measure of sum of squares (SS), it can be shown as

Test static = $SS_{\text{Model}}/SS_{\text{Residual}}$

And the total variance is, $SS_{\text{Total}} = SS_{\text{Model}} + SS_{\text{Residual}}$

Where, model and residual indicate expected (systematic) variance and random (unexpected) variance respectively.

Table 6 Comparison of xylanase activity of *Thielaviopsis basicola* MTCC 1467 with other fungal microorganisms

Microorganism	Substrate	Type of cultivation	Xylanase activities (IU/mi)	References
<i>T. harzianum</i> T4	Xylan, fruit stalk, extractives	Submerged	6.69, 5.696, 0.133	Medeiros et al. 2000
<i>Thermoascus aurantiacus</i>	Sugarcane bagasse, Corn straw	Solid state	11, 97	da Silva et al. 2005
<i>A. terreus</i>	1 % Xylan	Submerged	42	Geweely et al. 2006
<i>P. oxalicum</i>	Wheat bran	Solid state	16	Li et al. 2007
<i>P. clerotiorum</i>	Theat bran	Submerged	7.5	Knob and Carmona (2008)
<i>Fusarium solani</i> F7	Wheat straw	Submerged	78.32	Gupta et al. 2009
<i>T. harzianum</i>	Wheat straw	Solid state	146	Sanghvi et al. 2011
<i>Trametes versicolor</i>	Tomato pomace	Solid state	50 Ug ⁻¹	Iandolo et al. 2011
<i>A. tubingensis</i> JP-1	Sugarcane bagasse	Solid state	17	Pandya and Gupte 2012
<i>Thielaviopsis basicola</i>	Rice straw	Submerged	59.64	Present work

F ratio is measured as the ratio of mean sum of squares (MS)

$$F \text{ ratio} = MS_{\text{modal}} / MS_{\text{Residual}}$$

Where mean sum of squares (MS)=sum of squares (SS)/degrees of freedom (df)

The F ratio can also be calculated using Pearson or sample correlation coefficient (r)

$$F = r^2 / (1 - r^2)(n - 2)$$

To obtain the optimized levels or composition of each factor, the predictive analysis based on statistical calculations is shown in Table 5. Statistical analysis of the xylanase production data in the above experimental designs revealed that among all the selected factors, Rice straw contributed the maximum impact (16.109 %) and KH_2PO_4 showed the least impact (9.41 %) on the overall enzyme production under the selected fermentation conditions (Table 5).

Final medium for xylanases production by *T. basicola* MTCC 1467 is, rice straw 4 %, MgSO_4 0.1 g, KH_2PO_4 0.1 g, peptone 2 g, and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 mg, MnSO_4 0.15 mg, $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ 0.2 mg for 100 ml of the medium at pH 7. To confirm these results, experiments were carried out using these nutrient concentrations, and it was observed that the xylanase activity obtained was 59.64 IU/ml as compared to 59.638 IU/ml predicted using MINITAB software for the same composition. This showed that the experimental value almost matches with predicted values. The final optimized medium produced 59.64 IU/ml of xylanase activity at 9 days of incubation period as compared to 38 IU/ml before optimization [data not shown] and the activity was improved by ~161 % in the medium optimized by orthogonal matrix method. The obtained xylanase activity by *T. basicola* MTCC 1467 using rice straw is comparable to reported xylanase activities (Table 6). However, the conditions explored for the production of xylanase in these studies are different but the production titers of this enzyme from the present study are quite comparable with the previous reports.

Conclusions

Xylanases play a vital role in the production of various bio-based products of commercial significance. Of the different hydrolytic enzymes, xylanase have attracted attention because of their diverse range applications. In the present study, production of xylanase by *T. basicola* MTCC 1467 using rice straw, an agricultural waste as sole carbon source, was studied and the impact of critical fermentation factors at different concentrations at individual and interactive levels were studied using Taguchi L_{16} orthogonal array. The

experimental design determined the influence of individual factors helped in establishing the relationship between variables and operational conditions as well as the optimal levels for the best performance. Significant variation in enzyme production was noticed among assigned factor levels. Evaluating the relative influence of each factor at individual level, rice straw had the major influence on the production of xylanase. Validation of the findings of the experimental design demonstrated a significant (~161 %) improvement in xylanase production by *T. basicola* MTCC 1467 under optimized set of conditions.

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