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Studies on Production, Optimization and Purification of Uricase from *Gliocladium viride*

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Five different fungal strains belonging to *Gliocladium and Gliomastix* species were initially screened for their uricase producing capability, among which *Gliocladium viride* MTCC 3835 was identified to produce highly active uricase. Statistical designs were applied to optimize uricase production. Using Plackett–Burman design, peptone, yeast extract and $CuSO_4$ were found to have significant effect on enzyme activity. Box-Behnken design was used to find the optimal concentrations of significant variables, which were as follows, Peptone-12.71g/L, Yeast Extract-10.57g/L, $CuSO_4$-0.0762g/L. Maximum activity of 84.92 U/ml was observed experimentally, which is 1.344 times higher than the activity got in basal medium. Crude uricase was further purified using three-phase partitioning (TPP), and the significant factors like inorganic phase saturation, ratio between organic phase and crude enzyme, operating temperature were optimized. Crude enzyme saturated with 50%(w/v) with ammonium sulphate and at crude enzyme to *t*-butanol ratio of 1:1(v/v) at 30°C resulted in 80.109% yield of uricase with 1.44 fold purification.

Keywords*: Gliocladium viride,* Uricase, Plackett–Burman, Box-Behnken, TTP,

Uricase (EC 1.7.3.3, UC) is an enzyme belonging to the class of the oxido-reductases and catalyses the oxidation of uric acid to allantoin, and thus plays an important role in the purine degradation pathway. Uricase in its conjugated form can be used as a therapeutic enzyme for the treatment of hyperuricemia and gout (Nancy et al. 2006). It is also used as a clinical reagent for the determination of blood and serum uric acid concentrations (Ademek et al. 1989). Further, immobilized Uricase can be used as a uric acid biosensor (Fatma, 2008) and its recombinant form (Rasburicase®) is effectively used to prevent acute tumoral lysis syndrome (Cannella and Mulkis, 2005). *Aspergillus flavus* native uricase enzyme is available for clinical use and manufactured in France and Italy as Uricozyme® (Bomalaski et al, 2002). Thus, uricase turns out to be a very important therapeutic enzyme which is immensely required in its purest form with high activity.

Microorganism, animals and higher plants are capable of producing uricase on its own, but human being cannot produce uricase because of the mutation in the fifth exon of uricase gene. So human being has to depend on the other easily available sources like microbes. In literature, it has been reported that various microorganisms are highly capable of producing uricase, where

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optimization of fermentation medium to produce high active uricase and its purification has been the prime areas of the research in those fields. The complexity of the interaction of medium components with the metabolic activity of the cell and also the production of enzyme is the big hindrance for a satisfactory detailed modeling. Statistical experimental design has been successfully used for several decades and it can be adopted at various stages of optimization techniques, such as screening experiments for targeted response. The Plackett–Burman design (Liu et al. 2003) and response surface methodology with various designs turn out to be good choices in applying statistical designs to bioprocessing, as they efficiently provide the best and most accurate results with meager implementation of man power. (Vohra and Satyanarayan, 2003; Abdel-Fatteh, 2002).

Purification of crude enzyme has become inevitable due to its commercial demand in its purist form. Though many traditional purification techniques like salting out, ion-exchange, affinity chromatography, and gel filtration have been used to purify enzyme, most of them involved a number of steps and furthermore the scale up of these methods are difficult and moreover expensive. In view of this, an attempt is made to develop simple, economical and more ease (easy) and efficient methods of separation and purification of uricase. Three-phase partitioning (TPP) is the method which uses an aqueous inorganic medium and an organic medium to purify the crude protein either in the bottom aqueous medium or in the interface. Kosmotropy, salting out, co-solvent precipitation, isoionic precipitation, osmolytic electrostatic forces, conformation tightening and protein hydration all contributes for the enzyme segregation in the interface.

In this present study, different fungal species belongs to *Gliocladium* and *Gliomastix* genera were used to produce highly active uricase using submerged fermentation technique in shake flasks. The strains were prior induced using suitable inducing agent in the basal medium. The basal medium was further optimized using statistical experimental design to identify the significantly influencing variables and optimum composition of significant variables. In this present work, Plackett-Burman design and Box-Behnken design has been used to optimize the medium composition for the production of highly active therapeutic enzyme 'uricase'. The statistical analysis and data interpretation is done using statistical tool Design Expert® 8.0. Ammonium sulphate and *t*-butanol system has been used in TPP to partially purify the uricase enzyme. All the TTP experiments were carried out using crude enzyme. Various parameters required for the efficient separation and purification of uricase like inorganic phase saturation, ratio between organic phase and crude enzyme, and operating temperature were optimized to get high fold purification and yield.

Material and Methods

Microorganisms

Five different fungal strains were used in this present study to find there efficacy to produce uricase enzyme. All the five fungal strains (*Gliomastix sp* MTCC 3869, *Gliocladium viride* MTCC 3835, *Gliocladium catenulatum* MTCC 2340, *Gliomastix murorum var.polychroma* MTCC 3830, *Gliocladium roseum* MTCC 4940) were purchased from Microbial Type Culture Collection (MTCC), Chandigarh, India. Purchased strains were stored on potato dextrose agar slant under refrigeration and periodically sub-cultured.

Induction Medium

The purchased fungal strains were prior induced in solidified induction medium to acclimatize the strains for the production of Uricase enzyme. Uric acid was used as an inducing agent in the induction medium. The

solidified uric acid induction medium or the basal medium was prepared using sucrose (20 g/L), uric acid (3 g/L), di-potassium hydrogen phosphate, magnesium sulphate heptahydrate (0.5 g/L), sodium chloride (0.5 g/L), ferrous sulphate (0.01 g/L) and agar (15.0 g/L). The pH of the induction medium was adjusted to 6.8 using 0.1N NaOH solution and was maintained at 32^{o}C for seven days.

Fermentation or Basal Medium

In this present study, submerged fermentation method in shake flasks was used to produce uricase enzyme. Two 10mm discs were cut and removed from the six day old fungal strain of the solidified induction medium plates. Then the two discs were inoculated in a 50mL basal fermentation medium. The basal fermentation medium was prepared using sucrose (20 g/L), uric acid (2 g/L), di-potassium hydrogen phosphate (1 g/L), magnesium sulphate heptahydrate (0.5 g/L), sodium chloride (0.5 g/L), ferrous sulphate (0.01 g/L). The pH of the fermentation medium was adjusted to 7.5 using 0.1N NaOH solution. The fermentation medium was incubated for 7 days at 30°C in an orbital shaker at 150rpm. 5 mL of fermentation medium samples were drawn periodically to measure the enzymatic activity. For determining the enzyme activity, the mycelia of the fungus was collected by centrifugation at 8000 rpm at 4°C for 10 minutes. The cell free supernatant was then used to determine the enzymatic activity.

Enzyme assay

The enzymatic assay was carried out by the method described by Mahler *et al* (1955). A 3 mL of 20mM boric acid buffer solution having pH of 9.0 was added with 75µL of 3.57mM uric acid solution and 20µL of cell free supernatant (used as crude enzyme), at 25°C. Blank solution was separately prepared by adding 20µL of buffer solution instead of the cell free supernatant. The blank and the test solutions were incubated at 25°C for ten minutes. The reduction in the uric acid concentration in the test sample was measured using UV-visible spectrophotometer at 293nm. The difference between the absorbance of the test and blank was equivalent to the decrease in the uric acid concentration during the enzymatic reaction. Thus, one unit (U) of enzyme activity was defined as the amount of uricase required to convert 1 µmol of uric acid into allantoin per minute at 25 °C and at a pH of 9.0, considering the milli molar extinction co-efficient of uric acid (ε) at 293nm as 12.6 $mM^{-1}cm^{-1}$(Machinda and Nakanishi, 1980).

Statistical designs

In this present study, Design Expert ® 8.0 was used to perform the medium optimization. Two different optimization techniques were used to optimize the fermentation medium composition. Initially Plackett-Burman method was used to screen and identify the most significant media components for the production of uricase enzyme. Next Box-Behnken method was used to identify the optimum quantity of the identified significant media components to produce high activity uricase enzyme.

Plackett–Burman design

Plackett-Burman technique was initially used to optimize the submerged fermentation process in shake flasks. Fermentation medium compositions were initially screened to identify the significant variables which will significantly influence the production of Uricase enzyme. Based on the Plackett-Burman factorial design, each factor was examined in two level, namely low level (-1) and high level (+1) (Plackett and Burman, 1946). In the present study, seven variables were tested at two levels and a total of eight experiments were conducted. Medium components like sodium chloride,

magnesium sulphate, zinc sulphate, were considered as dummy variables with constant concentrations in all the trials, these variables are required for the growth and metabolic activity of the *Gliocladium virde* **(Table 1)**. 100 µL of spore suspension of six day old *Gliocladium virde* from the uric acid induction medium was transferred to the 50 mL of production media for the Plackett–Burman design, whose compositions are listed in **Table 2**.

Table 1: Media components and test levels for Plackett–Burman experiment

Sl. No.	Components	Codes	Low Level (g/L)	High Level (g/L)	Constant Level of Dummy Variables (g/L)
1	Sucrose	X_1	10	30	-
2	Glucose	X_2	10	30	-
3	Peptone	X_3	5	20	-
4	Yeast Extract	X_4	1	10	-
5	K_2HPO_4	X_5	1	3	-
6	$CuSO_4.5H_2O$	X_6	0	0.15	-
7	$FeSO_4$	X_7	0.01	0.2	-
8	$MgSO_4.7H_2O$	X_8	-	-	0.5
9	NaCl	X_9	-	-	0.5
10	$ZnSO_4$	X_{10}	-	-	0.15
11	Uric Acid	X_{11}	-	-	3

Table 2: Plackett–Burman experimental design for evaluating factors influencing uricase production using *Gliocladium virde*

Runs	Sucrose g/L(X1)	Glucose g/L(X2)	Peptone g/L(X3)	Yeast Extract g/L(X4)	K_2HPO_4 g/L(X5)	$CuSO_4$ g/L(X6)	$FeSO_4$ g/L(X7)	NaCl g/L(X8)	$MgSO_4$ g/L(X9)	$ZnSO_4$ g/L(X10)	Uric Acid g/L(X11)	Response Activity U/mL
1	1	-1	-1	1	-1	1	1	-	-	-	-	73.16
2	1	1	-1	-1	1	-1	1	-	-	-	-	16.14
3	1	1	1	-1	-1	1	-1	-	-	-	-	44.98
4	-1	1	1	1	-1	-1	1	-	-	-	-	69.92
5	1	-1	1	1	1	-1	-1	-	-	-	-	83.12
6	-1	1	-1	1	1	1	-1	-	-	-	-	63.76
7	-1	-1	1	-1	1	1	1	-	-	-	-	58.89
8	-1	-1	-1	-1	-1	-1	-1	-	-	-	-	20.67

Table 3: The main effect, % contribution and p-values of each factor (Plackett-Burman)

Variables	Main Effect	% Contribution	F-Value	p-Value	Sum of Square	Degrees of freedom
Sucrose(X_1)	1.05	0.052	-	-		
Glucose(X_2)	-10.26	5.00	22.69	0.0176	210.70	1
Peptone(X_3)	20.80	20.54	93.22	0.0024	865.70	1
Yeast Extract(X_4)	37.32	66.11	300.1	0.0004	2786.16	1
K_2HPO_4 (X_5)	3.30	0.52	-	-		
$CuSO_4$ (X_6)	12.73	7.69	34.88	0.0097	323.95	1
$FeSO_4$ (X_7)	1.38	0.091	-	-		

Plackett–Burman experimental design does not describe interactions among the variables and it is used to screen and evaluate the important variables that would significantly influence the response. Plackett–Burman experimental design is based on the first order model, which is as follows:

$$y = \beta_o + \sum \beta_i X_i \quad (1)$$

where, Y is the response (enzyme activity), β_o is the model intercept and β_i is the linear coefficient, and X_i is the level of the independent variable. **Table 2** represents the design matrix of the Plackett–Burman experiment. All the experiments were carried out in duplicates and the averages activity is reported as the final response in **Table 2**.

Box–Behnken design

The first three variables of highest effects and highest percentage contribution were considered as significant variables, which were deduced by the Plackett-Burman experimental design as shown in **Table 3**. They were further analyzed at three levels of concentration to find out the most optimal values for producing highly active uricase enzyme. The three levels were codes as -1, 0 and +1 representing low, middle and high concentrations respectively as shown in **Table 4**. To describe the nature of the response surface in the experimental design, a three level Box-Behnken design was applied (Box and Behnken, 1960). According to the design, seventeen combinations were conducted and their observations were fitted to the following second order equation as represented in Eq 2. **Table 5** represents the design matrix of the 17 trial Box–Behnken experiment. For predicting the optimal point, a second order polynomial function was fitted to correlate a relationship between independent variables and its response (Uricase activity), which also gave the interactive effects of the variables, which is as follows,

$$y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (2)$$

where Y is the predicted response, βo model constant, X_1, X_2 and X_3 are independent variables, β_1 ,β_2, β_3 are linear coefficients, β_{12}, β_{22} and β_{33} are cross product coefficients and β_{11}, β_{22} and β_{33} are quadratic coefficients. The quality of the fit of the polynomial model equation is expressed by the coefficient of determination R^2.

Table 4: The levels of variables for the Box–Behnken optimization experiment

Variable	Variable Code	-1	0	+1
Peptone	X_1	5	12.5	20
Yeast Extract	X_2	5	10	15
$CuSO_4$	X_3	0	0.5	1

Data analysis and optimization

The data of the enzyme activity of each trial was subjected to analysis, using statistical tool Design Expert® 8.0 for Plackett–Burman and the Box–Behnken experiment. Design Expert® 8.0 uses least square regression analysis to fit a model equation to the given data set. For the response surface, a simplex search algorithm was used to find the optimal points of the factors.

Purification of Uricase

In this present study, three phase partition was used to purify the crude enzyme. Ammonium sulphate and *t*-butanol was used in three phase partition to purify the enzyme. The crude enzyme solution (supernatant) obtained by centrifuging the fermentation broth was brought to 30, 50 and 70% (w/v) saturations using ammonium sulphate. To this solution, *t*-butanol was

added in order to obtain 1:0.5, 1:1 or 1:1.5 (v/v) ratio of crude enzyme to *t*-butanol at the specified temperature. After phase separation incubation for 1 h at various temperature conditions 30, 45 and 60°C, the mixture was centrifuged at 2000×*g* for 5 min to facilitate separation of phases. All the three phases were analyzed for uricase activity. The specific activity of each phase was determined and the phase that gave the highest specific activity was chosen. The interfacial layer gave the highest uricase recovery. The best conditions which resulted into maximum recovery were used as standard purification procedure.

Table 5: Box–Behnken factorial experimental design, showing the response of uricase enzyme activity as influenced by Peptone, Yeast Extract and CuSO4.

Run	Variable X_1 Peptone g/L	Variable X_2 Yeast Extract g/L	Variable X_3 $CuSO_4$ g/L	Measured Activity (U/mL)	Predicted Activity (U/mL)
1	-1	-1	0	62.86	64.0105
2	1	-1	0	71.891	71.2103
3	-1	1	0	72.457	73.1377
4	1	1	0	82.097	80.9465
5	-1	0	-1	62.971	62.9546
6	1	0	-1	71.633	73.4479
7	-1	0	1	75.692	73.8771
8	1	0	1	78.376	78.3924
9	0	-1	-1	63.107	61.9729
10	0	1	-1	79.628	78.9636
11	0	-1	1	76.801	77.4654
12	0	1	1	78.204	79.3381
13	0	0	0	81.011	*82.8936*
14	0	0	0	83.348	82.8936
15	0	0	0	80.973	82.8936
16	0	0	0	84.412	82.8936
17	0	0	0	84.724	82.8936

Results and Discussion

Production in the basal production medium

Out of all the fungal strains tested in the basal media, *Gliocladium virde* showed highest uricase activity which is as shown in **Figure 1**. The enzymatic activity obtained by the *Gliocladium virde* in the basal medium was 63.14 U/mL on the 5 day incubation, where uric acid was used as a sole nitrogen source.

Optimization of Incubation time

100 µL of spore suspension recovered from the six day old induction medium culture of *Gliocladium virde* was transferred to

50 mL production medium for the Plackett–Burman design, whose composition are as tabulated in **Table 2**. After inoculation, enzyme activity was determined on second, third, fourth and fifth day. Fourth day activity was chosen for further data analysis, since fourth day was identified to be optimal incubation day, which gave the highest activity.

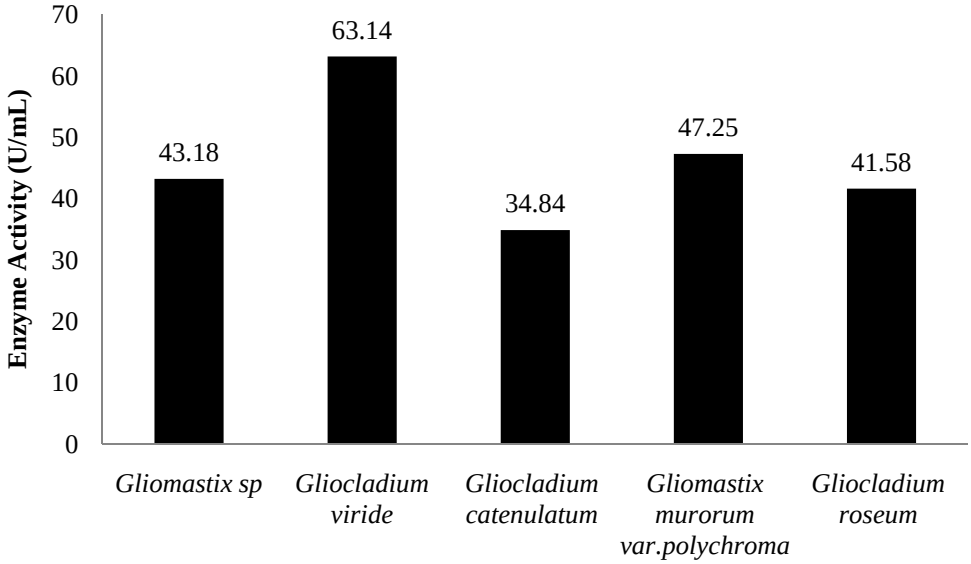

Figure 1: Enzyme Activities of the Five Screened Fungal species

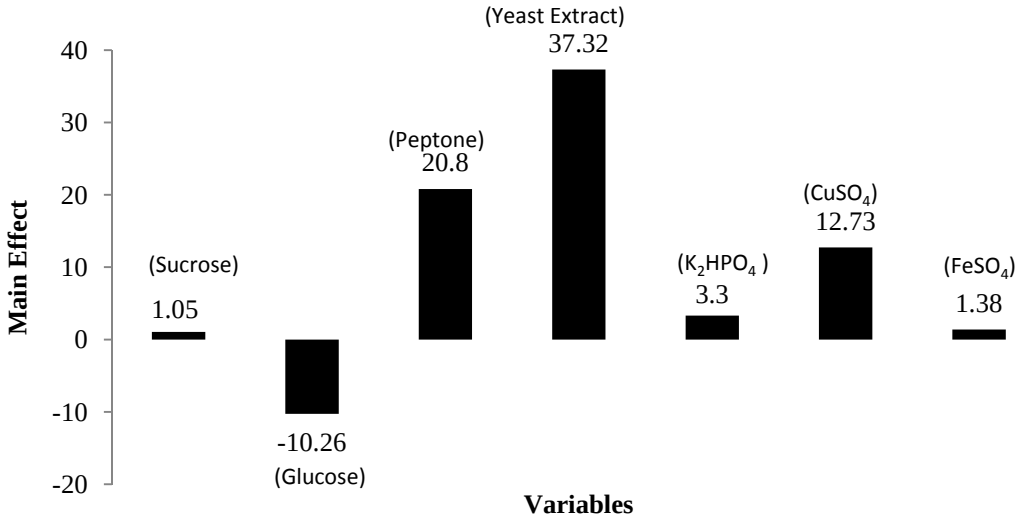

Figure 2: Pareto Chart showing the main effects of the variables on enzymatic activity

Optimization of uricase production by multi-factorial experiments

Evaluation of the factors affecting uricase productivity

In the first approach, the Plackett-Burman experimental design was used to identify the most significant media components to produce high activity uricase. Seven different variables were chosen to perform this optimization process, where the activity values of the fourth day old cultures were considered. The main effect of each variable upon uricase activity was estimated as the difference between both averages of the measurements made at the high level (+1) and at the low level (-1) of that factor. The data in **Table 2** shows a wide variation of the enzymatic activity, which reflects the variations caused due to the presence of different media components at low and high levels considered in various combinations. The Pareto chart representing the main effects of various media components is shown in **Figure 2**.

Based on the magnitude of main effect and percentage contribution of the media components, which were obtained from the data analysis, Yeast Extract, Peptone, $CuSO_4$, K_2HPO_4, $FeSO_4$, sucrose showed positive effect on the enzymatic activity, indicating that higher concentrations of these components could increase the activity, and glucose which contributed negatively to the enzyme activity will fetch a higher activity at lower concentrations. The first three significant variables, Yeast extract, Peptone and $CuSO_4$ were selected to be the most significant variables and were selected for further analysis using Box-Behnken experiment.

In particular, peptone and yeast extract have meat extracts which are rich in purines, which may be acting as media components, potentially inducing uricase productivity. The induction effect of meat extract for uricase production has been proved and reported previously by several researchers. Machinda and Nakanishi in 1980 have studied the induction effects using other purines like adenine, guanine, hypoxanthine, theobromine, uric acid and theophyline, in which uric acid was proved to be the best inducer for uricase production and hypoxanthine being the next. Watanabe *et al* (1969) studied the effects of purine bases like adenine, guanine, hypoxanthine, xanthine on the uricase productivity by *Streptomyces* species. In their work, when meat extracts were omitted from the media, a significant reduction in the uricase production and uric acid consumption was observed, while the cell growth was unaffected. Their results indicated that the purine bases, present in meat, like hypoxanthine might have effects on the uricase production by *Streptomyces* species. Glucose was found to have a negative effect on uricase activity, indicating that less concentrations of glucose support higher activities and glucose has to be used at lower concentrations. Glucose turned out to be a medium component effecting uricase activity negatively even in case of *Pseudomonas aeroginosa* during media optimization using Plackett-Burman experimentation (Yaseer et al. 2005).

The Polynomial model describing the relationship between the seven factors and the uricase activity could be presented as follows:

$$Y_{Activity} = 53.84 + 0.52X_1 - 5.13\,X_2 + 10.40\,X_3 + 18.66\,X_4 + 1.65\,X_5 + 6.36\,X_6 + 0.69\,X_7 \quad (3)$$

The p-Value of the Plackett-Burman model was 0.0013 (less than 0.05), which indicates that the model is significant.

Optimization of Significant variables by Box-Behnken Design

The most significant variables (peptone, yeast extract, and $CuSO_4$) identified

using the Plackett-Burman statistical method was further subjected to the Box-Behnken method to optimize the magnitude of those variables. **Table 5** shows the design matrix for the Box-Behnken design. **Table 6** represents the Analysis of Variance table for the response surfaces. **Figure 3** shows the interaction effects of each of the two variables on the enzyme activity. For predicting the optimal point within experimental constrains, a second-order polynomial function was fitted to the experimental results of uricase activity, which is as follows

$$Y_{activity} = 82.89 + 3.75\,X_1 + 4.72\,X_2 + 3.97\,X_3 + 0.15\,X_1X_2 - 1.5\,X_1X_3 - 3.78\,X_2\,X_3 - 6.42\,X_1^2 - 4.15\,X_2^2 - 4.31\,X_3^2 \quad (4)$$

In **Table 5**, the reported measured activity values were obtained experimentally, while the reported predicted values were generated by the second order polynomial equation (**Equation 4**), where X_1, X_2, X_3 are the concentrations of peptone, Yeast Extract and $CuSO_4$ respectively. At the model level, the correlation terms (R and R^2) are estimated. The correlation between the measured and predicted values was done by the estimating correlation coefficient R. The more the value of R is near or equal to 1, the better is the fit. The value of correlation coefficient R, was determined using Design Expert® 8.0 and was found to be 0.964. The value of determination coefficient R^2 is 0.929 for uricase activity, being a measure of fit of the model, indicates that 7.1 % of the total variations are not explained by the enzyme activity, which may have been caused due experimental errors.

Table 6: ANOVA for Response Surface Quadratic Model

Source	Sum of Square	Degrees of freedom	F-value	*p*-value	Co-efficients
Model	842.52	9	24.53	0.0002	82.89
X_1	112.63	1	29.51	0.0010	3.75
X_2	177.92	1	46.62	0.0002	4.72
X_3	125.88	1	32.99	0.0007	3.97
X_1X_2	1	0.093	0.8805		0.15
X_2X_3	1	8.93	0.1698		-1.49
X_3X_1	1	57.14	0.0061		-3.78
X_1^2	1	173.39	0.0003		-6.42
X_2^2	1	72.52	0.0033		-4.15
X_3^2	1	78.16	0.0027		-4.31

Table 7: Overall purification of Uricase from *Gliocldium viride* MTCC 3835

Steps	Uricase Activity (U/mL)	Total Protein (mg/mL)	Specific Activity (U/mg)	Purification Factor (Folds)	Percentage Yield (%)	Concentration Factor (-)
Crude Extract	84.21	0.589	142.977	1	100	1
Three phase partitioning	67.46	0.326	206.649	1.44	80.109	1.81

The nature of the 3D surface plot (**Figure 3**) shows that the highest activity is obtained within the chosen ranges of the factors, and its optimum concentrations can be obtained by extrapolating the highest point on the surface plot. From the F**igure 3**, the optimum values of the significant factors from Box-Behnken design were found to be: Peptone-12.71g/L, Yeast Extract-10.57 g/L, $CuSO_4$-0.0762 g/L, which were obtained by choosing the highest point on the response surface and extrapolating the plot to the respective axes. The optimal value of the uricase activity from the regression equation was found to be 84.9176 U/mL (**Figure 3**).

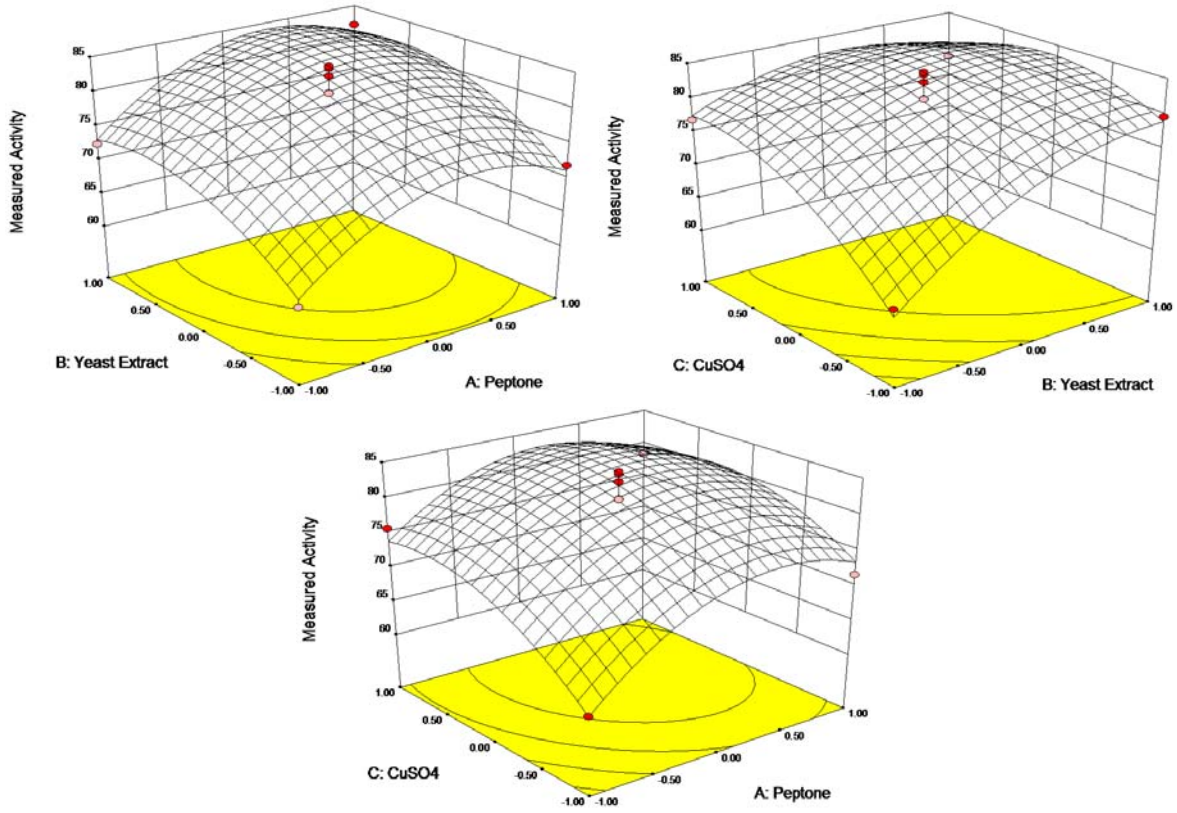

Figure 3: Uricase enzyme activity (U/mL) response surface from *Gliocladium viride* as affected by culture conditions.

Verification of the Model

Optimal concentrations of the factors, obtained from the optimization experiment were verified experimentally and compared with the predicted data. The measured uricase activity was 82.1 U/mL, where the predicted value from the polynomial model was 84.9 U/mL. The verification revealed a high degree of accuracy of the model of more than 96.7%, indicating the model validation under the tested conditions.

Three phase partitioning of uricase

In order to determine the suitable phase condition for the efficacy of TPP, optimization of various process parameters has to be investigated. The influence of different parameters, such as percent saturations of ammonium sulfate (w/v),

crude extract to t-butanol ratios and different temperature conditions were studied. In all these optimization experiment, uricase got segregated at the interface of the aqueous and organic phase, where the initial protein concentration of the crude extract was found to be 0.589 mg/ml. The effect of ammonium sulphate saturation on the purification fold and activity recovery was studies using three different percentage saturation 30, 50 and 70% w/v. From the data, it was observed that the increase in the percentage saturation increased the purification fold and beyond 50% saturation the purification fold decreased. Hence the ammonium saturation was fixed as 50% for further experiments.

As per the literature, t-butanol was selected as organic solvent, since t-butanol is a branched and relatively larger molecule; it does not diffusion easily in to the folded uricase molecule and hence the possibility of denaturing is limited. At fixed ammonium saturation (50% w/v), t-butanol molar ration was varied from 1.05 to 1 (v/v). Maximum fold purification and uricase activity recovery was observed at 1:1 t-butanol and to crude extract. Similarly, the optimal temperature for the maximum purification and activity was observed at 30^{o}C. Upon testing for the presence of uricase in all the three phases after three phase partitioning, it was observed that uricase was completely concentrated into the interfacial layer, and there was no uricase recovery from the aqueous salt phase and the organic phase. The uricase activity of the interfacial layer was measured to be 67.46 U/mL after three phase partitioning. **Table 7** represents the overall purification data of uricase from *Gliocladium viride* MTCC 3935.

Conclusion

Form this study, *Gliocladium viride* MTCC3835 was found to produce high activity uricase (63.14U/mL), which is higher than the activity reported in the literatures like, *Mucor hiemalis* (1.25U/mL) (Yazdi et al. 2006), *Aspergillus carbonarious* (0.16U/mL), *Botrytis fabae* (0.13U/mL), *Aspergillus sydowii* (0.093U/mL) (Mohmoud etal. 1996), *Aspergillus terreus*, *Trichoderma* sp and *Aspergillus flavus* (Abdel-Fattah and Abo-Hameed, 2002). Using Plackett-Burman design, it was found that peptone, yeast extract and CuSO4 have significant effect on the uricase activity. The optimal composition of the significant variables (peptone-12.71g/L, yeast extract-10.57 g/L, $CuSO_4$-0.0762 g/L) were found using Box-Behnken design. Finally the uricase activity after media optimization was found to be 82.1 U/mL, which was 1.344 times more than the basal medium. Crude uricase produced was purified using three-phase partitioning (TPP), and significant factors for protein partitioning like optimum percentage saturation of ammonium sulphate, the crude extract to t-butanol ratio (v/v) and the temperature for partitioning were optimized. Crude enzyme solution saturated to 50%(w/v) with ammonium sulphate and with a crude extract to *t*-butanol ratio of 1:1(v/v) at 30°C resulted in 80.109% recovery of uricase with a purification fold of 1.44.

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